INLEIDING

De voorjaarsbijeenkomst van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) vindt in 2007 plaats op 16, 17 en 18 april te Papendal.

Op dinsdagochtend wordt de bijeenkomst geopend met de plenaire sessie getiteld: 'Microbes under Stress Conditions'. Micro-organismen ondervinden net als mensen veel stress, zowel op de werkvloer als tijdens de uitoefening van hun hobby's. De manier waarop micro-organismen hiermee omgaan is onderwerp van een uitdagende sessie, dus komt allen uitgerust naar Papendal!
Vorig jaar zijn alle leden voor het eerst uitgenodigd een voorstel voor een thematische sessie in te dienen. Van deze mogelijkheid is uitgebreid gebruikgemaakt. De geselecteerde sessies zijn door de indieners enthousiast en professioneel ingevuld, hetgeen onder meer blijkt uit de vele positieve reacties die wij na afloop van de bijeenkomst ontvingen. Deze succesvolle formule herhalen we dit jaar en dit heeft wederom een bont, gevarieerd en veelbelovend programma opgeleverd. We hopen dat de geselecteerde sessies op een zelfde hoog niveau worden ingevuld als vorig jaar.

In 2006 werd voor het eerst de uitreiking van de posterprijs gevolgd door een groot feest voor alle geledingen van NVvM en NVMM. Wij zijn benieuwd of ook het feest dit jaar hetzelfde niveau zal halen.
Om de voorjaarsvergadering voor jonge microbiologische onderzoekers zo interessant mogelijk te maken, is er naast een feest en de mogelijkheid om een posterprijs te winnen, ook een tegemoetkoming in de kosten. Stichting Antonie van Leeuwenhoek heeft vorig jaar de verblijfskosten van jonge microbiologische onderzoekers die hun werk presenteren vergoed en zal dit in 2007 wederom doen. Zeer hartelijk dank hiervoor! De vrijstelling van inschrijfkosten voor deze jonge onderzoekers blijft uiteraard gehandhaafd.
Sinds twee jaar wordt de voorjaarsvergadering voorafgegaan door een dag voor de arts-assistenten in opleiding. Zij nemen dan deel aan een landelijke toets en volgen een aantal lezingen. Dit bevalt dusdanig goed dat we er vanuit mogen gaan dat de voorjaarsvergadering voortaan altijd vooraf zal worden gegaan door deze 'assistentendag'.

Wij wensen allen een inspirerende voorjaarsvergadering 2007.

Het programma van het ochtendsymposium ziet er als volgt uit:
- **Salivating for knowledge: Strategies used by Group A *Streptococcus* to survive under stress in the throat**
  J.M. Musser, Baylor College of Medicine, Houston, USA
- **Job-related stress in *Saccharomyces cerevisiae*: adaption to industrial process conditions**
  J.T. Pronk, Delft University of Technology & Kluyver Centre for Genomic of Industrial Fermentation, Delft
- **Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population**
  R. Andino, Mission Bay Genentech Hall, San Francisco, USA
- **SET regulation in asexual and sexual Plasmodium parasites reveals a novel mechanism of stage-specific expression**
  A.P. Waters, Leiden University Medical Centre, Leiden

Posterbeoordelingscommissie
Mw. Drs. L.M. Kortbeek, voorzitter
Dr. W. Bitter
Prof. dr. S. Brul
Dr. J. Kluytmans

De NVMM en de NVvM organiseren deze bijeenkomst in samenwerking met
Microbiële Oecologie, Technische Microbiologie en Mycologie
Nederlandse Vereniging voor Medische Mycologie
Nederlandse Werkgroep Klinische Virologie
Sectie Algemene Virologie
Sectie Levensmiddelenmicrobiologie
Secties Algemene en Moleculaire Microbiologie
Stichting Kwaliteitsbewaking Medische Microbiologie
Werkgemeenschap Microbiële Pathogenese
Werkgroepen Oost en West Medische Microbiologie
Werkgroep Epidemiologische Typeringen
Werkgroep Moleculaire Diagnostiek Infectieziekten

ZonMw

ALW

Congress Care

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Dates
16, 17 & 18 April 2007

Venue
Hotel en Congrescentrum Papendal
Papendallaan 3
Arnhem
Tel. 026 483 79 11

Website
Please check www.congresscare.com for up-to-date program information and www.nvmm.nl or www.nvvm-online.nl for more information on the NVMM or NVvM.

Language
The language will be English during the scientific sessions, unless stated otherwise.

Accreditation
The 'Wetenschappelijke Voorjaarsvergadering 2007' will be accredited by the NVMM with 8 points for day 1 (Tuesday April 17th) and 5 points for day 2 (Wednesday April 18th).

Name badges
All participants should wear their name badges throughout the conference.

Registration desk
The registration desk will be open on Monday, Tuesday and Wednesday during conference hours.

Poster session
Posters will be on display throughout the congress. The numbers on the poster boards correspond with the abstract numbers in the program/abstract book. Poster authors are requested to man their posters on Tuesday evening April 17th from 20:30 - 22:00 hours.

Poster price
Yakult Nederland sponsors the poster price for the best poster and the poster price ceremony with drinks. The price is € 250.
The poster price ceremony will be held on Tuesday April 17th at 22:00 hours. The winner has to be personally registered and present.

Young Investigator's Grant
The registration fee for presenting AIOS (oral or poster, presenting author only) will be waved.

Grant 'Antonie van Leeuwenhoek Stichting'
The 'Antonie van Leeuwenhoek Stichting' supports PhD-students and AIOS to attend the
'Wetenschappelijke Voorjaarsvergadering' and therefore they will sponsor the hotel costs (1 night, Hotel 2) for presenting PhD-students and AIOS (poster or oral, presenting author only). Please fill out on the registration form whether you would need a hotel room and please provide the congress secretariat with a written certification by the head of the department or employer stating you are a PhD-student or AIOS.

Dance Party 'Groot Microbiologie Feest'
The poster price ceremony will be followed by a dance party open for all participants.

Catering
Exhibition, lunch break, coffee/tea break. Coffee and tea will be available at all times at the exhibition. The lunch will be served at the exhibition during the lunch break.

Hotel rooms
If you have reserved a hotel room you may collect the room key as of 13:00 hours at the front desk of the hotel. Please make sure to check out before 10:00 hours.

Hotel en Congres Centrum Papendal
All participants receive a route description together with their confirmation of registration. For more info please check www.papendal.nl
Papendal taxi: The Papendal taxi will bring you from Central Railway Station Arnhem to Hotel en Congrescentrum Papendal (€ 6,50 per person). If you would like to use this service, please call 026-3210000 (mention the Papendal taxi). You have to pay at arrival at the hotel reception. At the end of congress you can order at the hotel reception a Papendal taxi to bring you to the railway station.

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Sponsor poster price and drinks on Tuesday evening, April 17th:

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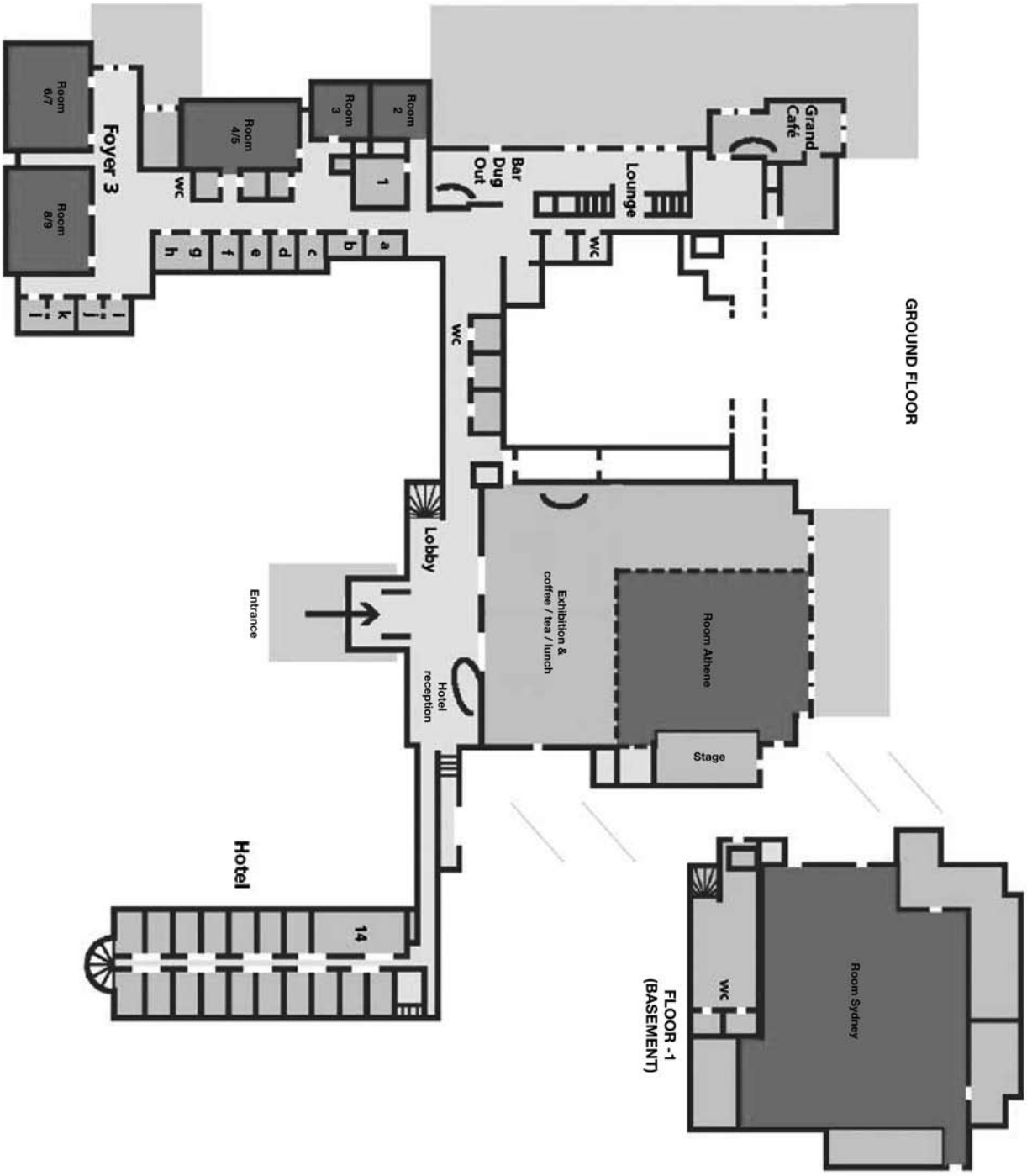

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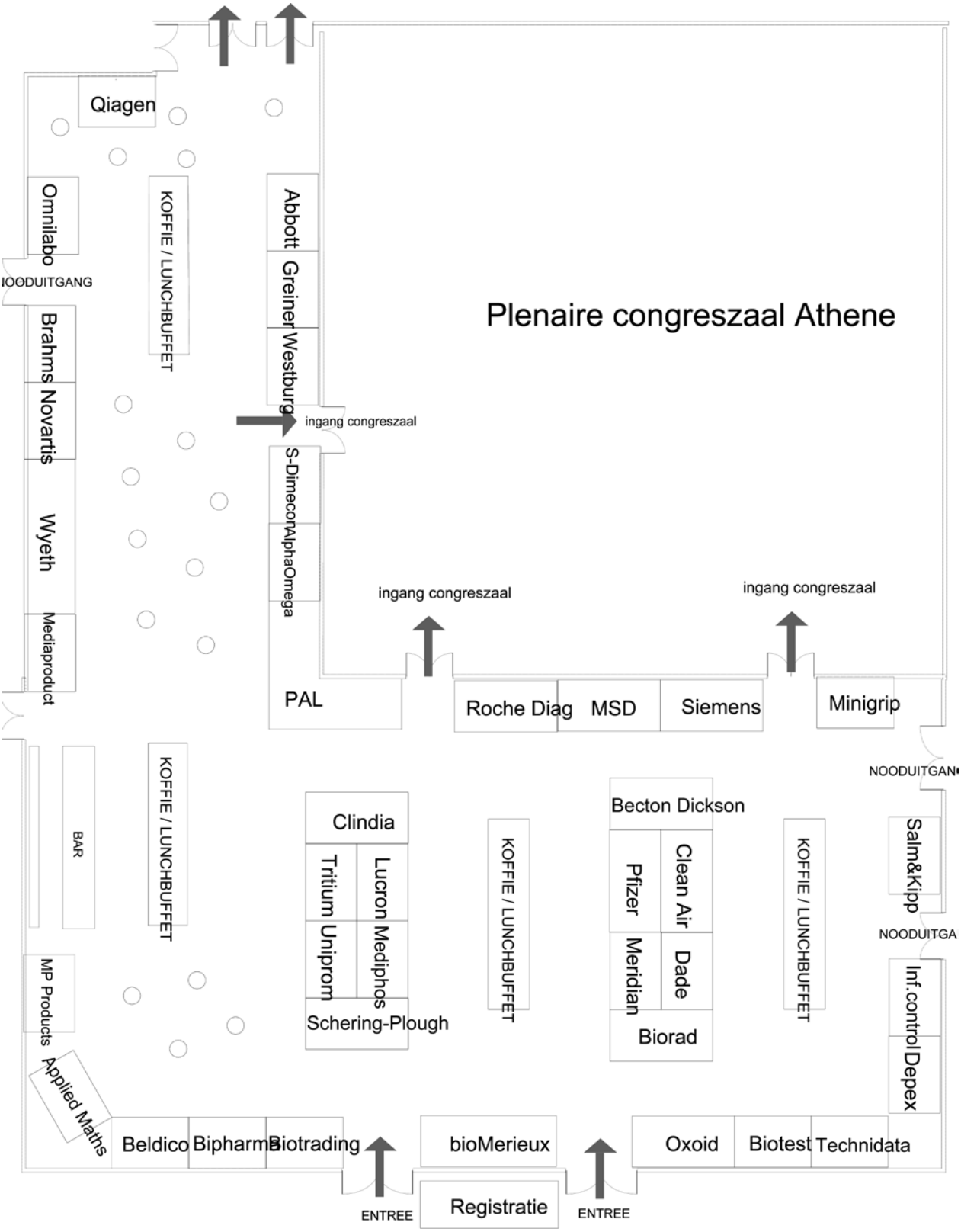

MONDAY 16 APRIL 2007

Room Sydney

12:00	Registration and lunch	
13:00 - 15:00	National Examination for medical microbiologists in training	
15:00 - 15:30	Coffee/tea	
15:30 - 16:15	Anaerobic infections and diagnostic laboratory practices	
	J. Brazier (Cardiff, UK)	
16:15 - 17:00	Rationele keuzes en doseringen van antibiotica	
	J.W. Mouton	
17:00 - 17:45	Toxoplasmose	
	L.M. Kortbeek	
18:30	Dinner	

TUESDAY 17 APRIL 2007

Room Athene **Plenary session 'Microbes under Stress Conditions'**

Chairmen: H. Wösten & J. Galama

09.30 - 10:15	J.M. Musser (Houston, USA)	O001
	Salivating for knowledge: strategies used by group A *Streptococcus* to survive under stress in the throat	
10:15 - 11:00	J.T. Pronk (Delft)	O002
	Job-related stress in *Saccharomyces cerevisiae*: adaption to industrial process conditions	
11:00 - 11:30	Break	
11:30 - 12:15	R. Andino (San Francisco, USA)	O003
	Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population	
12:15 - 13:00	A.P. Waters (Leiden)	O004
	SET regulation in asexual and sexual Plasmodium parasites reveals a novel mechanism of stage-specific expression	
13:00 - 14:00	Lunch	

Room Athene **Lunch sponsorsymposium Novartis**

13:00 - 14:00

Room Athene **Parallel session 'Vectors and vector borne diseases'**

Chairman: J. van der Giessen

14.15 - 14:30	L.S. van Overbeek	O006
	Natural factors influencing microbial composition in ticks	
14:30 - 14:45	P.R Wielinga	O007
	Longitudinal analysis of tick densities and *Borrelia, Anaplasma* and *Ehrlichia* infection of *Ixodes ricinus* ticks in different habitat areas in the Netherlands	
14:45 - 15:00	C.B.E.M. Reusken	O008
	Introduction of the Asian Tiger mosquito in the Netherlands: development of methods for surveying (imported) mosquitoes for arboviruses	
15:00 - 15:15	T.J. Schuijt	O009
	The *Ixodes scapularis* salivary protein Salp15 inhibits complement mediated killing of complement sensitive *B. garinii* strains	
15:15 - 15:30	G.T. Noordhoek	O010
	Borrelia burgdorferi in ticks and patients in a family practice on the Dutch North Sea island of Ameland	
15:30 - 16:00	Coffee/tea	

Room Sydney **Parallel session 'Diagnostics'**

Chairman: M. Wolfhagen

14:00 - 14:15	M. Herremans	O011
	Comparison of a *Treponema pallidum* IgM immunoblot with a 19S FTA-ABS test for the diagnosis of congenital syphilis	
14.15 - 14:30	C.E. Visser	O012
	Evaluation of 6 different methods to identify nonfermentative Gram-negative bacteria in CF patients: 4 biochemical and 2 molecular methods	
14:30 - 14:45	J. Top	O013
	Evaluation of a rapid test panel, the API Strep-20, the BD Phoenix and VITEK-2 automated instruments, and Raman spectroscopy for species identification of Enterococci	
14:45 - 15:00	A.P. van Dam	O014
	Diagnostic value of a positive galacto-mannan assay in broncho-alveolar lavage fluid	
15:00 - 15:15	M.G.R. Hendrix	O015
	Robotizing of the agar dilution method for susceptibility testing of bacteria according to CLSI guidelines	
15:15 - 15:30	G.J. Wisselink	O016
	Comparison of PCR-Reverse Line Blot analysis and traditional culture of dermatophytes in clinical samples	
15:30 - 16:00	Coffee/tea	

Room 2 **Parallel session 'Microbial surface 1'**

Chairman: H. Wösten

14:00 - 14:30	F.M. Klis Fungal cell surface proteins	O017
14:30 - 15:00	N. Dekker Function and metabolism of (1,3)-alpha-glucan in fission yeast	O018
15:00 - 15:15	W.R. Teertstra Repellents function in cell wall integrity and development by forming amyloids	O019
15:15 - 15:30	J. Grijpstra Capsule biogenesis in *Cryptococcus neoformans*	O020
15:30 - 16:00	Coffee/tea	

Room 3 **Parallel session 'Sectie onderwijs: Het onderzoek meester'**

Chairman: L. van Alphen

14:00 - 14:15	W. Hoekstra Introductie: Mastering microbiology	O021
14.15 - 14:30	H.H. Schalk Zeker weten? - Leren de kwaliteit van biologie-onderzoek te bewaken in het VWO	O022
14:30 - 14:45	J. Verhoef Modernisering van het onderwijs bij de opleiding tot arts-microbioloog	O023
14:45 - 15:00	C. Bruggeman Een nieuwe opleiding: de opleiding tot moleculair medisch-microbioloog	O024
15:00 - 15:15	M.W. Reij Distance learning for food safety microbiology	O025
15:15 - 15:30	A. van Goor & M.M. Immink Presentatie website van de Sectie Onderwijs van de NVvM	O026
15:30 - 16:00	Coffee/tea	

Room 4/5 **Parallel session 'Pathogenesis: stressmanagement'**

Chairmen: Y. Pannekoek & A. van der Ende

14:00 - 14:30	J. Vogel (Berlin, Germany) Exploring small noncoding RNAs of bacterial pathogens	O027
14:30 - 14:45	A.H.M. van Vliet Stress-responsive gene regulation in *Helicobacter pylori*: hierarchy or organised chaos?	O028
14:45 - 15:00	Y. Pannekoek Riboregulation in *Neisseria meningitidis*	O029
15:00 - 15:15	A.M. Rolloos The Sab adhesins of *Helicobacter pylori*: acid-responsive regulation of expression and their role in the modulation of the host immune response	O030
15:15 - 15:30	M. Zwietering Quantification of the effects of salt stress and physiological state on thermotolerance of *Bacillus cereus* ATCC 10987 and ATCC 14579	O031
15:30 - 16:00	Coffee/tea	

Room 6/7 **Parallel session 'Progress in microbiology'**

Chairman: S. Brul

14:00 - 14:15	S.C.M. Haaijer *Thiobacilli* as key players in iron sulfide-associated denitrification	O032
14.15 - 14:30	S.A. Dar Niche differentiation of coexisting sulfate reducing bacteria in a full-scale sulfidogenic bioreactor	O033
14:30 - 14:45	J. Wang Microbial mediated iron oxidation in circumneutral wetland environments	O034
14:45 - 15:00	G. Roeselers Phototrophic biofilms: primary productivity as a major determinant of microbial diversity	O035
15:00 - 15:15	J.L.W. Rademaker DNA-markers for determination of microbiological quality of milk	O036
15:15 - 15:30	M.J.R. Nout Volatile flavour formation by solid-state fermentation of soya beans by *Bacillus* spp.	O037
15:30 - 16:00	Coffee/tea	

Room 8/9 **Parallel session 'SKMM'**

Chairman: G.J.J. van Doornum

14:00 - 14:30	Vergadering over toekomst SKMM	
14:30 - 15:00	V. Fingerle (Munich, Germany) Lyme borreliosis serodiagnosis – misty results from the solid phase?	O039
15:00 - 15:30	K.P. Hunfeld (Frankfurt, Germany) Is serological testing a reliable tool in laboratory diagnosis of syphilis? Insights from the German proficiency testing program	O040
15:30 - 16:00	Coffee/tea	

Room Athene **Parallel session 'Medical microbiology'**

Chairman: M. Wolfhagen

16.00 - 16:15	J.J. Kerremans Rapid bacterial identification and antimicrobial susceptibility testing decreases antibiotic use and accelerates pathogen directed therapy	O041
16:15 - 16:30	R.P.H. Peters Quantification of *Streptococcus pneumoniae* DNA in blood samples from patients with invasive pneumococcal infection	O042

16:30 - 16:45	A.M Pettersson-Fernholm	O043
	Clinical evaluation of an internally controlled *Tropheryma whipplei* real-time PCR	
16:45 - 17:15	G.J.H.M. Ruijs	O044
	"Wie zeg je?... Dokter Ruijs? OK, verbindt maar door". Het (telefonisch) intercollegiaal consult in de medische microbiologie	
17:15 - 17:30	M.G.R. Hendrix	O045
	Real-time polymerase chain reaction and immuno-fluorescent-antibody assay for the detection of viral and atypical bacterial pathogens in children with lower respiratory tract infection	

Room Sydney **Parallel session 'MRSA: Trends and threats'**

Chairman: C. Vandenbroucke-Grauls

16:00 - 16:30	E. Tiemersma	O046
	Epidemiologie van MRSA in Europa en Nederland	
16:30 - 17:00	A. Voss	O047
	MRSA en bronnen buiten het ziekenhuis	
17:00 - 17:30	J. Kluytmans	O048
	Hoe verder – Impact op het Nederlandse beleid	

Room 2 **Parallel session 'Microbial surface 2'**

Chairman: H. Wösten

16:00 - 16:30	F.R. Mooi	O049
	Surface structures of the Gram-negative bacterium *Bordetella pertussis*: role in pathogenesis and adaptation to vaccines	
16:30 - 17:00	M.P. Bos	O050
	Biogenesis of the Gram-negative bacterial outer membrane	
17:00 - 17:15	W. de Jong	O051
	Identification of novel surface proteins in *Streptomyces coelicolor* using MALDI-TOF Mass Spectrometry	
17:15 - 17:30	A. Hendrickx	O052
	Expression of five putative LPXTG surface proteins enriched in clinical and outbreak associated *Enterococcus faecium* isolates	

Room 3 **Parallel session 'Global Harmonisation in microbial food safety'**

Chairman: B. Marthi

16:00 –16:05	B. Marthi	
	Introduction	
16:05 - 16:30	B.H. ter Kuile	O053
	From risks to methods to regulation	
16:30 - 17:00	M.H. Zwietering	O054
	Global harmonisation of micro-biological criteria for foods	
17:00 - 17:30	J.M.B.M. van der Vossen	O055
	Overview of methods in use and their improvements	

Room 4/5 **Parallel session 'Otitis Media: pathogens and vaccines'**

Chairman: J. Hays

16:00 - 16:15	J. Hays	O056
	Otitis Media – getting an earful!	
16:15 - 16:45	E.A.M. Sanders	O057
	Do pneumococcal vaccines prevent Otitis Media?	
16:45 - 17:15	C. Aebi (Bern, Switzerland)	O058
	Moraxella catarrhalis vaccine development	
17:15 - 17:30	S. Verhaegh	O059
	Studies into outer membrane protein expression in various *Moraxella catarrhalis* populations	

Room 6/7 **Parallel session 'Phage biology & phage technology'**

Chairman: P.J.A. Haas

16:00 - 16:30	J.M. Musser (Houston, USA)	O060
	Molecular dissection of group A *Streptococcus* epidemic waves: the restless tide of phages	
16:30 - 16:45	W.J.B. van Wamel	O061
	Bacteriophages provide *Staphylococcus aureus* a toolbox to counteract the human innate immune system	
16:45 - 17:15	H. de Haard	O062
	Identification of therapeutic drug compounds with phage display	
17:15 - 17:30	M.A.P. van Bergen	O063
	Phage therapy of Salmonella infected broilers	

Room 8/9 **Parallel session 'WOGIZ: infectieziekten in de openbare gezondheidszorg: de nationale, regionale en lokale aanpak'**

Chairman: W. Dorigo

16:00 -16:05	R.A. Coutinho	O064
	Belang van een versterking van de regionale infectieziektebestrijding	
16:05 -16:30	P. Schneeberger & H. van den Kerkhof	O065
	Regionale infectieziektebestrijding in de praktijk: arts- microbioloog ontmoet arts-infectieziekten	
16:30 - 17:00	B. Mulder & D. Notermans	O066
	Kaas als bron van *Salmonella typhimurium* faagtype 560 uitbraak in Twente: de samenwerking in de praktijk	
17:00 - 17:30	R. van Essen & D. Veenendaal	O067
	SOA diagnostiek in Noord-Holland: Pionieren in de polder: de start van een Sense poli in de regio & Public health and open sexually transmitted disease outpatient clinic in small city areas in the Netherlands	

Room Athene **Plenary session**

Chairman: S. Brul

17:40 - 17:55 Kluyver lecture

17:55 - 18:20 Announcement Kiem price winner

Restaurant **Conference dinner**

18:30 - 20:30

Room Sydney **Poster session & drinks (sponsored by Yakult)**

20:30 - 22:00

20:30 - 21:15 Presentations odd poster numbers

21:15 - 22:00 Presentations even poster numbers

22:00 **Presentation Yakult Poster Price**

As of 22:15 **Dance party 'Groot microbiologiefeest'**

WEDNESDAY 18 APRIL 2007

Room 8/9 **Breakfast symposium Merck Sharp & Dohme**

07.30 - 07:45 Ontvangst en ontbijt

07:45 - 08:15 J.W. Mouton
Detectie en confirmatie van ESBL's

08:15 - 08:45 A.T. Bernards
Observation in the Netherlands of ESBL's, the ONE-study

Room Athene **Parallel session 'Metagenomics of microbial communities'**

Chairman: M. Jetten

09:00 - 09:30 M. Horn (Vienna, Austria) O070
Chlamydial symbionts of amoeba

09:30 - 10:00 J.H.J. Leveau O071
LIL-FISH in the big metagenomic pond

10:00 - 10:15 M.J. Foti O072
Diversity and activity of sulfate reducing bacteria along a salinity gradient in soda lakes

10:15 - 10:30 P. Kovatcheva-Datchary O073
RNA stable isotope probing - direct identification of starch fermenting bacteria in the human colon

Room Sydney **Parallel session 'Sequence based typing: current state and prospects'**

Chairmen: L. Dijkshoorn & D. Duim

09:00 - 09:30 E. Sadowy (Warsaw, Poland) O074
MLST - principles and applications for Gram-positive bacteria

09:30 - 10:00 M. Koopmans O075
Molecular typing to unravel international foodborne viral transmission

10:00 - 10:15 R. Ikawaty O076
A novel and quick MLVA (Multi-Locus Variable Number of Tandem Repeat Analysis)-based typing system for *Staphylococcus aureus*

10:15 - 10:30 B. Zwart O077
Loco-regional outbreaks of *Salmonella typhimurium* in the Zaanstreek-Waterland area

Room 2 **Parallel session 'Antiviral treatment: Testing for resistance during antiviral therapy 1'**

Chairman: A. Vossen

09:00 - 09:30 R.A. de Man O078
Testing for HBV drug resistance

09:30 - 10:00 A.M.J. Wensing O079
Testing for HIV drug resistance

10:00 - 10:30 C.J. Schinkel O080
Testing for HCV drug resistance

Room 3 **Parallel session 'Molecular studies in oral microbiology'**

Chairman: W. Crielaard

09:00 - 09:30 V. Zijnge O082
Molecular approaches in identification of complex microbial communities

09:30 - 10:00 D. Kara O083
Effect of *Veillonella parvula* on the protein expression of *Streptococcus mutans* grown in a biofilm

10:00 - 10:15 M.L. Laine O084
Biomedical informatics in chronic infectious and inflammatory disease research: periodontitis as a case study

10:15 - 10:30 V. Menke O085
The presence of Barrett's epithelium is associated with a specific bacterial flora in the esophagus

Room 4/5 **Parallel session 'Protein secretion and secreted proteins'**

Chairmen: A. Bart & A. van der Ende

09:00 - 09:30 S. Albers O086
Protein secretion in the *Archaea*: multiple paths towards a unique cell surface

09:30 - 09:45 P. van Ulsen O087
Protein secretion and secreted proteins in pathogenic *Neisseriaceae*

09:45 - 10:00 W.S.P. Jong O088
Limited tolerance towards folded elements during secretion of the autotransporter Hbp

10:00 - 10:15 C. Belzer O089
Bile acid resistance is essential for urease mediated gall-stone formation by *Helicobacter* species

10:15 - 10:30 I. Jongerius O090
Convertase inhibition by *Staphylococcus aureus*

Room 6/7 **Parallel session 'Emerging systemic infections – new agents of disease, new hosts promoting disease, and new virulence factors 1'**

Chairman: S. de Hoog

09:00 - 09:30	G. Walther Mushrooms as agents of human disease	O092
09:30 - 09:45	R. Horré (Bonn, Germany) Near drowning associated brain infection by Scedosporium, a unique disease entity	O093
09:45 - 10:15	J. Guillot (Maisons-Alfort, France) Diversity of Pneumocystis in animals, implications of host specificity for human infection	O094
10:15 - 10:30	F. Verduyn Lunel Candida antibodies can precede invasive candidiasis in patients with hematological malignancies who have undergone multiple courses of chemotherapy	O095

Room 8/9 **Parallel session 'Werkgroep Oost & West - Tuberculose: nieuwe ontwikkelingen 1'**

Chairman: R.W. Vreede

09:00 - 09:30	W. de Lange Casuïstiek: de vele gezichten van tuberculose	O096
09:30 - 10:00	F. Vlaspolder Tuberculosediagnostiek op sputum: is kweek altijd noodzakelijk? (pro)	O097
10:00 - 10:30	A.G.M van der Zanden Tuberculosediagnostiek op sputum: is kweek altijd noodzakelijk? (contra)	O098

Room 4/5

10:30 - 11:00	Vergadering werkgroep Microbiële Pathogenese
10:30 - 11:00	Coffee/tea break

Room Athene **Parallel session 'New microbial discoveries'**

Chairman: M. Jetten

11:00 - 11:30	M. Strous Nitrate dependent anaerobic oxidation of methane	O099
11:30 - 12:00	M. Konnecke (Oldenburg, Germany) Nitrification in the Ocean: isolation of novel ammonium oxidizing crenarcheota	O100
12:00 - 12:15	E.W. Vissers Freshwater Crenarchaeota, their ecology and ecophysiology with regard to climate change	O101
12:15 - 12:30	A.J.M. Stams Characterization of a benzene-degrading chlorate-reducing microbial community	O102

Room Sydney **Parallel session 'Evolutionary genetics and population biology of microorganisms'**

Chairman: R. Willems

11:00 - 11:30	B.G. Spratt (London, UK) Multilocus sequence typing and the population and evolutionary biology of bacteria	O103
11:30 - 12:00	L.M. Schouls Impact of nationwide vaccination against *Haemophilus influenzae* serotype b (Hib) on the composition of the circulating Hib population	O104
12:00 - 12:15	J. Top Emergence of ampicillin resistant *Enterococcus faecium* (AREfm) in the Netherlands	O105
12:15 - 12:30	A. Bart Molecular epidemiology of cutaneous Leishmania isolates from Dutch patients	O106

Room 2 **Parallel session 'Antiviral treatment: Testing for resistance during antiviral therapy 2'**

Chairmen: M.C.W. Feltkamp & A. Vossen

11:00 - 11:30	M.T. van der Beek Testing for HSV and CMV resistance	O107
11:30 - 11:45	H.C. Gelderblom Early prediction of response during high dose interferon induction therapy in difficult-to-treat chronic hepatitis C patients	O108
11:45 - 12:00	M. Jonges Antiviral susceptibility of influenza viruses in the Netherlands	O109
12:00 - 12:15	A. Meijer Evaluation of the NA-Star® kit for determination of oseltamivir susceptibility of influenza viruses	O110
12:15 - 12:30	M. van Poelgeest HPV-specific T-cell responses in relation to clinical impact of Imiquimod treatment in patients with high grade VIN	O111

Room 3 **Parallel session 'Bacterial population heterogeneity: Impact on survival 1'**

Chairman: T. Abee

11:00 - 11:30	M. Loessner (Zurich, Switzerland) *Listeria monocytogenes* phages and pathogen control	O112
11.30 - 12.00	J. Hylckama-Vlieg Lactic acid bacteria diversity and heterogeneity	O113
12:00 - 12:15	R. Moezelaar Population heterogeneity and stress survival in *Listeria monocytogenes*	O114
12:15 - 12:30	E.J. Gaasbeek DNA repair mechanisms in *Campylobacter jejuni*	O115

Room 4/5 **Parallel session 'Pathogenesis'**

Chairman: B. Zaat

11:00 - 11:15	A. de Greeff Expression profiling of udder tissue in response to acute clinical *Streptococcus uberis* infection	O116
11:15 - 11:30	J. Bestebroer Staphylococcal superantigen-like protein 5 inhibits chemokine-induced cell activation	O117
11:30 - 11:45	P.C.R. Godschalk Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain-Barré and Miller Fisher Syndromes	O118
11:45 - 12:00	A. Paauw The High Pathogenicity Island of an outbreak *Enterobacter cloacae* strain contributes to virulence	O119
12:00 - 12:15	N.N. Driessen Biosynthesis and immunosuppressive activity of mannose-capped mycobacterial lipoarabinomannan	O120

Room 6/7 **Parallel session 'Emerging systemic infections - new agents of disease, new hosts promoting disease, and new virulence factors 2'**

Chairman: J. Meis

11:00 - 11:15	J. Zhao Alkylbenzene assimilation, a new virulence factor?	O122
11:15 - 11:30	E. Kuijper Systemic Zygomycete infections, an emerging disease entity	O123
11:30 - 11:45	M. Sudhadam Detection of *Exophiala dermatitidis*, an emerging brain pathogen	O124
11:45 - 12:00	P. Verweij Emerging Aspergillus species resistant to current antifungals	O125
12:00 - 12:15	W. van de Sande The antifungal effect of amphotericin B, itraconazole, voriconazole and caspofungin on conidia and hyphae of *Aspergillus fumigatus*	O126
12:15 - 12:30	C.C. van Leer Markers specific for zygomycetes detected in BAL fluid of patients with suspected invasive fungal infection	O127

Room 8/9 **Parallel session 'Werkgroep Oost en West - Tuberculose: nieuwe ontwikkelingen 2'**

Chairman: J.A. Kaan

11:00 - 11:30	B. Mulder Interferon gamma releasing assays: ervaring in het laboratorium / Comparison of Interferon-gamma assays and TST results and the use in the routine laboratory diagnosis of latent or clinical Tuberculosis	O128
11:30 - 12:00	E.M.S. Leyten Interferon gamma releasing assays versus huidtesten in de praktijk	O129
12:00 - 12:30	R. van Altena Tuberculose: paradoxale reacties en reconstitutiesyndroom	O130
12:30 - 14:00	Lunch	

Room 2

12:30 - 14:00 **BBC-MMO vergadering**

Room Sydney

12:45 - 14:00 **Ledenvergadering NVvM**

Room Athene **Parallel session 'Case presentations'**

Chairman: G. Kampinga

14:00 - 14:15	J.J.C. De Vries Positive blood culture with *Plasmodium falciparum*	O131
14:15 - 14:30	R.P. Schade Thoracic actinomycosis, presenting as a subcutaneous abscess	O132
14:30 - 14:45	J.J.C. de Vries Retrospective analysis of 13 cases of extraintestinal *Campylobacter* disease and literature review	O133
14:45 - 15:00	E.M. Mascini A multidisciplinary approach to control an outbreak of MRSA in a long-term care facility for mentally disabled persons	O134
15:00 - 15:15	A.J. van Griethuysen A rare cause of prosthetic hip infection with unexpected consequences	O135
15:15 - 15:30	R.P. Schade Systemic cryptococcosis presenting as a solitary skin lesion in an organ transplant recipient on tacrolimus therapy	O136
15:30 - 16:00	Coffee/tea break	

Room Sydney **Parallel session 'Clinical epidemiology'**

Chairman: J. Kluytmans

14:00 - 14:15	M.J.A. de Regt High acquisition rates of ampicillin-resistant CC17 *E. faecium* (ARE) during hospitalization in a Dutch hospital	O137
14:15 - 14:30	J.S. Kalpoe Dissemination of *Bacillus cereus* in a pediatric intensive care unit traced to non-disposable ventilator air-flow sensors	O138
14;30 - 14:45	L.E. Willemsen Transmission of highly resistant *E. coli* (HR-EC) between patients in a Dutch hospital: Does the WIP guideline for highly resistant micro-organisms work?	O139

14:45 - 15:00	R.F. Gerritsen	O140
	Presence of highly resistant micro organisms (HRMO's) in long term care facilities in the Twente, eastern Achterhoek and Friesland regions	
15:00 - 15:15	M.J. Mooij	O141
	High prevalence of integron class 1 in highly resistance *Enterobacteriaceae* (HRE)	
15:30 - 16:00	Coffee/tea break	

Room 2 **Parallel session 'ICT: Implicaties van een landelijk EPD'**

Chairman:C.H.E. Boel

14:00 - 14:30	L. Mook	O143
	Stand van zaken in Nederland rondom EPD	
14:30 - 15:00	C.J.N.M. van der Palen	O144
	E-labs: new ways for sharing patient information across health care organizations	
15:00 - 15:30	P. Kabel & G. Ruijs	O145
	Topklinische EPD's: van Elisabeth tot Isala	
15:30 - 16:00	Coffee/tea break	

Room 3 **Parallel session 'Bacterial population heterogeneity: Impact on survival 2'**

Chairman: T. Abee

14:00 - 14:30	C.W. Michiels (Leuven, Belgium)	O146
	Stress resistance and SOS response in *Escherichia coli*	
14:30 - 15:00	M. Ehling-Schulz (Munich, Germany)	O147
	Emetic *Bacillus cereus*	
15:00 - 15:15	L. Hornstra	O148
	Germination of *Bacillus subtilis* spores; the effects of thermal preservation on spores	
15:15 - 15:30	M. Nierop-Groot	O149
	Identification of genetic polymorphisms involved in survival of *Campylobacter jejuni* in the poultry meat chain	
15:30 - 16:00	Coffee/tea break	

Room 4/5 **Parallel session 'Staphylococci: resistance and virulence'**

Chairman: A.C. Fluit

14:00 - 14:30	A.C. Fluit	O150
	Community-acquired methicillin-resistant *Staphylococcus aureus*	
14:30 - 15:00	M.J.J.B. Sibbald	O151
	Mapping the secretome of *Staphylococcus aureus*	
15:00 - 15:15	A.M. Stemerding	O152
	The discovery of a staphylococcal Fc gamma receptor inhibitory protein	
15:15 - 15:30	E van Duijkeren	O153
	Methicillin-resistant *Staphylococcus aureus* strains isolated from pigs on different kinds of pig farms	
15:30 - 16:00	Coffee/tea break	

Room 6/7 **Parallel session 'WMDI: Investigating the differential diagnosis by a targeted molecular approach: Where do we stand?'**

Chairmen: E. Claas & R. Schuurman

14:00 - 14:30	H.G.M. Niesters	O154
	From maximal to optimal molecular diagnostics. Is there a road back?	
14:30 - 15:00	P.H.M. Savelkoul	O155
	Molecular diagnostics: expanding applications	
15:00 - 15:15	T. Schuurman	O156
	Rapid and sensitive detection of 5 gastro-intestinal pathogens using two internally controlled multiplex Real-Time PCRs.	
15:15 - 15:30	N.M. van Maarseveen	O157
	Two internally controlled multiplex Real-Time PCRs for diagnosis of viral gastroenteritis	
15:30 - 16:00	Coffee/tea break	

Room 8/9 **Parallel session 'Sneldiagnostiek parasitaire infecties'**

Chairman: L.M. Kortbeek

14:00 - 14:15	R. van Doorn	O158
	Sneldiagnostiek malaria	
14:15 - 14:30	H. Schallig	O159
	Sneldiagnostiek *Leishmania*	
14:30 - 14:45	Ingeleid door L.M. Kortbeek	
	Discussie over gevolgen voor de dagelijkse praktijk van de implementatie van sneldiagnostiek van malaria	
14:45 - 15:00	B. Mulder	O160
	Sneldiagnostiek *Cryptosporidium*	
15:00 - 15:15	T. Mank	O161
	Sneldiagnostiek *Giardia*	
15:15 - 15:30	Ingeleid door T. Mank & B. Mulder	
	Discussie over gevolgen voor de dagelijkse praktijk van de implementatie van sneldiagnostiek *Cryptosporidium* en *Giardia*	
15:30 - 16:00	Coffee/tea break	

Room Athene **Ledenvergadering NVMM**

16:00 - 18:00

O001

Salivating for knowledge: strategies used by group A *Streptococcus* to survive under stress in the throat

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The ability of microorganisms to respond to stress by altering gene expression in specific host environments is essential for survival and pathogenesis. Many human pathogens persist for prolonged periods in particular host niches, and therefore must be able to adapt to a variety of stressors. Delineation of the molecular underpinnings of bacterial stress response required for persistence in humans is critical to developing therapeutic agents that inhibit genes or gene products contributing to pathogen survival, thereby interrupting infection. Group A *Streptococcus* (GAS) has a predilection for inhabiting the human oropharynx. The organism causes most cases of bacterial pharyngitis and colonizes up to one-half of all school age children in non-epidemic periods. Although GAS has been known to be the major cause of bacterial pharyngitis for more than 80 years, we have only a rudimentary understanding of the molecular mechanisms used by this pathogen to respond to stress and survive in the human oropharynx. We recently initiated study of GAS-saliva interaction to gain insight into GAS activity during upper respiratory tract infection. By analyzing the transcriptome of GAS grown in human saliva, we discovered a key two component gene regulatory system (SptR/SptS) that is crucial for persistence of GAS in saliva. SptR/SptS controls expression of multiple genes encoding proteins involved in complex carbohydrate acquisition and utilization pathways, resulting in optimized persistence of GAS in human saliva. In related research, we analyzed the transcriptome of GAS during an 86-day infection protocol in 20 monkeys with experimental pharyngitis. Specific genetic programs contributing to colonization, acute, and asymptomatic phases of disease were identified. Temporal changes in the GAS transcriptome were integrally linked to the phase of clinical disease and host defense response. Knowledge of the gene expression patterns characterizing each stressful phase of pathogen-host interaction provides new avenues for targeted investigation of proven and putative virulence factors and genes of unknown function, and will assist vaccine research.

O002

Job-related stress in *Saccharomyces cerevisiae*: adaptation to industrial process conditions

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The yeast *Saccharomyces cerevisiae* is applied on an enormous scale in industrial biotechnology. Despite its long use in industrial fermentation, description of *S. cerevisiae* as an 'industrial' or even 'domesticated' microorganism should not obscure the fact that its metabolic and regulatory networks are predominantly the result of aeons of evolution in natural environments. In addition to its role in industry, *S. cerevisiae* has become a model eukaryote for modern biology. In view of the enormous body of information that is available from labs around the world, it all too easy to consider laboratory cultivation as 'normal' rather than as extremely artificial. What we call 'stress' is therefore very much dependent upon our perspective.

The use of –omics techniques has enabled biologists and biotechnologists to study the adaptation of *S. cerevisiae* to a wide range of chemical and physical stimuli. This research increases our understanding of cellular regulation and contributes to the design and construction of yeast strains that are better suited for existing and novel biotechnological processes. Hitherto, genome-wide analysis of the cellular responses of *S. cerevisiae* to such stimuli has been largely focused on transcriptome analysis. As will be illustrated with a few examples, this approach has great diagnostic value for industrial processes. It is often assumed that mRNA profiles give a direct indication of the contribution of the corresponding gene products to cellular fitness. By discussing the response of *S. cerevisiae* to anaerobiosis, it will be illustrated that this correlation is not always straightforward.

Temperature is a particularly interesting process parameter in industrial fermentation, because it affects the catalytic properties of all enzymes in metabolic networks. We have recently studied the responses of *S. cerevisiae* to suboptimal cultivation temperature in steady-state chemostat cultures. Our research focused on the question how this yeast maintains its glycolytic activity at low temperature: via increased synthesis of enzymes or via changes in the intracellular concentration of low-molecular-weight metabolites and effectors. A multi-level analysis indicated that the latter mechanism ('metabolic regulation') is the predominant mechanism by which yeast controls the flux through glycolysis at low temperature. In evolutionary terms, this predominant reliance on metabolic regulation of a central pathway, which represents a significant fraction of the

organism's cellular protein, may be advantageous to limit the need for protein synthesis and degradation during diurnal temperature cycles. This strategy may also offer an explanation for a characteristic property of *S. cerevisiae* that is of great importance for several of its industrial applications: the apparent 'overcapacity' of its glycolytic pathway at higher ('normal'...) cultivation temperatures.

O003

Diversity, cooperation, and viral mutual aid

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An RNA virus population does not consist of a single genotype; rather, it is an ensemble of related sequences, termed quasispecies. High mutation rates of RNA viral replication create a 'cloud' of potentially beneficial mutations at the population level, which afford the viral quasispecies a greater probability to evolve and adapt to new environments and challenges during infection. Using poliovirus as our model we developed strategies to increase or reduce the mutation rate of the viral polymerase thus changing the levels of genomic diversity in the viral population. In infected animals, reducing or increasing viral diversity leads to loss of neurotropism, and an attenuated pathogenic phenotype. These findings suggest that quasispecies diversity is finely tuned to ensure evolutionary survival of the virus and is a biological determinant for the outcome of poliovirus infection. Our study uncovered a surprising property of the virus population, in which different variants within the quasispecies experiment a cooperative interaction so that some variants allow others to enter the brain. Furthermore, while the viral population with restricted genomic diversity replicate robustly in small intestine, we were unable to isolate viruses from feces of infected mice. This observation suggests that quasispecies diversity plays an important role in virus spread from individual to individual. Interestingly, Sabin vaccine strains are restricted quasispecies, suggesting that population diversity is, at least in part, the basis of attenuation in poliovirus vaccine strains. Thus, altering the structure of the quasispecies result in attenuation of the virus and may provide a novel, rational and general approach for the development of safe live-attenuated virus vaccines.

O004

Functional analysis of a *Plasmodium berghei* gametocyte repressor complex

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Meiosis and its attendant generation of genetic diversity in *Plasmodium* is dependent on the transmission of male and female haploid sexual precursor cells (gametocytes) to the mosquito vector where fertilization takes place; the diploid zygote then transforms into a polarized cell that is motile and known as an ookinete which penetrates the midgut into the surrounding epithelium.
This early sexual developmental program depends on the transcription and storage, rather than translation, of certain mRNAs already in blood-stage gametocytes. Assembly into and maintenance of mRNAs in translationally quiescent mRNPs is known as translational repression (TR) and depends on the activity of an evolutionarily conserved DEAD-box RNA helicase, Plasmodium DOZI (Development of zygote inhibited). A DOZI::GFP fusion protein localizes to distinct positions in the cytoplasm of female gametocytes that contain transcripts known to be translationally repressed. Analyses of DOZI::GFP immunoprecipitation eluates by mass-spectrometry identified numerous proteins that potentially also reside in this *Plasmodium* repressor complex, a number of which have now been analysed by gene disruption.
The annotated *Plasmodium* genome contains relatively few obvious transcription factors and our data suggest that TR and mRNA turnover can be key influences on stage specific gene expression in *Plasmodium*. This developmental programme reflects a response to a selective need to produce a polarised cell quickly whilst undergoing meiosis which is not thought to support accurate gene transcription. The stored mRNA species effectively form a smoking gun that might be exploited to prevent transmission of the parasite to the mosquito vector thereby interrupting the parasite life cycle.

O006

Natural factors influencing microbial composition in ticks

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Wageningen University, Wageningen

Lyme disease is an emerging disease in the Netherlands and elsewhere in the world, and the number of people suffering from Lyme disease grows every year. Documented number of people in the Netherlands contacting general practitioners with erythema migrans (first indication of Lyme disease) after tick bites increased from 6,000 in 1994, to 12,000 in 2001 and to 17,000 in 2005. Possible reasons that Lyme disease is emerging are: increase in the number of tick bites and increased public awareness about the risks associated with tick bites. The natural vector for the causative agent, *Borrelia burgdorferi* sensu lato is the common sheep tick (Ixodes ricinus) which resides in natural habitats like woody areas. Floral and faunal compositions of these areas are

considered to be important and our hypothesis is that the composition of the natural area (habitat) influences the total microbial composition in ticks including the *B. burgdorferi* s.l. infection rate. The microbial community associated with ticks may play an important role in successful colonization and transmission of *Borrelia* species after blood meals. In total 180 ticks (60 per area) were collected from 3 different areas in the Netherlands: Amsterdamse Waterleiding Duinen (AWD), Ede and Veldhoven. DNA extracts from these ticks were analyzed for the presence of *Borrelia* species, via a reverse line blot approach whereas the composition of the microbial community was determined by PCR-DGGE with bacterial primers. Further, the floral composition of these areas as well as the structural and chemical composition of the leaf litter layer (important for survival of ticks) was determined. The effect of environmental parameters on microbial community composition was determined by multivariate analysis. The floral composition and chemical and structural composition of the leaf litter layers in the 3 different areas were different. Forty ticks (22.2%) were infected with *Borrelia* species: 33 (18.3%), 1 (5.5%), 1 and 1 were, respectively, infected with *Borrelia afzelii*, *Borrelia garinii*, *Borrelia valaisiana* and *B. burgdorferi* s.l. and 3 were double infected with: *B. burgdorferi* sensu stricto and *B. garinii*, *B. valaisiana* and *B. garinii*, and *B. valaisiana* and *B. afzelii*. Twelve (20.0%) infected ticks were found in AWD, 18 (30.0%) in Ede and 10 (5.6%) in Veldhoven. PCR-DGGE fingerprint analysis revealed that the microbial composition in ticks from AWD differed from that in ticks from Ede and Veldhoven. Also, the microbial species diversity in ticks from AWD was higher than in ticks from Ede and Veldhoven. We conclude that the floral composition of the natural habitat and, or composition of the leaf litter layer play an important role on the microbial composition in ticks. Although we do not have conclusive evidence that the percentage of infected ticks differs in these areas, we determined strong local effects on the number of infected ticks. The existence of spatial hot spots in *Borrelia* species infected ticks is an intriguing fact and requires further attention. Information on environmental factors determining *Borrelia* species infections in ticks is important for management practices in forests and alerts for recreation in these areas.

O007

Longitudinal analysis of tick densities and *Borrelia*, *Anaplasma* and *Ehrlichia* infection of *Ixodes ricinus* ticks in different habitat areas in the Netherlands

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From 2000 to 2004, ticks were collected by blanket dragging in four habitat areas in the Netherlands: dunes (Duin & Kruidberg), heather (Koninklijke Houtvesterijen), forest (Koninklijke Houtvesterijen) and a city park (Bijlmerweide). Ticks densities were calculated and the infection with *Borrelia burgdorferi*, *Anaplasma* and *Ehrlichia* species was investigated by reverse line blot (RLB) analysis. The lowest tick density was observed in the heather area (1-8/100m^2). In the oak forest and city park densities ranged from 26-45/100m^2. The highest density was found in the dune area (139-551/100m^2). The infection rates varied strongly between the four areas and years, ranging between 0.8 ' 11.5% for *Borrelia* spp. and between 1-16% for *Ehrlichia/Anaplasma* spp. *Borrelia* infection rates were highest in the dunes, followed by the forest, the city park and heather area. In contrast, *Ehrlichia/Anaplasma* was found most in the forest and less in the city park. The following species were found: unspeciated *B. burgdorferi* sensu lato (2.5%); *Borrelia afzelii* (2.5%); *Borrelia valaisiana* (0.9%); *B. burgdorferi* sensu stricto (0.13%); *Borrelia garinii* (0.13%). For *Ehrlichia/Anaplasma* this was: unspeciated *Ehrlichia/Anaplasma* spp. (2.5%); *Anaplasma schotti* variant (3.5%); *A. phagocytophilum* variant (0.3%); and *Ehrlichia canis* (0.19%). *E. canis* is here reported for the first time in ticks in the Netherlands. *Borrelia lusitaniae, Ehrlichia chaffeensis* or the HGA agent were not detected. About 1.6% of the ticks were double infected with *Borrelia* and *Ehrlichia/Anaplasma*, which was more than predicted from the individual infection rates, suggesting hosts with multiple infections or a possible selective advantage of co-infection. Currently, we are further investigating this by determining the levels of several other micro-organisms (*Rickettsia* and *Babesia*) in these ticks.

O008

Introduction of the Asian Tiger mosquito in the Netherlands: development of methods for surveying (imported) mosquitoes for arboviruses

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In 2005 the presence of the Asian Tiger mosquito (*Aedes albopictus*) in greenhouses from importers of Dracaena sanderiana ('Lucky Bamboo') was established by the Dutch Plant Protection Service (PD). The insects were introduced through the import of these ornamental plants from South-East China. *Ae. albopictus* is a known vector for 22 different arthropod-borne (arbo) viruses, including dengue virus. Following a joint risk assessment for the Ministry of Health, Welfare and Sports, four intertwined studies were

started addressing the following questions: 1) What is the extent of the current introduction of *Ae. albopictus* to the Netherlands? 2) Has *Ae. albopictus* become established in the Netherlands? 3) Are the introduced mosquitoes carrier of dengue virus? 4) Are there indications that dengue virus has been transmitted to exposed individuals (employees of the importers or inspectors of the PD)?
To address research question 3, we established and validated a semi-nested RT-PCR for serotype-specific detection of dengue virus in mosquitoes. Mosquitoes were collected at two-weekly intervals using carbon dioxide mosquito traps. Upon determination the Ae. albopictus mosquitoes were stored in RNAlater®, transported to the RIVM and transferred to -80 °C until analysis. RNA was extracted using an optimized RNA isolation procedure based on the RNeasy RNA isolation kit by Qiagen®. As positive and negative controls, known infected and uninfected *Ae. albopictus* mosquitoes were tested in parallel. The experimental set-up, optimization, validation and analysis of field-caught mosquitoes will be discussed, specifically issues related to the analysis of mosquitoes for the presence of RNA viruses.

O009

The *Ixodes scapularis* salivary protein Salp15 inhibits complement mediated killing of complement sensitive *B. garinii* strains

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Introduction: The Lyme disease agent *Borrelia burgdorferi* is maintained in a tick-mouse cycle. In the US, *B. burgdorferi* sensu stricto is maintained in the *Ixodes scapularis* ticks, while the *B. burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii* strains in Europe are maintained in the *Ixodes ricinus* ticks. Feeding of ixodic ticks normally takes a few days to several weeks, which gives the host immune system time to interact with the feeding tick. The ticks have developed several mechanisms to evade both the innate and adaptive immunity, which enable them to take an effective blood meal. Tick saliva possesses proteins with immunosuppressive, anticomplement and antihaemostatic activity. Salp15, one of the tick salivary proteins, is known to inhibit T cell activation and proliferation by specifically binding to the CD4 coreceptor of the T cells. Salp15 appeared also to be favorable for the survival of *B. burgdorferi* in the host after transmission by the tick. Salp15 specifically interacts with the *B. burgdorferi* outer membrane protein OspC and gives protection against the borreliacidal antibodies directed. In this study, we investigate the role of the tick salivary protein Salp15 in the protection of *B. garinii* against a part of the innate immune response, direct killing by the complement system.
Methods: Complement sensitive *B.garinii* strains A87S and VSBP (2.5*10E5) were used. They were incubated with recombinant *Ixodes scapularis* Salp15 or BSA respectively for 30 minutes at 33°C. The spirochetes were then incubated with 25 µl of a 1:4 dilution of normal or heat-inactivated human serum without specific antibodies against *B. burgdorferi* sensu lato. After 1.5, 4.5 and 24 hours of incubation, the percentage of dead spirochetes were quantified by determination of spirochete motility and the extent of blebbing using dark-field microscopy.
Results: Since complement-mediated killing was apparently affected by the initial viability of the spirochetes, which varied between cultures, the experiment was repeated 13 times. Less complement-mediated killing was found when *B. garinii* strain A87S was preincubated with Salp15. After 1,5 hour incubation with serum 62% (range: 32-85%) of the spirochetes incubated with BSA were killed by complement, while 32% (range: 9-44%) spirochetes were killed in the presence of Salp15. This resulted in an average inhibition of killing of 48% by Salp15 ($p<0,001$). After 4,5 and 24 hours differences were less apparent: on average 90% of the spirochetes incubated with BSA were killed by complement, while 81% spirochetes were killed in the presence of Salp15 ($p<0,1$). Especially after 24 hours, counting was difficult due to lysis of most killed spirochetes. Experiments with strain VSBP confirmed this inhibition of killing by Salp 15.
Conclusions: Both *B. garinii* strains A87S and VSBP are initially protected against complement mediated killing when they are incubated with *I. scapularis* Salp15. After prolonged incubation, the protective effect is less apparent. Remaining questions are whether the binding of Salp15 to OspC diminishes complement mediated killing or whether Salp15 does this alone, and what would be the effect of *I. ricinus* Salp15, the natural counterpart of these strains.

O010

Borrelia burgdorferi in ticks and patients in a family practice on the Dutch North Sea Island of Ameland

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Introduction: Awareness of Lyme disease is increasing in the Netherlands. Consultations for tick bites and erythema migrans have increased three-fold from 1994 to 2005. We investigated the percentage infected ticks and the risk of subsequent development of clinical symptoms of Lyme disease in persons that visited a family practice on the island of Ameland which is sometimes described as a hot spot for *Borrelia burgdorferi* in ticks.

Methods: Removed ticks were treated with a lysis buffer and DNA was extracted with the manual silica based Boom method. A conventional PCR was performed followed by a microwell hydridisation assay, using an *OspA* specific probe. Inhibition of the PCR was monitored using a modified target. Six to 18 months after removing the tick, persons were contacted by phone and questioned about erythema migrans and other symptoms of a possible *B. burgdorferi* infection. Serology was available from a small subgroup (n=16) several months after removal of the tick.
Results: From January 2004 to December 2005, 110 ticks were tested. In 22 ticks (20.0%) *B. burgdorferi* DNA was detected. Eleven persons reported that the tick had been on the skin for more than 24 hours. Follow up information was available from 95 persons (86,4%). None of them reported having an erythema migrans or other systemic symptoms compatible with Lyme disease during the period following the tick bite. Seven persons reported a non-specific red discoloration of the skin on the site of the tick bite at some time during the first months following the tick bite. Of these, three were persons with a positive tick and four with a negative tick. Lyme serology was performed on eight persons with a positive tick, all of whom were negative. Of serology performed on eight persons with a negative tick, one was positive in IgG.
Conclusions: Infection with *B. burgdorferi* after a tick bite was not found in this group of persons in which, in the fast majority of cases the tick has been on the skin for less 24 hours, even though a relatively large percentage of ticks was positive for *B. burgdorferi*. These findings support the policy described in the Dutch CBO-guideline on Lyme borreliosis not to use prophylactic antibiotics in every person with a tick bite.

O011

Comparison of a *Treponema pallidum* IgM immunoblot with a 19S FTA-ABS test for the diagnosis of congenital syphilis

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We compared an in-house *Treponema pallidum* IgM immunoblot (IB) with a 19S Fluorescent Treponemal Antibody-Absorbtion (IgM) test (FTA-ABS) during routine use for the diagnosis of congenital syphilis in a national reference laboratory in a non-endemic setting. The overall agreement between the assays was high (97%) and 19S positive samples had at least two reactive bands in the IB. If the 19S is taken as the gold standard, the estimates sensitivity of the IB was at least 88% with a specificity of 97.2%. Analysis of the discrepancies revealed that the IB was positive with one or two specific bands in 2.8% of the cases, while the 19S was negative, possibly indicating higher sensitivity of the IB. We conclude that the IB is a sensitive method to detect contact with *T. pallidum* in neonates and can replace the 19S in routine laboratory screening for CS cases.

O012

Evaluation of 6 different methods to identify nonfermentative Gram-negative bacteria in CF patients: 4 biochemical and 2 molecular methods

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The identification of non-fermentative Gram-negative bacteria in Cystic Fibrosis (CF) patients is notoriously difficult. To evaluate our 'in house' biochemical determination method we compared it with 3 commercial biochemical methods: API 20NE and VITEK 2 (BioMerieux) and RapID NF plus (Remel) and 2 molecular methods: 'in house' 16S rRNA sequence analysis and FISH (fluorescent in situ hybridisation) for *Pseudomonas aeruginosa* and *Burkholderia cepacia* (SeaPro International).
We tested 69 clinical isolates from sputum samples of known CF patients. The sequence analysis based on the Michigan State University's Center for Microbial Ecology's database was considered to be the gold standard.
All 4 phenotypic (biochemical) methods were equal in their performance; correct identification in appr. 64%. FISH identified the *P. aeruginosa* and *B. cepacia* strains in all cases correctly. Phenotypical identification of non-fermentative Gram-negative bacteria in CF patients is not sufficient and identification of a first isolate should be confirmed by a molecular method.

O013

Evaluation of a rapid test panel, the API Strep-20, the BD Phoenix and VITEK-2 automated instruments, and Raman spectroscopy for species identification of *Enterococci*

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Introduction: The identification of vancomycin-resistant enterococci (VRE) has become an important component of infection-control programs. The differentiation between *vanA/B*-VRE (high-level and transferable vancomycin resistance in *Enterococcus faecium* and *Enterococcus faecalis* (Efe/Efa) and *vanC*-VRE (intrinsic low-level vancomycin-resistance in *Enterococcus casseliflavus* and *Enterococcus gallinarum* (Ecas/Egal) is relevant since in contrast to

vanA/B-VRE, *vanC*-VRE have not been implicated in outbreaks. Furthermore, vancomycin treatment failure has been associated with infections caused by *vanC*-VRE. Differentiation of *vanC*-VRE from other enterococcal species by adequate identification is therefore relevant since low-level resistance may not be detected using the CSLI breakpoints. In the current study, we evaluated a simple rapid test panel (RTP), the BD Phoenix and VITEK-2, the API Strep 20 using the APIweb analysis and Raman spectroscopy for their accuracy to identify clinical relevant enterococcal species and their sensitivities and specificities to distinguish Efe/Efa from *vanC* positive *Enterococci*.

Method: Ninety-six clinical enterococcal strains (8 different species) were analysed. A genotypic test based on the sequence of the *rpoA* gene was used as reference method. The phenotypic test panel provided a species identification within 4 hours, testing the reduction of lithmus milk, acidification of arabinose, hydrolysis of L-arginine, pigment production and motility. Raman spectroscopy is a identification method under development yielding results within 1 minute.

Results: The accuracy of identification of the different methods was for APIweb 81%, Phoenix 86%, VITEK-2 86%, RTP 91%, and Raman spectroscopy 96%. Although the accuracy of APIweb to identify Efe and Efa was very high (96%), the overall specificity was very low (42%), because 58% of Ecas/Egal isolates were misidentified as Efe. The best method to distinguish Efe/Efa from *vanC* positive species was the RTP with a sensitivity of 98.5% and specificity of 100%. The Phoenix and VITEK 2 revealed specificity to distinguish Efe/Efa from *vanC* positive species of 100% and 92% respectively.

Conclusions: All methods were comparable regarding the identification of *Enterococci*. However, from the routine laboratory tests the RTP was the most rapid and reliable method to distinguish Efe/Efa from Ecas/Egal. Raman spectroscopy is a very promising fast alternative. The API revealed a high percentage of false positive *E. faecium* identifications, which may result in unnecessary infection control interventions and unintended treatment of *vanC* positive Ecas/Egal infections with vancomycin .

O014

Diagnostic value of a positive galactomannan assay in broncho-alveolar lavage fluid

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The galactomannan EIA can be used as a diagnostic tool in the diagnosis of invasive aspergillosis. Originally, the test has been developed for serum, but broncho-alveolar lavage (BAL) fluid has also been used as an input material in this assay. Whereas initially an index/cut-off ratio for a positive test of 1.0 was proposed, an index/cut-off ratio of 0.50 has also been proposed as a cut-off for a positive test (Musher *et al.*, J Clin Microbiol 2004;42:5517-22). We retrospectively evaluated the diagnostic value of a positive galactomannan EIA on BAL fluid among patients admitted to our institute suspected for pulmonary aspergillosis.

From 2003 to 2006, 89 BAL specimens were tested in the galactomannan EIA. Of these, 19 showed an index/cut-off value over 1.0 and 13 additional specimens had an index/cut-off value between 0.50 and 1.0. Of these 13 specimens, three showed a index/cut-off ratio between 0.80 and 1.0 and 10 were between 0.50 and 1.0. These specimens originated from 32 different patients. Twenty-six had hematological malignancies, and 16 of them had received a hemopoietic stemcell transplant. Four patients were solid organ recipients and two patients had autoimmune disorders.

A diagnosis of definite invasive aspergillosis could be established in seven patients: three of them showed an invasive fungal infection after obduction, three others had a positive culture from the BAL specimen or a nasopharyngeal biopsy and one patient was diagnosed by the presence of hyphi in BAL fluid, in conjunction with typical abnormalities on CT-scan. Five other patients were diagnosed as probable cases based on a typical CT-scan pattern including halo-signs. Twelve patients had possible aspergillosis based on suspective, but atypical pulmonary infiltrates on CT-scan or chest X-ray. In eight patients aspergillosis was considered unlikely, showing no typical abnormalities on X-ray; in addition, most of these had improved in the absence of antifungal treatment.

All patients with definite or probable aspergillosis had an index-cut-off ratio of at least 0.80. All of them had a hematological malignancy. In contrast, six of the eight patients with no invasive aspergillosis had a cut-off value below 0.80; the other two patients who had no invasive aspergillosis, showing an index/cut-off ratio of 1.54 and 1.81, were solid organ transplant recipients.

In conclusion, an index/cut-off ratio of 0.50 is probably too low and results in a number of false-positive findings. Among our patients, an index/cut-off ratio of 0.80 would have been more appropriate. This is in accordance with a recent animal experimental study, in which an index/cut-off ratio of 0.75 was proposed as a treshold value (Francesconi *et al.* J Clin Microbiol 2006;44:2475-80). A positive result of the assay in patients who have no hematological malignancies should be interpreted with additional caution.

O015

Robotizing of the agar dilution method for susceptibility testing of bacteria according to CLSI guidelines

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Introduction: The agar dilution method according to the CLSI guideline M100-A18 is regarded as the golden

standard for susceptibility testing of bacteria. However, the procedure is laborious and therefore not useful for laboratories handling many isolates. It also seems to be very difficult to robotize this procedure. To overcome these problems we developed a robot for the multipoint inoculation of bacterial isolates on agars, overnight incubation of these plates and photographing with digital interpretation of the pictures obtained in terms of susceptibility patterns.

Methods: Agar dishes containing the appropriate antibiotics were inoculated using a multipoint inoculator, after which the plates were incubated and photographed at appropriate times post inoculation in a completely robotised way. The 1 Mega pixel pictures taken were digitally analysed using the Image Processing Toolbox of MatLAB® with in-house developed scripts in terms of growth or no-growth of bacterial colonies. Then these data were interpreted with an expert system to give the susceptibility pattern. The system generates these susceptibility data without any action from a technician. To validate this method, manual and robotized reading of the dishes were compared.

Results: Readings, both manual and digital, of approximately 15.000 plates were compared using the chi-square method. Results of good growing micro-organisms showed an agreement of 96 to 98% with kappa's ranging from 0.90 to 0.96. Results for weak growing (fastidious) micro-organisms showed an agreement of 94% and kappa's of 0.75. A sub-set analysis of discrepant results indicated that the robotized method was more accurate than the manual procedure.

Conclusion: Using this robot combined with vision technology it is possible to completely robotize the agar dilution method for susceptibility testing of bacteria. Comparing the manual and automated procedures the automated procedure even seems to perform better, while reducing hands-on time dramatically. The results of this procedure are available at 8 o'clock in the morning. The robotized method is now validated for bacterial identification purposes using chromogene agars and other substrate specific plates and the results are equally promising. More challenging is to analyze primary plates in this way for example: urine and fecal samples.

O016

Comparison of PCR-Reverse Line Blot analysis and traditional culture of dermatophytes in clinical samples

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Objectives: Traditionally, laboratory detection and identification of dermatophytes consists of culture on selective media and potassium hydroxide (KOH) tests. This process yields positive results within approximately 2-6 weeks, and negative results are generated after 6 weeks of incubation. Using PCR followed by Reverse Line Blot (PCR-RLB) analysis it becomes possible to obtain positive and negative results within 2-3 days. In this study we compared traditional culture with PCR-RLB analysis.

Methods: Two hundred and three clinical samples (187 nail, 16 skin) were analysed retrospectively by PCR-RLB after traditional culture. Samples were processed using QIAamp® DNA mini kit (Qiagen, Germany) with a separate pre-lysis step. PCR targeted the ITS region between the genes coding for 18S and 5.8S rRNA. PCR products were analysed using RLB [Bergmans *et al.*, submitted]. The membrane harboured 13 different probes to identify and discriminate between 9 different dermatophyte species within 3 genera, namely; *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton interdigitale, Trichophyton tonsurans, Trichophyton violaceum, Trichophyton verrucosum, Microsporum canis* (complex), *Microsporum audouinii* and *Epidermophyton floccosum*.

Results: Culture, KOH and PCR-RLB analysis yielded 37/203, 93/200 and 97/203 positive results respectively. Of the 37 culture positive samples, 35 scored positive in the PCR-RLB. The two PCR-RLB negative samples were identified by culture as *Fusarium* spp. and *Scopularulopsis brevicaulis*, which are non-dermatophytes. One sample identified as *S. brevicaulis* by culture yielded a positive PCR-RLB signal for *T. rubrum*. Sixty-two samples scored positive in PCR-RLB but remained negative in culture, 53 of these samples were KOH positive. Of the 97 PCR-RLB positive samples 79 were identified as *T. rubrum*, 14 as *T. interdigitale*, 3 as *Trichophyton* spp. The remaining sample could not be further identified. One sample showed inhibition in the PCR-RLB, this sample remained KOH and culture negative. Sensitivity for the PCR-RLB compared to culture for dermatophytes is 100% (34/34), compared to the KOH the sensitivity of PCR-RLB is 92% (86/93).

Conclusion: These data show PCR-RLB to be a fast and very sensitive method to detect and identify dermatophytes compared to traditional culture methods. Molecular assays are known to be more sensitive than culture or microscopic techniques. Therefore it is very likely that the PCR-RLB positive, culture negative samples are in fact positive.

O017

Fungal cell surface proteins

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The budding yeast *Saccharomyces cerevisiae* has a highly elastic wall, as vividly illustrated when the cell is transferred to a hypertonic medium and it rapidly shrinks, or when it

is transferred to a hypotonic medium and it visibly swells. Fixed cells, which cannot sustain turgor pressure any more, are generally considerably smaller than living cells. This elasticity is due to the presence of a continuous inner wall layer, which consists of moderately branched β-1,3-glucan molecules that laterally associate through the formation of multiple hydrogen bonds. The β-1,3-glucan network may be fortified by the covalent attachment of chitin chains to non-reducing ends of the β-1,3-glucan chains. This happens predominantly at the inside of the β-1,3-glucan network. The network seems to be further strengthened by so-called Pir-proteins, which are assumed to cross-link β-1,3-glucan chains through a recently described ester linkage. The skeletal inner layer is surrounded by an external protein layer, which mainly consists of glycosylated, GPI-dependent cell wall proteins (GPI-CWPs) emanating into the environment. GPI-CWPs are covalently linked to a strongly branched and thus water-soluble β-1,6-glucan molecule, which in turn is linked to a β-1,3-glucan chain, forming the protein-polysaccharide complex CWP-GPI_r → β-1,6-glucan → β-1,3-glucan. This complex has also been identified in the cell wall of many other ascomycetous fungi.

A powerful tool to analyze the composition and dynamics of the fungal cell wall proteome is mass spectrometry. Intriguingly, many ascomycetous fungi incorporate a wide variety of GPI-CWPs and other CWPs in their walls. For example, wild-type cells of *S. cerevisiae* and *Candida albicans* growing in rich medium express at least 20 different covalently bound CWPs. Several studies claim that the fungal cell wall also contains many proteins of cytosolic origin that have arrived there through a nonconventional export pathway. However, most of these studies are flawed because they extract living cells under conditions that cause cell leakage. The composition of the cell wall proteome depends on environmental conditions and even depends on the phase of the cell cycle. We will discuss possible functions of CWPs.

O018

Function and metabolism of cell wall α-glucan in fungi

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Morphology and integrity of yeast cells is maintained by a rigid cell wall, which prevents cells from lysis by counteracting turgor pressure. An excellent model yeast to study cell wall metabolism is the fission yeast *Schizosaccharomyces pombe*, since its complete genome is known, growth occurs at the cell ends generating a rod-like appearance, and mutants involved in cell wall metabolism can be engineered. Cell wall metabolism is highly dynamic, allowing for growth through new cell wall synthesis as well as cell separation through cell wall hydrolysis, both without compromising cell integrity.

The *S. pombe* cell wall comprises two layers, an outer layer of glycosylated mannoproteins, and an inner layer mainly composed of the carbohydrates β-glucan and α-glucan. Previously we characterized the chemical structure of α-glucan and showed that it consists of two building blocks each comprising two distinct constituents. Each building block is composed of (1,3)-α-glucan with some (1,4)-linked α-glucose residues at its non-reducing end (Grün *et al.*, 2005). Interestingly, we observed that α-glucan isolated from a temperature-sensitive mutant only consisted of one building block and was threefold decreased in level, rendering cell morphology temperature dependent. The point mutation was identified in the α-glucan synthase gene *Ags1*. In a recent study, the enzymatic function of Ags1p was investigated in more detail (Vos *et al.*, submitted). First, we overexpressed Ags1p in *S. pombe* cells, which led to accumulation of (1,4)-α-glucan as detected by iodine-staining. Second, site-directed point mutations of a highly conserved residue in Ags1p resulted in an abrogation of (1,4)-α-glucan accumulation. Finally we showed that (1,4)-α-glucan biosynthesis is an essential function of Ags1p. We propose that Ags1p functions in cell-wall α-glucan biosynthesis by specifically synthesizing the (1,4)-α-glucan constituent.

Not only cell-wall α-glucan biosynthesis is highly regulated, also α-glucan breakdown is strictly coordinated both in space and time. During cell division, after mitosis and cytokinesis have been completed, α-glucan must be broken down in order to physically separate the two daughter cells. Cell separation is initiated after full maturation of a three-layered septum, which is laid down in the middle of the cell. We identified and characterized two *endo*-(1,3)-α-glucanases, Agn1p and Agn2p, and investigated their specific roles in the *S. pombe* life cycle (Dekker *et al.*, 2004, 2007). Whereas Agn2p is specifically expressed during sporulation, Agn1p is expressed during vegetative growth. In-depth analysis showed that *agn1*$^+$ expression is regulated by the transcription factors Ace2p and Sep1p, and is synthesized during septum synthesis, in concert with the (1,3)-β-glucanase Eng1p (Dekker *et al.*, 2006). Absence of either Eng1p or Agn1p resulted in a cell separation defect, caused by a deficiency in primary septum or septum edging hydrolysis, respectively. We conclude that localized hydrolysis of α-glucan plays an intrinsic part of the life cycle of *S. pombe*.

Interestingly, the α-glucan synthases and hydrolases are highly conserved in many ascomyceteous and basidiomyceteous fungi, including the medically important pathogen *Cryptococcus neoformans*. We hope that in the future our work will aid in the rational design of antifungals directed against the enzymes involved in α-glucan metabolism.

O019

Repellents of *Ustilago maydis* function in cell wall integrity and development by forming amyloids

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Ustilago maydis contains one repellent and two class I hydrophobin genes in its genome. The repellent gene *rep1* encodes 11 secreted repellent peptides that result from the cleavage of a precursor protein at KEX2 recognition sites. The hydrophobin gene *hum2* encodes a typical class I hydrophobin of 117 aa, while *hum3* encodes a hydrophobin that is preceded by 17 repeat sequences. These repeats, called questellents, have a similar hydropathy pattern as repellents and are also separated by KEX2 recognition sites.

Gene *hum2*, but not *hum3*, was shown to be expressed in a cross of two compatible wild-type strains, suggesting a role of the former hydrophobin gene in aerial hyphae formation. Indeed, aerial hyphae formation was reduced in a f*hum2* cross, while it was further decreased in the f*hum2*f*hum3* cross. However, the reduction in aerial hyphae formation was much more dramatic in the f*rep1* cross. Moreover, colonies of the f*rep1* cross were completely wettable, while surface hydrophobicity was unaffected and only somewhat reduced in the f*hum2* and the f*hum2*f*hum3* cross, respectively. It was also shown that the repellents and not the hydrophobins are involved in attachment to hydrophobic surfaces. Deleting either or both hydrophobin genes in the f*rep1* strains did not further affect aerial hyphae formation, surface hydrophobicity and attachment. Apart from a role in aerial growth and attachment, repellents also function in cell wall integrity. A mutant with a deleted *rep1* gene was more sensitive to the cell wall perturbing agents Calcofluor white and Congo red.

Previously, it was shown that hydrophobins form highly surface active amyloid-like structures at hydrophilic-hydrophobic interfaces such as at the cell wall of aerial hyphae. Interestingly, repellents assemble into a similar structure. This was shown by transmission electron microscopy, X-ray diffraction, interaction with ThT and Congo red and surface tension measurements. However, in contrast to hydrophobins, the repellent already becomes amyloid in solution.

Taken together, we propose that repellents have taken over the function of hydrophobins in growth and development of *U. maydis* by assembling into amyloid fibrils within and at the outer surface of the hyphal cell wall.

O020

Capsule biogenesis in *Cryptococcus neoformans*

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The human pathogen *Cryptococcus neoformans* can cause meningo-encephalitis, especially in immunocompromised patients. A polysaccharide capsule is one of its main virulence factors and consists of two distinct polysaccharides, glucuronoxylomannan (GXM) and galactoxylomannan (GalXM). How capsular polysaccharides are synthesized, transported and assembled by the Cryptococ is largely unknown. The biosynthesis of GXM and GalXM was proposed to require a series of specific enzymes for each polysaccharide. The route for transport and assembly of GXM and GalXM, however, might be the same for both polysaccharides or, alternatively, be completely independent routes. We have investigated whether mutations in cap-genes, previously identified by Kwon-Chung *et al.*, affect only GXM or both GXM and GalXM biogenesis. Wild type H99 (serotype A), its four isogenic derivatives with deletions in four different cap-genes and a CAP67 mutant (serotype D), which is known to produce GalXM, were used. Using immunological and chemical analysis we demonstrate that GalXM is produced and secreted by all Δ*cap* mutants but that these mutants do not secrete intact GXM. However, some typical GXM sugars are still detected in the extracellular milieu. The lack of intact GXM on the cell surface is not due to a defect in (1-3)-glucan, previously shown to be required for GXM attachment, since purified GXM from the wild type strain re-associates to the cell wall of all four Δ*cap* mutants. The wild type and all Δ*cap* mutants also secrete lipids, amongst others glucosylceramides. We are currently investigating the relation between this lipid (vesicle) secretion and GXM and GalXM biogenesis.

O021

Mastering microbiology

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From its very beginning microbiology attracted researchers from various disciplines like biology, chemistry, medicine (dentistry, veterinary science) and (bio)technology. Microbiology as we experience it nowadays is an umbrella for various sub-disciplines like medical microbiology, oral microbiology, veterinary microbiology, pharmaceutical microbiology, technical microbiology, food microbiology, microbial ecology, molecular microbiology and so on.

In my presentation, I will elaborate on the consequences of the complex disciplinary characteristics for educating microbiology at high school and university.

Concepts in microbiology are continuously developing and so are the various technical means to approach the research questions. With respect to that but also with respect to the great societal impact of microbiology , I will expand on the role of post academic education of microbiologists and the role of the NVVM and NVMM thereby.

O022

Are you certain? – Learning to ensure the quality of biology research in pre-university education

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In Dutch pre-university biology education, students often conduct their own research with enthusiasm, but that enthusiasm does not guarantee quality. Learning to do research means learning to ensure its quality. To ensure the quality of scientific research one needs to understand the *concepts of evidence*, the empirical argumentation which plays a role in every phase of research.

Two teaching and learning strategies have been designed and implemented to see whether they are good enough for learning to ensure quality of research. A dilemma in the didactical practice of conducting research in school is whether reflection upon its quality should take place in and as a consequence of (problems within) one's own research, or by taking a step aside and instead posing an other problem and applying it to one's own research. Both approaches have been used. In the *explicit teaching and learning strategy* reflection is stimulated by giving reflection tasks to students, for which they have to interrupt their own research: reflection-on-action. In the *implicit teaching and learning strategy* criteria are only raised – either by the teacher or by the students – when it is demanded by the quality of the research: reflection-in-action.

Starting from Galperins model of spiral formation of mental actions, utterances of teacher and students with respect to the concepts of evidence were categorised as *problematising, describing, explaining, generalising* and *applying*. The role of the teacher appeared to be crucial, the explaining and generalising particularly took place in conversations between teacher and students. In their mutual conversations the students mainly rested on the concrete level of describing and problematising their research. The reflection tasks on their own did not stimulate students to generalise, but the conversations with the teacher about the tasks did a bit more than the conversations about the own research of the students. Therefore, reflection-on-action, by taking a side-step from their own research, seems to stimulate the spiral of description, explanation and generalisation a little more than reflection-in-action alone. Put otherwise, recontextualizing the concepts of evidence in an additional context seems to stimulate the formation of mental actions a little more than staying in the context of the own research.

The lesson to be learned is that in learning to ensure the quality of research talking about it is crucial, on the concrete level as well as on the explanatory and general level.

O023

Modernisering van het onderwijs bij de opleiding tot arts-microbioloog

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In de geneeskunde heeft opleiden en opgeleid worden van oudsher een centrale plaats ingenomen. Elke arts is op zijn tijd student of docent. Er zijn weinig beroepen waar *leren* zulk een belangrijke rol bij de beroepsbeoefening speelt. Tijdens de beroepsbeoefening kan het leren via reflectie over het medisch handelen gaan, over medisch handelen dat heeft plaatsgevonden of over medisch handelen dat gaat worden verricht.

Elke arts draagt zijn kennis en ervaringen uit aan studenten, basisartsen, verpleegkundigen, etc.

De vervlechting van weet- en nieuwsgierigheid in het medisch handelen heeft geleid tot de hoge kwaliteit van de gezondheidszorg. Zo is en was de specialistische opleiding een opleiding van artsen die van elkaar leren en elkaars ervaringen delen. In Nederland heeft dat altijd goed gewerkt, dankzij redelijk kleinschalige opleidingsinrichtingen, de liefde bij vele opleiders voor het vak en de drang te leren en te doceren. Hierdoor hebben wij over het algemeen goede artsen en medisch-specialisten opgeleid.

De laatste decennia hebben wij echter allerlei maatschappelijk veranderingen kunnen waarnemen. Het natuurlijke gezag van de arts is verminderd. De arts moet in een mondige wereld veel meer laten zien dat hij een goede arts is. De opvattingen over wie een goede arts is, zijn veranderd. Kwaliteit moet geborgd zijn. Veel artsen hebben het gevoel dat de maatschappij steeds meer over hun schouder meekijkt als zij hun vak uitoefenen. Of wij het appreciëren of niet, vele artsen accepteerden niet meer dat zij 24 uur per dag, zeven dagen in de week, 365 dagen per jaar arts zijn. De toekomstige arts moet steviger in zijn schoenen staan. In onze informatiemaatschappij wordt de arts tevens overspoeld met informatie die hij slechts selectief kan opnemen. Terwijl de maatschappij echter van hem verwacht dat hij al die informatiestromen kent. De Amerikanen noemen dat de 'tattered social contract between medicine and society needs to be repaired'. (Cox & Irby. NEJM 2006;335:1375).

Door de grote veranderingen in de maatschappij is met name in Nederland het onderwijs al vanaf de zestiger jaren

van de vorige eeuw in beweging. Met veel vallen en opstaan verandert de wijze van onderwijs geven. De Mammoetwet uit 1960 gaf de stoot. Het middelbaar en basisonderwijs veranderde; het studiehuis werd ingevoerd. De universitaire opleiding veranderde. Het raamplan 1996 had tot gevolg dat ook het onderwijs aan geneeskundestudenten aan alle universitaire medische centra in Nederland werd geherstructureerd. De eerlijkheid gebiedt om te vermelden dat lang niet alle betrokkenen tevreden zijn over al die veranderingen. De voortrekkers van nieuwe curricula mogen en kunnen deze kritiek niet negeren door eenvoudig te wijzen op onkunde of conservatisme van een aantal docenten (hoofdartikel NRC 20 juni 2006). Veranderingen zijn soms doorgeschoten. De gedachten die twintig jaar geleden leefden over de toekomst van onderwijs zijn lang niet altijd bewaarheid. Daarom is draagvlak creëren voor nieuwe vormen van onderwijs belangrijk.
Nadat de nieuwe curricula voor de vorming van de student tot basisarts waren ingevoerd, werd het duidelijk dat ook de vervolgopleidingen moesten worden vernieuwd omdat de aansluiting van specialistische opleiding op de basisopleiding verloren dreigden te gaan.
In oktober 2002 verscheen het rapport: 'De arts van straks' van de Commissie Meyboom en in juli 2003 het rapport 'De zorg van morgen' van de Commissie Legrand. In deze rapporten worden aanbevelingen gedaan voor herstructurering van de artsenopleiding. Mede op grond van deze rapporten nam het Centraal College voor de Medische Specialismen (CCMS) de regie om de wetenschappelijke verenigingen te helpen tot een uniform stramien te komen voor de opleiding. Het gaat dan om eindtermen waarin de competenties moeten zijn beschreven. In competenties komen kennis en kunde, reflectie en creativiteit samen. Competenties beperken zich niet tot competentie die specifiek zijn voor een bepaald medisch specialisme. Er zijn ook vakoverstijgende competenties noodzakelijk om een goede specialist te zijn en volgens advies van het College moeten deze worden gebaseerd op de competentie-indeling zoals beschreven in de CanMEDS 2000.

O024

Een nieuwe opleiding: de opleiding tot moleculair medisch-microbioloog

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Binnen de laboratoriumdiagnostiek van infectieziekten neemt de moleculaire diagnostiek de laatste jaren een prominente plaats in. De verwachting is dat dit de komende jaren nog zal toenemen. Bij de virologie ziet het er zelfs naar uit dat moleculaire technieken de komende jaren de kweek en identificatie van virussen helemaal zullen (kunnen) vervangen.
Om ervoor te zorgen dat de kwaliteit van de moleculaire diagnostiek van hoog niveau is en blijft, is het noodzakelijk dat er goed gekwalificeerde academici worden aangesteld in deze laboratoria.
Om dit te bereiken is er in 2005 vanuit de NVMM een commissie, de commissie Kopopleiding moleculair medisch-microbioloog (MMM), ingesteld. Deze commissie heeft een plan voor de MMM-opleiding uitgewerkt.
Volgens dit plan is de MMM-opleiding een vervolg op de opleiding tot medisch-microbiologisch onderzoeker (MMO). De opleiding duurt twee jaar en zou in eerste instantie kunnen worden gevolgd in de academische centra waar een volledig uitgerust laboratorium voor moleculaire diagnostiek van infectieziekten aanwezig is. In deze opleiding zal het accent vooral liggen op de moleculaire diagnostiek van infectieziekten en op de moleculaire typering van microörganismen. De plannen zijn om eind 2007 te starten met deze opleiding.
Meer informatie over deze plannen zullen in de lezing aan de orde komen.

O025

Distance learning for food safety microbiology

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Introduction: The development and the production of safe food require skilled personnel with an up-to-date knowledge of microbial pathogens, hygiene, preservation, and state-of-the-art monitoring methods. Classical courses to keep knowledge and skills up-to-date require the participant to be away from the work place, and may involve extensive travelling costs. The European Chair in Food Safety Microbiology at Wageningen University has developed a modular distance learning program allowing participants to study topics relevant to the management of microbial hazards in food and food production from their own computer.
Methods: Each module of the course is composed of a limited amount of illustrated printable texts, describing the theory. Theory only, however, does not motivate participants to actively study the materials. The majority of the materials therefore consists of a short piece of theory, followed by a closed question concerning a practical application of that theory, encouraging the participant to actively study and solve the problem. A crucial addition is the extensive feedback to each question. Irrespective of the correctness of the answer given, feedback is generated automatically by the computer program explaining the

problem and the answers extensively. Apart from the short closed questions, some modules contain a digital case study, in which the participant is given a particular role in food safety management and has to complete an assignment. Relevant background reading materials are supplied throughout the modules. The program as a whole aims to provide good awareness of the concepts and terminology underlying specific aspects of managing microbial hazards in food and food production.

Results: The following modules have been developed to date: 1) Food Related Hazards, 2) Preservation, 3) Good Hygienic Practices, 4) Hygienic Design, 5) HACCP and 6) Sampling & Monitoring.

All modules are based on established good practices and mainly follow Codex Alimentarius principles and guidelines. Each module can be taken independently. The total distance learning programme takes approximately 4 to 5 weeks of study time.

Modules have so far been used on a trial basis by over 150 Wageningen University students and by more than 200 external participants, from academia, (inter)governmental organisations and food industry residing on the various continents around the world. Since January 1st, 2007 the complete programme is offered by Wageningen Business School on a non-profit cost basis as part of its postgraduate education programme (accessible via http://www.wbs.wur.nl/UK/). The programme is relevant for people directly involved in food production, manufacturing and catering, but also for academia and (inter-)government staff.

Conclusion: The Food Safety Management distance learning programme offers a flexible learning solution, allowing the participant to learn at any time and place. It offers a special opportunity to those who live too far or do not have time to come to face to face courses and it allows the participants studying the materials at their own pace.

O027

Small regulatory RNAs and their targets in the model pathogen *Salmonella typhimurium*

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Small non-coding RNAs (sRNAs) are an emerging class of post-transcriptional regulators of bacterial gene expression. Such RNAs are typically 50-250 nucleotides long, and are expressed from free-standing genes found in the inter-ORF regions of bacterial chromosomes. Most sRNAs studied to date have been found to act on trans-encoded mRNAs to modulate their translation and stability. Recent systematic searches indicate that non-pathogenic *Escherichia coli* may express several hundred sRNAs. The abundance and functional roles of these molecules in bacterial pathogens, however, remain largely unknown.

We have identified and studied more than 25 sRNAs in *Salmonella enterica* serovar *Typhimurium*. These sRNAs are expressed from the *Salmonella* core genome as well as from the *Salmonella* pathogenicity islands. Nearly all of them have homologues in related bacterial species. Northern hybridizations with RNA extracted from several growth conditions showed that many of these sRNAs are upregulated under conditions relevant to *Salmonella* virulence.

We have taken a large-scale approach to identify the cellular targets of >25 *Salmonella* sRNAs. Our results indicate that several hundred *Salmonella* mRNAs may be directly regulated by sRNAs at the post-transcriptional level. We have also identified a regulatory network in which sRNAs control the expression of major *Salmonella* outer membrane proteins (OMPs). Two of these OMP-regulating sRNAs are induced by anti-microbial peptides, and their transcription is dependent on the alternative stress sigma factor, RpoE.

The results of this first comprehensive study of small non-coding RNAs and their functions in a bacterial pathogen will be presented. In addition, the roles of the sRNA-binding protein, Hfq, for *Salmonella* virulence will be discussed.

O028

Stress-responsive gene regulation in *Helicobacter pylori*: hierarchical structure or organized chaos?

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Bacteria are versatile organisms that are able to persist and thrive in inhospitable environments. The colonisation of multiple environmental niches requires rapid concerted responses to stressful conditions, and this is often governed by 'master switches' that control subsets of niche-specific regulons and factors. *Helicobacter pylori* chronically colonises the human gastric mucosa despite the acidic conditions occuring there, and the aggressive immune response to the infection. Comparitive genomics using three *H. pylori* complete genome sequences has indicated a paucity of regulatory proteins, and this was originally seen to lead to a limited capacity for gene regulation, and has led to debate on which regulatory system could act as master switch. The concept for needing a master switch is now being challenged, since *H. pylori* is capable of regulating the transcription of at least ~20% of its genome in response to diverse stresses like acidity, nutrient limitation, oxidative stress, metal availability and temperature, despite the paucity of regulators. Many genes respond to multiple stresses, and thus the stress-responsive regulons of *H. pylori* display significant overlap. Here it is proposed that *H. pylori* directs its stress-responses via efficient use of the few regulatory systems

available, through a complex network of direct regulation, unidirectional and bidirectional regulatory cascades, and regulation of transcription by multiple regulatory proteins. Transcriptional regulation in *H. pylori* might thus resemble organised chaos, rather than transcriptional hierarchy, and the resulting absence of a master switch in *H. pylori* reflects the colonisation of a single, variable niche. *H. pylori* uses its 'simple' regulatory circuits to generate a complex regulatory network, and the resulting sensitive modulation of transcription contributes to the chronicity of *H. pylori* infection, allowing colonisation of a niche that lacks competing bacteria.

O029

Riboregulation in *Neisseria meningitidis*

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A riboregulated-network, in which small non-coding RNAs (sRNA) regulate the stability and thus translation of transcripts (mRNA) has recently been discovered in prokaryotes. sRNAs are encoded in intergenic regions (IGRs) and interact with the 5' or 3' untranslated region (UTR) of target mRNAs. In many cases the conserved prokaryotic protein Hfq has emerged as the key modulator of riboregulation. Hfq facilitates base pairing between sRNA and target mRNA, which may decrease ribosome binding or unmask the RNaseE cleavage site leading to mRNA degradation. Alternatively, the base pairing improves ribosome binding leading to mRNA stability. Thus, translation is affected either negatively or positively. The ferric uptake regulator (Fur), a transcriptional regulator, is fundamental for iron homeostasis in many prokaryotes. Fur inhibits expression of genes crucial to iron-acquisition by means of binding to a specific target sequence (Fur-box) in their promoters, but also acts as a postive regulator and affects the production of factors that store or contain iron. Crosstalk between the riboregulated network and the Fur regulated network has been demonstrated; the expression of a particular class of sRNAs has been shown to be Fur-regulated. These sRNAs often contain a Fur box in the promoter region and indeed interact with target mRNAs encoding proteins involved in iron homeostasis.

The strictly human pathogen *Neisseria meningitidis* possess a variety of genes involved in the adaptation to different environments encountered in the host, among them many genes necessary for iron acquisition. All three available genome sequences of *N. meningitidis* contain a gene with significant homology to *hfq*. An *hfq* deletion mutant in *N. meningitidis* is severely hampered in growth indicating the presence of an Hfq-dependent riboregulated-network in *N. meningitides*.

To explore whether riboregulation contributes to posttranscriptional regulation in *N. meningitidis*, IGRs of serogroup B strain MC58 were queried for sequences that include a putative promoter, the presence of a Fur box, followed by a 50 to 500 nt spacer and a potential terminator structure. This *in silico* approach led to the identification of nine putative sRNAs. Assessment by Northern blot analyses showed that one of these, sNmb3 (± 200 nt), localized between the loci NMB2073 and NMB2074, was shown to be repressed by iron. Initially, putative target mRNAs of sNmb3 were identified in the genome sequence of MC58 *in silico*, by searching for complementary sequences between the 5' UTR of protein encoding genes and regions of sNmb3. A significant hit was found with the 5' UTR of an operon encoding the iron-containing enzyme succinate dehydrogenase (*sdhCDAB*). The interaction of sNmb3 and its putative target mRNA was assessed *in vivo*, using a recent developed GFP-reporter system in *Escherichia coli*. The 5' UTR of *sdhCDAB* was translational fused to GFP (target fusion), transformed to *E. coli* and cells were either cotransformed with a plasmid expressing a nonsense (control) sRNA or sNmb3. Bright fluorescence of cells was observed in case of co-expression of the nonsense sRNA. A major reduction in fluorescence, compared to the control, was observed in cells cotransformed with the construct expressing sNmb3, indicating that expression of sNmb3 results in translational repression of the target fusion, most likely through an interaction with the 5' UTR of *sdhCDAB*.

In conclusion: These data provide evidence for a regulatory role of a sRNA in *N. meningitidis*, indicating the existence of a functional riboregulated network interlinked with the Fur regulated network in this strictly human pathogen.

O030

The Sab adhesins of *Helicobacter pylori*: acid-responsive regulation of expression and their role in the modulation of the host immune response

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Introduction: The human gastric pathogen *Helicobacter pylori* is the major cause of gastritis and peptic ulcer disease and is also associated with the development of gastric cancer. One factor allowing colonization by *H. pylori* is adhesion to the gastric epithelium via specific adhesins. Expression of several *H. pylori* adhesins is subject to phase variation (which randomly determines the 'on'/'off' status

of a gene), and may also be acid-responsive. Two of the important adhesins are SabA/SabB. Our aim was to study the regulation of expression and role in infection of the Sab adhesins of *H. pylori*.

Methods: *H. pylori* strains G27 and 26695 were grown for six hours in medium with the acidity adjusted to pH 7.0 or 5.5. Regulation of transcription was assessed using Northern hybridization. The phase variation status of sabA and sabB was assessed by nucleotide sequencing of the coding regions. Mongolian Gerbils were infected with *H. pylori* strain 7.13 and the isogenic sabA and sabB mutants. The gerbils were sacrificed 12 weeks after administration of *H. pylori* and the severeness of inflammation was assessed in the gastric mucosa.

Results: Transcription of the sabA and sabB genes decreased when *H. pylori* strains G27 and 26695 were grown in acidic conditions (pH 5.5) compared to pH 7.0. This was independent of the 'on'/'off' status of the genes (G27: sabA 'on', sabB 'on' vs 26695: sabA 'off', sabB 'off'). The gerbils colonized with the sabB mutant showed a significant increased level of acute inflammation (p=0.040), surface degeneration (p=0.025) and dysplasia (p=0.040) when compared to the wild-type strain. Neither SabA nor SabB was required for colonization by *H. pylori* of the gerbil gastric mucosa.

Conclusion: Acid-responsive modulation of adhesin expression may allow *H. pylori* to regulate its adhesion properties, which would enable the bacterium to escape unfavorable environmental conditions. Regulation of Sab transcription seems independent of phase variation, and this may allow regulation of adhesin expression at both the cellular and the population level. Finally, absence of SabB expression results in increased inflammation, suggesting that SabB has immunosuppressive activity. Concerted modulation of bacterial gene expression and host immune response are likely to contribute to the chronicity of *H. pylori* infection.

O031

Quantification of the effects of salt stress and physiological state on thermotolerance of *Bacillus cereus* ATCC 10987 and ATCC 14579

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Introduction: The adaptive stress response in microbes has a practical importance for food safety in minimal processing, as the adaptive response to a first hurdle may render the cell more resistant to subsequent hurdles. *Bacillus cereus* acquires enhanced thermal resistance through multiple mechanisms. Two *B. cereus* strains, ATCC 10987 and ATCC 14579, were used to quantify in detail the effects of salt stress and physiological state on thermotolerance.

Methods: Cultures were exposed to increasing concentrations of sodium chloride for 30 minutes, after which their thermotolerance was assessed at 50 C. Linear and non-linear survival models, which cover a wide range of known inactivation curvatures of vegetative cells, were fitted to the inactivation data and evaluated. The fitting performances of the different models were compared, taking into account the biological variation, i.e. the day-to-day variation. After selection of the most adequate model(s) for fitting the inactivation data of both strains, the number of parameters of the selected model was reduced when certain inactivation phenomena (e.g. shoulder, tailing) were not present. The remaining model parameter estimates were used to quantify and to compare the different experimental conditions.

Results: The thermotolerance of exponential-phase cells of both strains was highly increased by preexposure to lethal and non-lethal sodium chloride conditions, resulting in a lower inactivation rate for strain ATCC 10987 compared to the experimental conditions without preexposure to salt, and an additional shoulder period for strain ATCC 14579. The adaptive stress response was influenced by physiological state; the effect of preexposure to salt was less pronounced for transition- and stationary-phase cells than for exponential-phase cells for both strains. Based on statistical indices and model characteristics, the biphasic models with shoulder were selected and used for quantification of the effects of salt stress and physiological state on thermotolerance. The statistical analysis showed that the maximum adaptation to salt stress in exponential-phase cells resulted in a thermotolerance comparable to that of transition- and stationary-phase cells.

Conclusions: This study showed that primary kinetic models can be used to reliably estimate the effects of various stress conditions on the number of surviving organisms. The model characteristics and model flexibility influence the selection of the most suitable model(s) for quantification. Quantification of the adaptive response might be instrumental in understanding adaptation mechanisms and allows the food industry to develop more accurate and reliable stress integrated predictive modelling to optimize minimal processing conditions.

O032

Thiobacilli as key players in iron sulfide-associated denitrification

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Nitrate-driven microbial oxidation of iron sulfides poses a potential threat to freshwater ecosystem quality. Potentially

toxic trace metals associated with iron sulfide minerals will be mobilized. Produced sulfate may stimulate microbial sulfate reduction leading to sulfide toxicity and internal eutrophication. Reformation of iron sulfide minerals closer to the surface of soils and sediments increases the likelihood of oxic iron sulfide oxidation during periods of drought and thereby acidification and trace metal release. In the Netherlands, soils and sediments in freshwater ecosystems are often enriched in iron sulfide minerals and nitrate influxes occur frequently due to agricultural activities. It is therefore likely that anoxic iron sulfide mineral oxidation will occur. The microbiology of this process is mostly unknown and therefore our studies were aimed at gaining knowledge on the involved microbial players in freshwater ecosystems.

Culture-dependent and culture-independent approaches were combined to elucidate the microbiology of nitrate-driven oxidation of iron sulfides in the freshwater nature reserve Het Zwart Water. An enrichment culture was derived from material, rich in acid volatile sulfur (AVS) from this ecosystem by anoxic batch incubations with thiosulfate and nitrate. Activity on iron sulfide and nitrate was determined. The dominant bacterium in the enrichment was identified with 16S rRNA gene sequence analysis and FISH. An anoxic bioreactor study was performed with AVS-rich material from Het Zwart Water. Pyrite (FeS_2) and iron sulfide (FeS) were first tested as electrondonors for microbial denitrification. Subsequently, batch and continuous cultivation experiments were performed with iron sulfide and nitrate and/or nitrite. After 18 months of iron sulfide-fed operation, a 16S rRNA gene based clone library was constructed with reactor material and FISH analyses were performed to investigate the microbial community. Soil samples were collected at Het Zwart Water from different depths and their AVS, soluble iron, sulfate and nitrate contents determined and subsamples were fixed for FISH analysis. In addition, groundwater from Het Zwart Water was analyzed as indicated above.

The enrichment culture oxidized thiosulfate and iron sulfide at the expense of nitrate. The culture was dominated (99% based on FISH) by a *Thiobacillus* species (98% sequence identity to *Thiobacillus denitrificans*). Iron sulfide stimulated microbial denitrification in the bioreactor study whereas pyrite did not. *Thiobacillus*-like bacteria made up 50% of the total microbial community. Most probably these were the functional group performing the nitrate-driven sulfide oxidation which was identified as the dominant redox process in the reactor. The soil profile analyses showed coinciding AVS content and presence of *Thiobacilli* and provided indications for anoxic iron sulfide oxidation in the soil. The molecular analyses of the groundwater further confirmed the abundance of *Thiobacilli* in Het Zwart Water (43% of all clones, 23±9% of total community based on FISH).

The results from our studies indicate that 1) *Thiobacilli* are likely key players in anoxic iron sulfide mineral oxidation in freshwater ecosystems. In addition 2), the type of iron sulfide mineral available seems an important factor in the occurrence of anoxic iron sulfide mineral oxidation.

O033

Niche differentiation of coexisting sulfate reducing bacteria in a full-scale sulfidogenic bioreactor

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A combination of culture-dependent and independent methods were used to study the coexistence of sulfate reducing bacteria (SRB) in an upflow anaerobic sludge bed (UASB) reactor treating sulfate-rich wastewater. From the highest serial dilution that showed growth, 26 strains of SRB were randomly picked and isolated. Repetitive enterobacterial palindromic (rep) PCR and whole cell protein profiling revealed a low genetic diversity with only two genotypes identified among the 26 strains obtained in pure culture. The low genetic diversity among the strains suggests the absence of microniches within the reactor, which might be due to a low spatial and temporal microheterogeneity. Total 16S rDNA sequencing of the representative strains L3 and L7 indicated their close relatedness to genus *Desulfovibrio*. Besides the difference in motility, the two strains differed in as many as five physiological traits, which might allow them to occupy distinct niches and thus coexist within the same habitat. For SRB community characterisation within the reactor, whole cell hybridisation with fluorescently labeled oligonucleotide probes was performed. The isolated strains *Desulfovibrio* strain L3 and *Desulfovibrio* strain L7 were the most dominant representing (30-35%) and (25-35%) respectively of the total SRB community. *Desulfobulbus* like bacteria contributed for 20-25%. Among acetate oxidizers the *Desulfobacca acetoxidans* specific probe targeted approximately 15-20% of the total SRB. The whole cell hybridization results thus revealed a coexistence of different species of SRB that can be enriched and maintained on a single energy source in a full-scale sulfidogenic reactor.

O034

Microbial mediated iron oxidation in circumneutral wetland environments

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It has been reported that microbial iron oxidation plays an important role in neutral wetland environments. However, little is known about the mechanism of this process, mainly due to the difficulties in obtaining pure culture of these microbes. This study aims to enrich and isolate iron-oxidizing bacteria from three contrasting iron-rich locations in freshwater and brackish wetland environments. 16S RNA gene fragments of the enrichment were amplified and cloned. The clones were grouped by DGGE analysis and representatives were sequenced. Specific oligonucleotide probes will be developed to study the distribution and abundance of iron oxidizers in environmental samples. Their activities will be studied in model systems, including oxygen-limited chemostats, artificial root systems, and gnotobiotic plant systems. Special emphasis will be given to the competitive abilities of iron-oxidising bacteria under oxygen-limited conditions in comparison to chemical and other oxygen-consuming microbial processes.

O035

Phototrophic biofilms: primary productivity as a major determinant of microbial diversity

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The effect of biodiversity on ecosystem processes has become a major focus in ecology. Various ecological studies have shown that primary productivity, describing the rate of energy capture and its transformation into biomass by organisms, has a major effect on species diversity. A common pattern is that diversity peaks at intermediate levels of productivity. Most studies have been carried out with particular well-studied taxonomic groups such as terrestrial plants. Microbial ecological field studies on productivity-diversity relationships are only scarcely documented.
We conducted an experiment using freshwater phototrophic biofilms growing on the surface of submerged poles as a model ecosystem. Since the light intensity in the water column always decreases with depth, these biofilms grow and develop under a constant light gradient. The diversity of oxygenic phototrophs, as judged by Shannon Weaver indices calculated on the basis of denaturing gradient gel electrophoresis (DGGE) profiles, was determined for samples from different depths. Photosynthetic activity, determined *in situ* with the pulse amplitude modulated (PAM) fluorescence technique, was used as a measure of productivity.
Here we show, for the first time, that diversity of oxygenic phototrophs varies along a gradient of primary productivity. We observed that diversity increased linearly with the productivity measured along the poles. Surprisingly, we observed that this correlation is absent on poles submerged in water with lower nitrate concentrations, suggesting that these biofilms experienced nitrogen limitation instead of light limitation.

O036

DNA-markers for determination of microbiological quality of milk

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Presence of bacteria associated with poor hygiene or with bovine mastitis was determined in farm tank milk using DNA-based methods. The aim was to evaluate the correlation of qualitative and quantitative bacterial counts by DNA-markers with the cause of contamination.
Fast and quantitative real time PCR-tests were selected for these (groups of) bacteria and were assessed for specificity using 30 reference strains.
Random samples from raw milk with a low total bacterial counts (TBC) (n=42), high TBC (n=25) and high somatic cell count (SCC) (n=25) were analysed. The results showed 16 PCR positive samples, three with high TBC and thirteen with a high SCC. Three samples with a high TBC comprised bacteria associated with poor hygiene *Pseudomonas*, *Escherichia coli* and *Enterococcus*. Thirteen samples with high SCC comprised *Pseudomonas*, in three samples together with *E. coli* , in three samples together with *Enterococcus* , in three samples together with the mastitis associated *Staphylococcus aureus* and in three samples together with the environmental mastitis associated *Streptococcus uberis*. *Streptococcus agalactiae* was not found in any of the samples.
In conclusion, in 16 samples raw milk two or three members of the dominant bacterial flora associated with poor hygiene or (environmental) mastitis were identified proving the suitability of the use of DNA-markers in a real time PCR assay for specific analysis of the microbiological quality of raw milk. Apparently, different (groups of) bacteria constitute the dominant members of the population in 22 of the 25 high TBC samples, negative for al six PCR tests. For a complete picture of the dominant bacterial flora additional tests are needed. A full set of specific test may enable advising farmers on hygiene measurements and specific mastitis treatments. Moreover, analysis of milk of individual cows could be used for mastitis diagnosis.

O037

Volatile flavour formation by solid-state fermentation of soya beans by *Bacillus* spp.

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Introduction: Traditional alkaline Bacillus fermented foods include Japanese Natto, Indian Kinema, and Soumbala from Burkina Faso. Whereas the latter is usually prepared from locust beans, Natto and Kinema are soya bean products. Bacillus fermented foods are locally preferred for their flavour and texture, and are used as condiments. During earlier research we isolated a collection of *Bacillus* strains from Kinema and Soumbala (Sarkar *et al*., 2002). We investigated major volatile flavour compounds and flavour profile groups in relation to phenotypic and genotypic properties of the strains tested.

Methods: Microbiological: *Bacillus* cultures (35 strains including *Bacillus alvei, Bacillus badius, Bacillus cereus, Bacillus licheniformis, Bacillus mycoides, Bacillus subtilis*, and *Bacillus thuringiensis*) were isolated from both Kinema and Soumbala and kept on Nutrient Agar (Oxoid) slants or at '80 °C. Soya beans were rinsed, soaked, sterilized, and inoculated with 3 log units/g beans, and fermented 24 h at 42 °C.

Analysis of volatiles: Volatile compounds were analyzed by gas chromatography, using flame ionization detection (FID), mass spectrometry and olfactometry (sniffing port detection). Use was made of static headspace sampling (HS), SPME (solid-phase micro-extraction) and dynamic headspace sampling with the use of purge and trap (Tenax).

Results: In addition to low levels of 5 volatiles originating from cooked soya beans, the predominating volatiles produced by *Bacillus* strains included diacetyl, 1-pentene-3-ol, 3-OH-2-butanone, 2,5 dimethylpyrazine, trimethyl 1,5 pentanediol, 1-hexanol, 2 ethylbutanoic acid, trimethylpyrazine, acetic acid, dimethyl 2,3 pentanediol, 2,3 dimethyl 2 butanol, dimethylpropanoic acid, 2,3 butanediol, dimethylbutanoic acid, trimethylbutanoic acid, and methoxyphenyl oxime. Related to the distribution pattern of volatiles, 4 flavour profiles were distinguished, including 'sweet butter', 'sweet sour marmelade', 'heavy soft', and 'sour'. Compared with 21 genotype clusters obtained by RAPD-PCR fingerprinting, clusters with higher genotypical similarity resulted in similar volatile patterns and flavour profiles. Some clusters of highly similar genotypes from Soumbala as well as from Kinema also had very similar volatile patterns and flavour profiles.

Conclusion: Some key volatiles such as 2,5 dimethylpyrazine and trimethylpyrazine produced by all 23 strains of *B. subtilis* (Owens *et al*., 1997)were not produced by strains of *B. alvei* or *B. badius* or *B. licheniformis*. Genotypic similarity is reflected in flavour components produced. This points to key enzymes in the metabolism of e.g. pyrazines that may have diverse encoding or expression according to genotype. Further research will be required to identify the key factors determining the flavour profile of Bacillus fermented soya beans.

039

Lyme borreliosis serodiagnosis – misty results from the solid phase?

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Tick-borne diseases, especially Lyme borreliosis, increasingly gain public awareness as shown by reports in the daily press, set up of numerous self-help groups or by establishment of an unmanageable number of information sources in the internet. Caused by public informations like 'typically chronic course', typically hard to diagnose and to treat' or 'in most instances negative serology', broad uncertainty is present even for medical professionals regarding diagnosis and therapy.

Lyme borreliosis, a multisystem disorder caused by at least four different species in the *Borrelia burgdorferi* sensu lato complex, predominantly involves skin, nervous system, joints, and heart. Based on available guidelines, quality standards or expert opinions this disease is in contrast to the public opinion in most cases good to diagnose and efficiently to treat. The first step in diagnosis of Lyme disease is sampling of anamnestic and clinical data: is there sufficient evidence for this disease? Only if yes, serological tests are indicated and often mandatory to substantiate this diagnosis.

Beside standardization and proper validation of available serological tests, major problems for Lyme borreliosis serodiagnosis in Europe are still the broad heterogeneity of *Borrelia* species and subtypes involved in human disease, the differential- or even only in vivo expression of immunodomiant antigens, and differentiation of specific from unspecific antigens in immunoblot. To overcome such problems, recombinant antigens of *B. burgdorferi* s.l. are a promising alternative for serodiagnosis. Here, only specific, diagnostic relevant antigens or even homologues of different strains can be combined, truncated proteins with higher specificity can be used, and standardization, evaluation and interpretation is much more reliable and easy.

We have continuously improved and standardized our Western immunoblot using recombinant antigens of different strains belonging to the three main human pathogenic European species *B. burgdorferi* s.s., *Borrelia afzelii*, and *Borrelia garinii*. The actual version is based on 18 antigens/homologues of the antigens p100, p58, BmpA, VlsE, OspC, p41i, and DbpA. Performed in line blot format, this method enables reactivity assessment even of every single homologous protein. Comparison of the line blot to our in-house recombinant Western blot and whole cell lysate blot revealed a significant increase in sensitivity for IgG and IgM detection especially for early manifestations while specificity remained

unchanged. The increase in sensitivity depends on both, the new line blot technique and on additional recombinant antigens. To our oppinion this line blot offers a useful improvement for serodiagnosis of Lyme borreliosis.

O040

Is serological testing a reliable tool in laboratory diagnosis of syphilis? Insights from the German proficiency testing program

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The accuracy of diagnostic tests is critical for successful control of epidemic outbreaks of syphilis. The reliability of syphilis serology in the nonspecialist laboratory has always been questioned, but actual data dealing with this issue are sparse. Here, the results of eight proficiency testing sentinel surveys for diagnostic laboratories in Germany between 2000 and 2003 were analyzed. Screening tests such as *Treponema pallidum* hemagglutination assay (mean accuracy, 91.4% [qualitative], 75.4% [quantitative]), *T. pallidum* particle agglutination assay (mean accuracy, 98.1% [qualitative], 82.9% [quantitative]), and enzyme-linked immunosorbent assays (ELISAs) (mean qualitative accuracy, 95%) were more reliable than Venereal Disease Research Laboratory (VDRL) testing (mean accuracy, 89.6% [qualitative], 71.1% [quantitative]), the fluorescent treponemal antibody absorption test (FTA-ABS) (mean accuracy, 88% [qualitative], 65.8% [quantitative]), and immunoblot assays (mean qualitative accuracy, 87.3%). Clearly, immunoglobulin M (IgM) tests were more difficult to manage than IgG tests. False-negative results for samples that have been unambiguously determined to be IgM and anti-lipoid antibody positive accounted for 4.7% of results in the IgM ELISA, 6.9% in the VDRL test, 18.5% in the IgM FTA-ABS, and 23.0% in the IgM immunoblot assay. For negative samples, the mean percentage of false-positive results was 4.1% in the VDRL test, 5.4% in the IgM ELISA, 0.7% in the IgM FTA-ABS, and 1.4% in the IgM immunoblot assay. On average, 18.3% of participants misclassified samples from patients with active syphilis as past infection without indicating the need for further treatment. Moreover, 10.2% of laboratories wrongly reported serological evidence for active infection in samples from patients with past syphilis or in sera from seronegative blood donors. Consequently, the continuous participation of laboratories in proficiency testing and further standardization of tests is strongly recommended to achieve better quality of syphilis serology.

O041

Rapid bacterial identification and antimicrobial susceptibility testing decreases antibiotic use and accelerates pathogen directed therapy

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Introduction: Rapid identification and susceptibility results are a prerequisite for good patient care by providing an earlier opportunity for pathogen directed therapy. In this study, we examined whether accelerated bacterial diagnostics had an effect on mortality and/or antibiotic usage.

Methods: In patients of the Erasmus MC, Rotterdam, the Netherlands, were randomly assigned at the time they had a positive culture from a normally sterile body fluid, to either the intervention (rapid) arm or the control arm. The intervention consisted out of a fast automated method for bacterial identification and antimicrobial susceptibility testing (the VITEK 2 system, bioMérieux, Marcy-l'Étoile, France) combined with direct inoculation of blood cultures into this system. In the control arm our standard method was used: the Vitek 1(bioMérieux, Marcy-l'Étoile, France) system inoculated from subculture plates. Patients were prospectively followed for 4 weeks after inclusion. Of all included patients, microbiological culture data, age, sex, duration and place of stay, and mortality data were collected. From the first 1000 patients included, we collected underlying diseases, antibiotic usage, and infections during the hospital stay.

Results: 1498 patients were included and randomized: 746 in the rapid arm, 752 in the control arm. There were no significant differences between the two groups regarding the baseline patient characteristics at inclusion. No differences existed in the mix of infecting organisms at the time of inclusion, as well as the infections defined by these cultures. In the rapid arm 71.8% of patients had bacteraemia, versus 69.4% in the contol arm (N.S.). The difference in time between the rapid and control arm from randomization until a result of the laboratory was available, was on average 22 hours for susceptibility results and 13 hours for identification (p-value <0.0001). Same day susceptibility results were available for 393 of 747 (53%) patients in the rapid arm versus 2 of 752 (<1%) in the control arm (p-value <0.001). There was no statistically significant difference in mortality rate: In the rapid arm 130 out of 739 patients (17.6%) died; in the control arm 112 out of 738 patients (15.2%) died (p-value=0.21). There were significantly more changes of antibiotic therapy on the day of randomisation in the rapid group (p-value=0.006) and significantly more changes on day two after randomization in the control group (p-value=0.02). Rapid bacterial

diagnostics reduced antibiotic usage in the rapid arm with 6 defined daily doses (DDD's) as compared to the control arm (p-value=0.001)

Conclusion: In our hospital, rapid bacterial identification and antimicrobial susceptibility results did not reduce mortality, but it did lead to a significant reduction in antibiotic use and a significantly earlier switch to pathogen directed antibiotics.

O042

Quantification of *Streptococcus pneumoniae* DNA in blood samples from patients with invasive pneumococcal infection

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Introduction: *Streptococcus pneumoniae* is the most prevalent cause of community-acquired pneumonia (CAP) and meningitis (CAM) in adults in the Netherlands. Invasive infection is associated with increased mortality, and rapid diagnosis is essential. Blood cultures, the diagnostic gold standard, have a relatively low yield and may take two days to become positive. We evaluated the clinical use of real-time PCR for direct detection and quantification of bacterial DNA in blood samples, without prior cultivation.

Methods: Whole blood samples were obtained simultaneously with blood cultures from patients suspected of CAP or CAM. Bacterial DNA was isolated blood samples with the QIAamp DNA Mini Kit (Qiagen, Germany). PCR amplifications were done on a TaqMan 7000 System (Applied Biosystems, USA) with a PCR assay that targeted the autolysin A gene of *S. pneumoniae*. A quantitation curve was included to calculate the bacterial DNA load (BDL). PCR results were compared to blood culture outcome and clinical data.

Results: In total, 130 blood samples from 63 patients were tested with PCR. The sensitivity of PCR for detection of *S. pneumoniae* in blood samples was 80% and the specificity 94%, table 1. PCR amplifications were positive in 11/14 patients (79%) with bacteraemia and negative in 44/48 patients (92%) without bacteraemia. With PCR, 4 additional cases were identified compared to blood culture, all of whom had a microbiologically proven source of *S. pneumoniae* infection (including 1 case of meningitis). Blood samples from patients with a positive PCR signal and negative blood culture result had been obtained more frequently under antimicrobial treatment than blood samples from patients with positive blood cultures (p=0.04). When PCR results were combined with blood culture outcome, the detection rate of invasive *S. pneumoniae* infection increased from 22% to 29%. The median *S. pneumoniae* BDL was 24 cfu equivalents/mL (range 3.9-683). No association was found between BDL and clinical characteristics.

Conclusion: 1) Quantification of bacterial DNA in blood samples adds to the diagnosis of invasive pneumococcal disease, also in patients receiving antibiotics. 2) However, to ensure clinical applicability, the sensitivity of the our assay for detection of bacterial DNA in blood samples should be improved. 3) Determination of BDL provides a quantitative measure of bacteraemia, but the clinical value needs to be further determined.

O043

Clinical evaluation of an internally controlled *Tropheryma whipplei* Real-Time PCR

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Introduction: Whipple's disease is a rare systemic disease with a multitude of different symptoms, including arthralgia, diarrhea, weight loss, myalgia, low-grade fever, neurological abnormalities, and myocarditis. The disease is caused by the gram-positive bacterium *Tropheryma whipplei*, belonging to the family of *Actinomycetes*. Diagnosis is confirmed mostly with periodic acid-Schiff (PAS) staining of small bowel biopsies, and lately also with 16S RNA polymerase chain reaction (PCR) on various clinical specimens. Culturing of *T. whipplei* is laborious and is not feasible in routine laboratories. In this study, we developed a sensitive real-time PCR method for the detection of *T. whipplei* from various clinical specimens.

Methods: The target for the real-time PCR is a *T. whipplei* specific repetitive chromosomal sequence. An internal control (IC), containing the same primer sequences but a different probe sequence, was constructed and integrated in the chromosome of an *Escherichia coli* K12 strain. The sensitivity of the test was assessed by testing known positive specimens (biopsies, liquor and blood) and comparing with an in-house 16S rRNA PCR test. The specificity was assessed by testing 10 different bacterial species. Furthermore, 20 control colonic biopsies of patients were tested for the presence of *T. whipplei*.

Results: The amount of internal control was optimised for spiking into clinical samples All the clinical samples (17/17) which were positive in the 16S rRNA real-time PCR were also positive in the newly developed test. However, the new PCR was on average 100 times more sensitive The absolute sensitivity of the new PCR was determined as at least 1 colony forming units (cfu). This was based on the cultured IC due to the inability to culture *T. whipplei*. The test was

shown to be specific for *T. whipplei*, since none of the bacterial cultures were positive in the test. Unexpectedly, 3 of the 20 control biopsies tested were positive in the test. The presence of *T. whipplei* in one biopsy was confirmed with the 16S rRNA PCR and with sequencing. The signal of the other two biopsies was below the detection limit of the 16S rRNA test. Clinical symptoms of these 3 patients data were in agreement with some of the characteristic of Whipple's disease.

Conclusions: 1) A highly sensitive and specific *T. whipplei* real-time PCR was developed, based on a repetitive sequence. The test uses an internal control with the same primer sequences but a different probe sequence. 2) With this test 3 additional positive out of 20 control biopsies were detected from patients with gastrointestinal symptoms. This finding suggests that infection with *T. whipplei* may be more common than currently diagnosed.

O044

'Wie zeg je?... Dokter Ruijs? OK, verbindt maar door'. Het (telefonisch) intercollegiaal consult in de medische microbiologie

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Communicatie als dé sleutelvaardigheid in de medische microbiologie. Wat zijn de taken van de arts-microbioloog zoal, die hij in zijn beroepsuitoefening vervult? Een van de belangrijkste wordt gevormd door het 'intercollegiaal consult'. De evidence is schaars over het intercollegiaal consult bij de behandeling van patiënten met een infectieziekte (in de medische microbiologie), en daarnaast bemoeilijken de verschillen tussen de Amerikaanse setting en die in (noordwest) Europa generaliserende conclusies.

Van het intercollegiaal consult vindt veel telefonisch plaats (ca. 80%). Het vergt een aanzienlijk deel van de werktijd. Het uiteindelijke doel van het consult is een verbetering in het beloop van de infectieziekte van de patiënt, maar is daarnaast een sociale interactie tussen de voerders van het telefoongesprek. De wetten van het telefoongesprek gelden ook hier en passeren de revue.

Wat is er bekend over de effecten op het beloop van de infectieziekte van de patiënt? Er is slechts een handjevol onderzoeken over het uiteindelijke effect gepubliceerd, met wisselende resultaten.

Concluderend: Communicatie is dé sleutelvaardigheid in de medische microbiologie. Het telefonisch intercollegiaal consult is een belangrijke tool voor de arts-microbioloog. Die tool moet met zorg en liefde worden gehanteerd. Het effect van het telefonisch intercollegiaal consult op de 'patient outcome' is echter moeilijk aan te tonen.

O045

Real-time polymerase chain reaction and immuno-fluorescent-antibody assay for the detection of viral and atypical bacterial pathogens in children with lower respiratory tract infection

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Introduction: Accurate detection of viral and atypical bacterial pathogens is important in young children admitted to a pediatric department presenting with lower respiratory tract infection (LRTI) both to guide therapy and to prevent nosocomial spread. Standard methods such as the immuno-fluorescent-antibody assay (IFAA) and viral culture have a low sensitivity and specificity. To overcome these problems a real-time polymerase chain reaction (RT-PCR) for the detection of these pathogens was developed and used.

Methods: samples from children aged under 5 presenting at our hospital suspected of having LRTI were tested using a standard IFAA capable of detecting: respiratory syncytial virus A and B (RSV), influenza A, parainfluenzavirus 1-4, and adenovirus, and a PT-PCR to test for (RSV), influenza A and B, parainfluenzavirus 1-4, rhinovirus, adenovirus, human metapneumovirus (hMPV), *Mycoplasma peumoniae* and *Chlamydia pneumoniae*. Tests were performed on nasopharyngeal washings.

Results: a total of 283 patients were included, of whom 78 (28%) were positive for RSV by IFAA. RT-PCR confirmed all these positive results. In addition, 23 (8%) samples were IFAA positive for non-RSV viruses and 182 (64%) were IFAA negative. In samples negative for RSV by IFAA, RT-PCR detected: rhinovirus (100 samples), RSV (25 samples), hMPV (18 samples), adenovirus (15 samples), influenza A (4 samples) and parainfluenzavirus 1-4 (6 samples). Of samples negative for RSV by IFAA 138 (67%) were positive by RT-PCR. More than one virus was detected in 20 (7%) children.

Conclusion: RT-PCR for pediatric children with LRTI was found to be superior to IFAA, increasing the diagnostic yield almost threefold.

O046

Epidemiologie van MRSA in Europa en Nederland

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To date, meticillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections, primarily among immunocompromised patients. As data from the European Antimicrobial Resistance Surveillance System (EARSS) show, MRSA accounts for an important part

of *S. aureus* bacteremias in Europe. In 2005, generally, central European countries reported MRSA proportions between 5% and 25%, whereas all Southern countries reported higher levels, of which eight reported proportions of over 40%. In North Europe, most countries report resistance rates of below 5%, which is thought to be due to a combination of low antibiotic pressure and strict 'find and isolate' policies. However, consistent to the increase in many European countries, MRSA rates have also increased in Northern Europe, where MRSA rates have been low and fairly stable for many years. In the Netherlands, MRSA proportions have recently increased from 0.34% in 1999 to 0.93% in 2005. Similar trends were reported for Denmark (from 0.28% to 1.70%) and Finland (0.95% to 2.91%) over the same period of time.

The increase in MRSA proportions in recent years is reflected by the increasing number of MRSA strains received at the National Institute for Public Health and the Environment (RIVM), which runs the national MRSA database. Whereas the number of strains received per year increases, the proportion of these strains that can be related to important traditional risk factors i.e., having been hospitalized or working abroad, decreases. With the recent emergence of MRSA strains at different occasions and settings outside healthcare facilities, evidence accumulates that the epidemiology of MRSA in the Netherlands is now rapidly changing. For example, the MRSA ST 398 strain, related to cattle farming, was first received at RIVM in 2003 and comprised 25% of all strains received in the last quarter of 2006. The above mentioned trends may complicate proper implementation of the Dutch guidelines. At the same time, strict adherence to the guidelines seems to be more important than ever, because MRSA proportions in the Netherlands may be approaching a critical threshold. Examples from Germany and the United Kingdom show that MRSA proportions can increase from less than 5% to over 20% in just a few years time.

The changing epidemiology of MRSA in Europe and in the Netherlands will be discussed using data from the international EARSS system and the national MRSA database.

O048

What's next: impact on the Dutch control strategy

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For many years, the Netherlands uses the so called 'Search and Destroy' (S&D) strategy to control MRSA in hospitals. Thereby a very low incidence has been maintained, despite a high incidence in the surrounding countries. Since clonal dissemination is the mechanism for the spread of MRSA, control of MRSA depends largely on the prevention of transmission from known carriers. Antibiotic usage probably plays a role as well by applying selective pressure that gives resistant strains an advantage over their susceptible ancestors, but control of antibiotic usage alone will not control the spread of MRSA. An active policy to find carriers of MRSA and prevent further transmission from these carriers is the core measure for the control MRSA.

The first outbreaks of MRSA in the Netherlands were described in the 1980s. They originated from the transfer of patients from hospitals abroad. S&D was applied and controlled the outbreaks. Subsequently a national control policy was defined by the Working Part on Infection Control (WIP) and has been based mainly on patients from foreign hospitals and control of spread within the hospital. However, recently there is an increase of patients with MRSA who do not belong to the known risk groups. This is caused partly by the emergence of MRSA in the community. In addition an extensive reservoir in people who work with pigs and calves has emerged over the last 3-4 years. Therefore the groups at increased risk of MRSA carriage have increased significantly. This increases the cost of screening and isolation. It is unlikely that the reservoirs in the community and in animals will be controlled in the coming years. To adapt the control policy in the most optimal way, more information regarding the risk groups, transmissibility of the veterinary strains and its virulence are urgently needed. The implementation of rapid diagnostic screening tests may help to remain in control of MRSA in a cost-effective way. If MRSA will spread in the community on a large scale, the current control policy has to be reconsidered. To have timely information on the epidemiology of MRSA the national surveillance has to be intensified.

O049

Surface structures of the Gram-negative bacterium *Bordetella pertussis*: role in pathogenesis and adaptation to vaccines

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Bordetella pertussis is the causative agent of pertussis or whooping cough. Before the introduction of vaccination, pertussis was one of the major causes of child death. Vaccines were introduced in the 1950s in many countries and have significantly reduced morbidity and mortality due to pertussis. Despite this initial success, pertussis has reemerged in vaccinated populations and it is presently the least controlled vaccine-preventable disease. Worldwide,

pertussis remains one of the top ten causes of death, mainly in unvaccinated children. Very high infection frequencies have been observed, even in vaccinated populations. E.g. sero-epidemiological studies suggest that 1 million people are infected by *B. pertussis* every year in the Netherlands. Although many of these infections are mild or subclincial, the high circulation rate of *B. pertussis* is a significant health risk for infants too young to be completely vaccinated. The persistence of *B. pertussis* despite high vaccination coverage is particularly surprising in view of its low genetic diversity. However, *B. pertussis* is a well-armed pathogen and produces a large number of virulence factors which target host tissues and foil host immune responses. Some of these virulence factors are antigenically polymorphic and highly directional changes have been observed in their allele frequencies suggestive of bacterial adaptation. Interestingly, variation is also observed in the regulation of virulence factors and in the 1990s strains have emerged which show an enhanced production of pertussis toxin. Both the antigenic changes and the changes in gene regulation have been observed after the introduction of vaccination, suggesting they represent adaptations to vaccination. Elucidation of the function of *B. pertussis* surface structures, their variability and their temporal changes may lead to improved pertussis vaccines.

O050

Biogenesis of the Gram-negative bacterial outer membrane

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Gram-negative bacteria are surrounded by two membranes, an inner membrane (IM) and an outer membrane (OM), which are separated by the periplasm containing the peptidoglycan layer. The two membranes are quite different in terms of structure and composition. Whereas the IM is a phospholipid bilayer, the OM is an asymmetrical bilayer, consisting of phospholipids and lipopolysaccharides (LPS) in the inner and outer leaflet, respectively. This makes the OM an effective permeability barrier rendering the bacteria inherently resistant to many antibiotics. The IM and OM also differ with respect to the structure of their integral membrane proteins. Whereas integral IM proteins typically span the membrane in the form of hydrophobic α-helices, integral OM proteins (OMPs) generally consist of antiparallel amphipathic β-strands that fold into cylindrical β-barrels with a hydrophilic interior and hydrophobic residues pointing outward to face the membrane lipids. Both membranes also contain lipoproteins, which are anchored to the membranes via an N-terminal lipid tail. All the constituents of the OM are synthesized in the cytoplasm or at the cytoplasmic face of the IM; therefore they have to be transported across the IM and through the periplasm to reach their destination and to assemble into the OM.

Our research on OM biogenesis focuses particularly on the transport and assembly processes of the β-barrel OMPs and LPS. We identified an OMP, designated Omp85, that is required for the insertion of OMPs into the outer membrane. We demonstrated that Omp85 is essential for the viability of the bacteria, and that, upon depleting the bacteria of Omp85, all other OMPs fail to insert into the outer membrane and accumulate as aggregates in the periplasm. Also, we found that Omp85 recognizes its OMP-substrates by a species-specific C-terminal motif. Interestingly, Omp85 homologues are present not only in all other Gram-negative bacteria, but also in mitochondria, where they are also essential for the assembly of OMPs. Thus, the process of OMP assembly appears evolutionary conserved.

For our studies on LPS biogenesis, we use *Neisseria meningitidis* as a model organism. This bacterium has turned out to be very instructive for these studies since it has the unusual property of being able to live without LPS. This trait enabled us to identify a protein that is essential for the transport of LPS to the cell surface. This protein, designated Imp, is not essential for the viability of *N. meningitidis*. An *imp* mutant produced much less LPS than its parental strain, and the residual amounts of LPS produced were not accessible at the bacterial cell surface. Thus, Imp is most likely responsible for the flip-flop of the LPS across the outer membrane to the cell surface. Other components of the LPS transport pathway are being identified by searching for Neisserial mutants with similar phenotypes as the *imp* mutant.

Thus, using other model systems besides the classical model organisms, *Escherichia coli* and *Salmonella enterica*, has yielded much progress in the field of OM biogenesis. These studies and other recent progress in the field will be discussed.

O051

Identification of novel surface proteins in *Streptomyces coelicolor* using MALDI-TOF mass spectrometry

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Streptomyces coelicolor is a soil-dwelling filamentous bacterium with a complex lifestyle. After a period of submerged growth, hyphae grow into the air to form chains

of spores that, after dispersal, can colonize a substrate elsewhere. Differentiation in *Streptomyces* is accompanied by a drastic change in the surface characteristics of hyphae: submerged growing hyphae are hydrophilic while aerial mycelium and spores are strongly hydrophobic. This surface hydrophobicity is mediated by the chaplins and the rodlins. Chaplins are a class of small secreted proteins that assemble into small amphipathic fibrils at the surface of aerial hyphae. Rodlins align these fibrils into a mosaic of rods called the rodlet layer.

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) was applied on intact spores to identify other proteins at the surface of the cell wall. Using this method we found the chaplins, the rodlins, the small peptide SapB as well as several other unknown proteins. The molecular weight of one of these proteins matched the deduced molecular weight of the secreted protein encoded by gene SCO6161. Deletion of SCO6161 resulted in mutants that were severely affected in sporulation even after prolonged incubation. Taken together, this indicates that like rodlins and chaplins SCO6161 is involved in aerial growth by playing a role in cell wall assembly.

MALDI-TOF MS analysis of spores of the chaplin mutant revealed proteins that were not detected in the wild-type strain. The mass of one of these proteins matched the deduced molecular weight of the secreted protein SCO6973. This protein is predicted to have three ankyrin-motifs. This motif is commonly found in eukaryotes and mediates protein-protein interactions. Possibly, SCO6973 is located beneath the rodlet layer and is involved in organization and stabilization of this film which is composed of rodlins and chaplins.

O052

Expression of five putative LPXTG surface proteins enriched in clinical and outbreak associated *Enterococcus faecium* isolates

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Introduction: *Enterococcus faecium* (*Efm*) is an opportunistic nosocomial pathogen causing serious infections in immuno-compromised patients. In these patients *Efm* is responsible for urinary tract infections, surgical site infections, bacteremia and endocarditis. In the *Efm* population structure, based upon multilocus sequence typing, hospital outbreak associated and most invasive isolates cluster in a genogroup, designated clonal complex-17 (CC17). To gain insight in the adaptive mechanisms that may have favoured the emergence of CC17 *Efm* we aimed to identify putative LPXTG cell wall anchored proteins (CWAP) that are found specifically enriched in CC17 and to detect their mRNA expression levels.

Methods: Using PCR and Southern hybridizations 131 *Efm* isolates (40 CC17 and 91 non-CC17) representing isolates from hospitalized patients (81), human volunteers (30), animals (12) and the environment (8) were screened for the presence of 22 putative CWAP genes identified from the *Efm* DO genome (a CC17 *Efm*). Reverse transcriptase (RT) PCR was used to detect mRNA transcripts of CWAP genes. Minimum Spanning Tree (MST) analysis was carried out for population modelling based on the presence and absence of 22 putative CWAP genes.

Results: CC17 *Efm* harbour on average 21 of the 22 analyzed CWAP genes while non-CC17 *Efm* contained only 15 of these genes. Five CWAP genes were found to be specifically enriched in CC17 *Efm* isolates and are highly homologous to microbial surface components recognizing adhesive macromolecules. These 5 CWAP genes were found in 28-40 (70-100%) of CC17 and in only 7-24 (8-26%) of non-CC17 isolates ($p<0.05$). MST analysis based on presence and absence of 22 CWAP genes revealed grouping of 40 CC17 together with 18 hospital-derived but evolutionary unrelated non-CC17 isolates in a distinct CWAP enriched cluster, suggesting horizontal transfer (HGT) of CWAP genes. Using RT-PCR, mRNA transcripts of the five CC17 enriched CWAP genes were detected after growth at 37 °C, demonstrating that these five genes are actively expressed.

Conclusions: We identified 5 putative CWAP genes enriched in CC17 which are possibly acquired via HGT. These 5 CWAP genes are expressed at transcriptional level, and therefore may encode for proteins that are anchored to the cell wall. We also show that infection and hospital-related CC17 *Efm* and 18 non-CC17 *Efm*, which are all clinically relevant cluster together based upon CWAP profile. This suggests that a specific CWAP profile may enhance its pathogenic potential and may contribute to its success in the hospital environment. The 5 putative CWAPs may serve as targets for immunotherapy to prevent and treat CC17 *Efm* infections.

O053

From risks to methods to regulation

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Food safety laws and regulations aim at reducing risks for individual consumers and society in general. The process of risk analysis is defined by Codex as hazard identification, risk assessment and risk communication. Methods and regulations are instruments for risk management. Risk management is not possible without prior risk assessment.

In practice this means that risks are first of all identified and assessed. The outcome of the assessment procedure is an advice to the risk manager, which usually spells out options to reduce the scrutinized risk, including control measures that may require test methods. The risk manager will decide which measures to take in order to reduce the risk and spell out which parameters will be monitored and the methodology to be used for that purpose. Recently introduced approaches for risk management include 'Microbiological criteria' and 'qualified Presumption of safety'. These require tailored methodology for monitoring. Methods to be used for managing risks must fulfil a number of demands. 1) They must be sufficiently reliable for law enforcement. 2) Ideally real time monitoring is possible. 3) They must be cost effective. 4) Worldwide application is possible.
It will be clear that at this moment very few methods fulfil all of these demands. In addition, there is usually more than one parameter to be monitored. All in all it can be very problematic to have adequate monitoring at a reasonable cost. Risk managers must carefully balance the wish to reduce risks with the costs involved to prevent causing the price of foodstuffs to rise more than strictly necessary.

O054

Global harmonisation of microbiological criteria for foods

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For both protection of public health and for fairness in trade, issues in microbiological food safety are of large importance. In the last decades, the approach in food safety is going more and more from 'checking' to 'control'. The idea behind HACCP, and also in the approaches currently under development (Food Safety Objectives), is to control risks at those places in the food chain, where it is the most efficient. Still criteria are needed, but these do not necessarily have to be related to the microorganisms themselves. For validation and verification of the approaches still microbiological methods are necessary, also if products are transported over borders, and little information of their history is available.
Setting harmonised criteria is not an easy task however due to the complexity of food chains, the complexity of the microbial responses, and the difficulty that infectious microorganisms can even pose a relevant risk if they are present in very low levels, especially considering very large amounts of product units. Furthermore large variabilities and uncertainties exist. Apart from these technical aspects, of course also many different stakeholders have many different interests, making balanced decisions very difficult and time-consuming to reach.
Since food products are nowadays traded world-wide, harmonisation is of utmost importance. For this it is important that the criteria are risk based and selected to result in an appropriate level of health protection, balancing the public health risk with all other relevant aspects like economics, food, quality, freedom of choice, and other risks.
To reflect issues and problems in harmonization, the example of food safety risks and criteria for baby food will be used. The problems of infant botulism through honey and *Enterobacteriaceae* in milk powder are managed in different ways, showing various aspects of these issues, the multitude of management options, the role of risk assessment, the difficulty in harmonising definitions and finally different microbiological criteria will be described.

O055

Overview of methods in use and their improvements

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Microbiological food safety and quality is of great importance for both the consumer and industry. Inevitably, this requires appropriate management tools that enable the control of all potential risks at relevant point in the food chain (HACCP). From a microbiological point of view, such control can be based on parameters such as physical and chemical parameters. However, microbiological examination is still essential for confirmation but also to meet requirements of specifications. In particular in processes and production practices for vulnerable food products of which product safety is difficult to manage, microbiological testing is common routine.
To date, the traditional procedures based on culturing and subsequent identification via immunochemistry and DNA probes are common practice. Such procedures often lack the speed required for making management decisions during production, and are therefore only useful for gaining retrospective information. During the last couple of decades, molecular biology made a jump forward which has resulted in several new approaches that improve microbiological analysis in terms of speed and sensitivity. The polymerase chain reaction for the specific amplification of DNA allowed a reduction in time for culturing. In those cases where the product flow is held in buffer for some time, measures could be taken based on this state of the art microbiological analysis.
Nevertheless, there is still a battle to win to come to at line monitoring. Therefore this presentation will also show what steps are taken to circumvent culturing and to improve the acceptance of novel molecular methods in the routine setting in the food industry. Moreover, new developments in the area of microarray technology

allow a further improvement in microbiological testing in future. Since biomarkers can be identified which correlate with virulence and survival factors, novel ways of microbiological testing can be introduced to control food safety.

O057

Do pneumococcal vaccines prevent otitis media?

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Otitis media is the most frequent childhood infection and the primary reason for a child under 3 years the age to consult a physician. Importantly, it is the most common indication for the prescription of antibiotics, and therefore contributing to spread of antibiotic resistant microbes, even in the Netherlands where it is estimated that 30% of all AOM cases result in antibiotic usage. By the age of 3 years, approximately 80% of all children have had at least one episode of OM. Recurrent AOM is defined as 3 or more episodes of AOM to have occurred in three months or 4 or more episodes in 12 months and affects 10-20% of all children by 12 months of age. In developing countries, OM occurs at even a much higher rate and contributes to increased mortality and severe disabilities. Perforation of the tympanic membrane, mastoiditis and otorrhea (consistent with CSOM) are common. Other OM complications include septic shock, meningitis, thrombosis and brain abscess. OM is responsible for the deaths of over 50.000 children under the 5 years of age annually, according to a WHO report about 15 years ago.

OM is a polymicrobial disease in which complex relationship between the different bacteria and viruses have been described. Of the 3 bacterial pathogens that predominate as the cause of AOM, Streptococcus pneumoniae is thought to be involved in 25-50%. Immunisation of children with polyvalent polysaccharide vaccines consisting of the capsular polysaccharides from pneumococcal serotypes most frequently involved in AOM, had no significant impact on the prevention of OM. Newer pneumococcal conjugate vaccines in which the 7 pediatric capsular polysaccharides are conjugated to proteins, were shown to induce good protective IgG antibody levels to prevent invasive pneumococcal disease and to reduce involvement in OM of vaccine serotypes by 57%. Also, nasopharyngeal carriage of pneumococcal serotypes was shown to decrease significantly. However, nasopharyngeal colonization by non-vaccine serotypes increased so that overall pneumococcal carriage rates in vaccinees remained similar to non-vaccinees. Also in OM, replacement by non-vaccine pneumococcal serotypes by 30% was shown in a large Finnish study, resulting in only a marginal reduction 6% of all-cause OM in general. In addition, an increase in Staphylococcus aureus AOM in children with recurrent AOM was shown after conjugate vaccination as well as a possible disturbance in the ecological balance between S. pneumoniae vaccine serotypes and S.aureus in the nasopharynx. In USA postmarketing studies on AOM after implementation of the conjugate vaccine, an increase in H.influenzae was observed as well as β-lactamase producing Gram-negatives, next to non-vaccine pneumococcal serotypes 6A and 19A. A more recent study in the Czech republic with a 10 (11)-valent pneumococcal vaccine conjugated to NTHi, showed a 50% reduction of pneumococcal OM, a 35% reduction of NTHi OM and an overall reduction of 33% of OM. However, in the Czech study, the overall incidence of OM was at least 10-times lower compared to the Finnish study as were involvement of S.pneumoniae, NTHi and MC, making the studies hard to compare.

A combination of vaccines including bacterial pathogens like S.pneumoniae, NTHi and M. catarrhalis may seem the most promising to prevent AOM, but other colonizing bacteria of the nasopharynx may come up. Viral vaccines like influenza and RSV may prove to be the most relevant to prevent bacterial disease. It may be concluded that additional future studies on bacterial and viral vaccines are still required to gain better insight in optimal prevention strategies for OM.

O058

Moraxella catarrhalis vaccine development

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Moraxella catarrhalis is an exclusively human respiratory tract commensal and mucosal pathogen and a major cause of acute otitis media in children and exacerbations of COPD in adults. Its relative contribution to the disease burden inflicted by otitis media is likely to increase in the near future, because the use of pneumococcal conjugate vaccines in infants increases the frequency of detection, the bacterial load in the nasopharynx and the incidence of otitis media caused by *M. catarrhalis* and other non-pneumococcal pathogens. However, *M. catarrhalis* otitis media is less severe and more frequently resolves spontaneously than pneumococcal otitis media.

The primary goal of vaccine development against *M. catarrhalis* is the identification of antigen preparations suitable for being included in an combination otitis media vaccine together with antigens of *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae* (NTHI). Major challenges include the fact that no suitable animal model for *M. catarrhalis* otitis media is available,

and that conventional, parenterally administered vaccines appear to afford only partial protection against mucosal infections (e.g., pneumococcal conjugate vaccines). Also, there is evidence to suggest that *M. catarrhalis* colonization in pharyngeal lymphoid tissue is much more frequent in healthy children than appreciated by surface swabbing. Thus, a vaccine with the potential to entirely eliminate *M. catarrhalis* carriage may not be desirable.

Passive and active immunization studies in a murine pulmonary clearance model provided the proof of principle that specific, bactericidal IgG serum antibodies are associated with protection against challenge with homologous or heterologous *M. catarrhalis*. Antigens used in active immunization studies were killed whole cells or purified or recombinant outer membrane proteins (OMP), respectively, or detoxified lipooligosaccharide (dLOS) covalently coupled to protein carriers. Based on their capacity to induce bactericidal antibodies against antigenically conserved, surface-exposed epitopes, a number of potential candidate vaccine antigens have been identified and include – among others – the UspA1 and UspA2 proteins, hemagglutinin (also known as *M. catarrhalis* IgD-binding protein), CopB, OMP CD, OMP G1b and LOS. OMP may not only be useful as individual subunit component in a combination vaccine, but may also severe as protein carrier for polysaccharide antigens of *S. pneumoniae* or LOS antigens of NTHI and *M. catarrhalis*.

As an alternative to traditional parenteral antigen delivery, intranasal immunization has been investigated in animal models using OMP (e.g., native and recombinant OMP CD) or dLOS and provided evidence of induction of both systemic and mucosal immune responses and protection against pulmonary challenge with *M. catarrhalis*.

Thus, immunization against *M. catarrhalis* appears feasible and has the potential of reducing the incidence of otitis media and COPD exacerbations caused by this pathogen. Clinical studies alone will unveil its true protective capacity and its impact on the microbial ecology of the human upper respiratory tract.

O060

Molecular dissection of group A *Streptococcus* epidemic waves: the restless tide of phages

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Although of tremendous importance to society, and a fundamentally interesting problem in biomedical research, the molecular genetic events contributing to bacterial clone emergence and epidemic waves are poorly understood. We have used group A *Streptococcus* (GAS) as a model human pathogen to study these processes. Historically, various techniques have been used to index genetic diversity among bacterial isolates for study of genomic diversity and strain evolution. Although many insights have been obtained, these studies have substantially underestimated genetic diversity among isolates due to limitations in the resolving power of the techniques applied, such as pulsed field gel electrophoresis, multilocus enzyme electrophoresis, and multilocus sequence typing. Moreover, convenience rather than population-based strain sampling generally has been used, thereby further limiting our understanding of epidemic waves. We have recently used genome-wide analysis methods to gain new information about molecular processes contributing to clone emergence and epidemic waves. Serotype M3 GAS strains (n=320) cultured from patients in a population-based study of invasive infections over 15 years and representing three distinct epidemic waves were studied by genome-wide analysis methods, including pulsed-field gel electrophoresis, whole-genome DNA-DNA microarray and PCR scanning, prophage genotyping, targeted gene sequencing, single nucleotide polymorphism genotyping, and genome resequencing. All variation in gene content was attributable to acquisition or loss of prophages, a molecular process that generated novel combinations of proven or putative virulence genes. Distinct serotype M3 genotypes experienced rapid population expansion and caused infections that differed significantly in character and severity. The molecular genetic analysis, combined with immunologic studies, implicated a four-amino-acid duplication in the extreme aminoterminus of M protein as a factor contributing to a new epidemic wave of serotype M3 invasive infections. In related studies, we discovered that a highly virulent clone of serotype M1 GAS now responsible for the vast majority of infections caused by this serotype in the United States, Canada, and Europe, has evolved through a series of horizontal gene transfer events that involved 1) the acquisition of prophages encoding streptococcal pyrogenic exotoxin A and extracellular DNases and 2) the reciprocal recombination of a 36-kb chromosomal region encoding the extracellular toxins NAD+-glycohydrolase (NADase) and streptolysin O (SLO). These horizontal gene transfer events were accompanied by significantly increased production of streptolysin O and NADase virulence factors. Virtual identity in the 36-kb region present in contemporary serotype M1 and M12 isolates suggests that a serotype M12 strain served as the donor of this region. Thus, genome-wide analysis of bacterial pathogens, coupled with conventional bacterial pathogenesis studies, proved to be crucial for understanding the molecular events contributing to clone emergence and epidemic waves. It is very likely that study of other microbial pathogens by the general strategies that we used will be a very fruitful line of investigation. Studies

of this type move us a step closer toward genome-wide personalized infectious diseases research, a discipline that will rapidly emerge in the coming 10 years.

O061

Bacteriophages provide *Staphlococcus aureus* a toolbox to counteract the human innate immune system

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The genes for the immune evasion molecules chemotaxis inhibitory protein of *Staphylococcus aureus* (*chp*), staphylococcal complement inhibitor (*scn*), staphylokinase (*sak*) and enterotoxin A (*sea*), cluster on the conserved 3' end of β-hemolysin (*hlb*) converting temperate bacteriophages (βC-Φs) forming an innate immune evasion cluster (IEC). Seven IEC variants were discovered, carrying different combinations of *scn*, *chp*, *sak* or *sea*, always in the same 5' to 3' orientation and on the 3'end of a βC-Φ. From most IEC variants we could isolate active bacteriophages by mitomycin C treatment, of which lysogens were generated in *S. aureus* R5 (broad phage host). All IEC carrying bacteriophages integrated into *hlb* as was measured by southern blotting of R5 lysogens. Large quantities of the different bacteriophages were obtained by mitomycin C treatment of the lysogens, bacteriophages were collected and used to re-infect all lysogenic R5 strains. In total 5 lytic families were found. Furthermore phage DNA was isolated and digested with EcoR1, revealing that one IEC variant can be found on different βI-Φs. βC-Φs carrying an IEC are mobile and very abundant, 90% of *S. aureus* clinical-, and nose-isolates carry one. In comparison with other mobile virulence factors, IEC encoding genes exhibit an exceptional high incidence among human isolated *S. aureus* strains. CHIPS and SCIN are small, excreted molecules that play a role in the staphylococcal defence against the human innate immune system. Both CHIPS and SCIN counteract crucial acute responses of our immune system such as complement activation, neutrophil chemotaxis and neutrophil activation. By studying gene expression via promoter–green fluorescent protein fusions, Northern blots and protein expression analyses, we found that CHIPS and SCIN are produced during the early (exponential) growth stages. Although the CHIPS and SCIN genes are expressed simultaneously, they are differently regulated by various *S. aureus* regulatory loci. However, the *sae* locus is crucial for up regulation of both CHIPS and SCIN. Because CHIPS and SCIN are both efficient modulators of neutrophil chemotaxis, phagocytosis and killing, their early expression is necessary for efficient modulation of the early immune response.

The large variety in both IEC and βC-Φs carrying this cluster shows that IEC is a very dynamic DNA element. It has spread successfully through the *S. aureus* population and probably will continue to do so. This provides *S. aureus* with a unique mechanism to adapt to, and counteract, the human host.

O062

Identification of therapeutic drug compounds with phage display

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Since the discovery of phage display by George Smith, who demonstrated the feasibility of displaying peptides on the surface of *Escherichia coli* bacteriophage M13, the method matured and has been explored extensively for the identification of antibodies, hormones, enzymes and other protein domains. The physical linkage between phenotype, i.e. the displayed protein domain, and genotype, i.e. the gene of the domain encoded by the encapsulated phage genome, permits the creation of repertoires, which are selected in a high throughput mode by binding to immobilized ligand, antigen or any other type of interacting molecule. With this selection procedure lead molecules can be discovered from fully synthetic repertoires or for antibodies from non-immune and immune libraries, but the method can be applied as well for the engineering of specificity, affinity and stabilty. The fisrt antibody product generated by phage display, the anti-TNF antibody Humira from Cambridge Antody Technologies/Abbott, is marketed successfully, while Lucentis, Genentech's Fab fragment directed against VEGF obtained by phage display based affinity maturation, was recently approved for clinical use, thus illustrating the importance of this type of display technology for obtaining fully human, in vitro affinity matured antibodies. During the presentation the principle of phage display will be explained, while at the end alternative display technologies, such as the recently developed B cell display based Nanoclone procedure, will be reviewed.

O063

Phage therapy of *Salmonella* infected broilers

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Bacteriophages (phages) are host specific viruses that can kill bacteria by infection and subsequent lysis of the bacterial cell. The use of phages to combat bacterial infections (phage therapy) has regained general interest

with the increase of antimicrobial resistance. In this study we evaluated the use of phage therapy for reduction of caecal *Salmonella* load in broiler chickens. *Salmonella* remains one of the main sources of human bacterial diarrheal illness. Despite the implementation of control methods, additional measures are required to further decrease the number of human infections. In this study, phages against frequently isolated Salmonella enterica serotypes in broiler chickens (including *Salmonella hadar* [SH] and *Salmonella tryphimurium* [ST]) were isolated from slaughterhouse wastewater. Subsequently they were purified, characterised, and selected for in-vivo animal experiments based on host range tests. Broiler chickens were separated into five groups and were orally infected with 10^5 colony forming units (cfu) per ml of *Salmonella* at day 6. Oral phage- and placebo treatment was done daily from day 20-22 with 10^{10} plaque forming units (pfu) per ml. Group 1) SH + placebo; Group 2) SH + phage; Group 3) ST + placebo; Group 4) ST + phage; Group 5) Phage treatment only. Each day five birds were sacrificed, followed by performing caecal *Salmonella* and phage counting. The variation in *Salmonella* colonisation of untreated birds was considerable, which makes it difficult to determine the effects of the treatment. However, a short term reduction for several animals was observed at day 22 for both serotypes. Although colonisation levels returned to the original level within one day, phage treatment may contribute in controlling Salmonella infections.

O064

Belang van de versterking van de regionale infectieziektebestrijding

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In de jaren zestig van de vorige eeuw waren velen van mening dat infectieziekten definitief waren bedwongen. De belangstelling voor dit vakgebied nam af, er werd nog maar weinig in geïnvesteerd. De opkomst van HIV/AIDS en andere (her)opduikende infectieziekten in de afgelopen 25 jaar heeft laten zien dat dit optimisme ongerechtvaardigd was, infectieziekten zijn terug van weggeweest. Dit resulteerde o.a. in de oprichting van het Centrum Infectieziektebestrijding (CIb) bij het RIVM. Eén van de taken van het CIb is het versterken van de lokale infectieziektebestrijding. In 7 regio's zijn nu regionale artsconsulenten en medisch-microbiologen aangesteld. In elk van de regio's zijn inhoudelijke projecten geformuleerd die moeten leiden tot betere samenwerking in de regio én met het CIb. Doelstelling is ook dat praktisch werkzame medisch-microbiologen en artsen infectieziekten op deze manier meer invloed krijgt op het onderzoeksprogramma van het CIb o.a. via de commissie openbare gezondheidszorg diagnostiek en microbiologie (COM).

O065

Project regionale ondersteuning infectieziektebestrijding

H. van den Kerkhof en P. Schneeberger
RIVM, Bilthoven

Gezien de nieuwe uitdagingen waarvoor de infectieziektebestrijding zich het laatste decennium van de afgelopen eeuw geplaatst zag, werd er vanuit het ministerie van VWS een toenemende noodzaak gevoeld dit systeem te versterken. Hiertoe is met subsidie van het Ministerie vanuit de koepel van de GGD'en (GGD Nederland) in 2001 het VISI-project (Versterking InfrasStructuur Infectieziektebestrijding) in gang gezet om de minister hierover te adviseren. In aansluiting hierop is in 2005 is het centrum infectieziektebestrijding (Cib hoofd prof. dr. R. Coutinho) opgericht, waarbinnen al snel de behoefte ontstond om de relatie met het perifere bestrijdingsapparaat (GGD'en i.s.m. de medisch-microbiologisch laboratoria) te verstevigen. Als vervolg op het VISI-project is ook het project regionale ondersteuning infectieziektebestrijding uitgewerkt. In het kader van dit project is Nederland in zeven regio's opgedeeld. In elk van deze regio's is een regionaal consulent-arts infectieziektebestrijding (RAC'er) aangesteld. Om de ondersteuning voor de medisch-microbiologen te structureren is per 1 november de Commissie Openbare Gezondheidszorg en Microbiologie (COM) gevormd en zijn zeven regionale consulenten artsen-microbioloog (COM'er) aangesteld. Beide groepen van zeven consulenten worden vanuit het RIVM ondersteund door vertegenwoordigers van de verschillende afdelingen van het Cib en door een tweetal coördinatoren: Peter Schneeberger voor de artsen-microbioloog, en Hans van den Kerkhof voor de artsen-infectieziektebestrijding.

Een belangrijk doel van het CIb is om een structuur te creëren die een centrale regie mogelijk maakt bij een landelijke crisis. Bij deze versterking van de infrastructuur zullen ook de medisch microbiologische laboratoria een belangrijke rol spelen.

In het kader van de versterking wordt voor de GGD'en gedacht aan de volgende aandachtsvelden:

- Regionale surveillance en epidemiologie
- Kwaliteitsprojecten als de organisatie van visitatie en van bijscholing
- Een beleid ten aanzien van de inzet van diagnostiek voor bestrijding
- Een beleid ten aanzien van externe ketenpartners in de regio
- Afspraken en afstemming ten aanzien van outbreak-management in de regio
- Het regelen van een deskundige 24-uurs bereikbaarheid
- Afstemming en normalisering van de ICT-structuur in de regio

- De implementatie van nieuw beleid en de begeleiding van onderzoek
- De signalering van inhoudelijke problemen uit de regio en de agendering hiervan in landelijke overleggen.

In het beroepsprofiel van beroepsgroep van de artsen-microbioloog staat belang van de *public health* duidelijk omschreven. Voor artsen-microbioloog is het evident dat zij met hun expertise en hun laboratoria een belangrijke rol vervullen voor de diagnostiek en de preventie van infectieziekten voor de publieke gezondheidszorg.
Leden van de commissie voeren werkzaamheden uit in de regio om de samenwerking met de regionale GGD'en en laboratoria te verbeteren en ondersteunen regionale onderzoeksprojecten. Binnen de commissie wordt meegedacht over oplossingen voor landelijke problemen die aangedragen worden door het CIb. Vanuit de regio zullen door zowel de GGD'en als vanuit de laboratoria verbeterpunten en nieuwe initiatieven voor onderzoek worden aangemeld via de nieuwe regionale structuur. Gedacht wordt onder andere aan projecten zoals kiemsurveillance, communicatiemiddelen tussen laboratoria GGD en RIVM, ISIS en verbetering van de protocollen. De commissie gaat functioneren als een trait d'union tussen het RIVM, GGD-en en de perifere laboratoria.

O066

Kaas als bron van *Salmonella typhimurium* faagtype 560 uitbraak in Twente: de samenwerking in de praktijk

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Salmonella typhimurium is een bekende bron van voedselgerelateerde infecties, in Nederland vaak gerelateerd aan varkens- en rundvlees. In Nederland loopt een surveillanceprogramma, waarbij (voormalige) streeklaboratoria alle *Salmonella*-isolaten inzenden naar het RIVM voor sero- en faagtypering.
Tijdens de eerste maanden van 2006 werd duidelijk dat een uitbraak gaande was van gastro-intestinale infecties met *Salmonella typhimurium* faagtype 560 (ST560). De meeste patiënten waren woonachtig in de regio Twente, hoewel zich ook gevallen voordeden in de rest van Nederland. In totaal werden in 2006 ruim 200 gevallen gemeld, waarvan meer dan 60% uit Twente afkomstig was. Het betrof een variant van het in Nederland voorheen zeldzame faagtype 560.
Ofschoon er al enige tijd een waarschijnlijke bron in beeld was, bleek bijzonder moeilijk om de bron van de infectie met volledige zekerheid vast te stellen. Door middel van telefonisch afgenomen vragenlijsten bij patiënten, uitgebreid microbiologisch onderzoek inclusief *multilocus variable number of tandem repeats analysis* (MLVA) en een case-controlstudie werd naar de bron gezocht. Uiteindelijk is in november 2006 de ST560 in boerenkaas van de verdachte kaasmakerij in Twente aangetoond.
Bij het zoeken naar en bestrijden van de bron is een intensieve samenwerking van verschillende partijen essentieel gebleken. De betrokken partijen waren de GGD Regio Twente, diverse onderdelen van het Centrum Infectieziektebestrijding (CIb) van het RIVM, de Voedsel en Waren Autoriteit (VWA), het Laboratorium Microbiologie Twente-Achterhoek, de GD en het Centraal Orgaan voor Kwaliteitsaangelegenheden in de Zuivel (COKZ).
Tijdens deze duo-presentatie zal met name worden ingegaan op de rol die het RIVM en het regionale laboratorium microbiologie hebben gespeeld bij de opsporing van de oorzaak van de uitbraak. Hierbij zal de rol van faag- en MLVA-typering worden belicht. Daarnaast zal worden stilgestaan bij de bevorderende en belemmerende factoren die een bijdrage hebben geleverd aan het uiteindelijk vinden van kaas als de bron van deze uitbraak.

O067

Pionieren in de polder, van soa- naar SENSE-poli bij GGD Flevoland

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GGD Almere, Almere

In de jaren 1990-1996 werd in toenemende mate de behoefte tot anoniem onderzoek en behandeling gesignaleerd voor seksueeloverdraagbare aandoeningen. Om aan deze kleine vraag te beantwoorden werd een eveneens kleine curatieve soa-poli gecreëerd bij de GGD-locaties Almere en Lelystad. Gestart werd met een poli van minder dan twee uur per week op beide locaties. Er werd weinig ruchtbaarheid gegeven aan het bestaan van de poli en alleen patiënten die zich spontaan melden met een hulpvraag bij de GGD werden gezien en onderzocht. Een en ander volgens een afsprakensysteem. De poli werd door de jaren heen bemand door de artsen-infectieziekten. Soms werd overleg gepleegd met en zo nodig doorverwezen naar de dermatologen in het Flevoziekenhuis en Zuiderzeeziekenhuis. De poli-onkosten werden gedragen door de GGD en de laboratoriumkosten werden gedeclareerd middels de VOMIL-regeling. Gaandeweg groeide de vraag naar onderzoek en behandeling. Dit voornamelijk door mond-op-mondreclame. Het patiëntenaantal groeide tot 1260 in 2006. Gedurende de jaren groeide de taken van de artsen-infectieziekten en slonk het FTE-cijfer. Dit

had tot gevolg dat de poli een onevenredig groot deel ging uitmaken van de werkzaamheden van de artsen-infectieziekten. Toen op 1 januari 2006 de nieuwe financieringsregeling van start ging voor SOA-onderzoek was dit een goed moment om de poliopzet te wijzigen. Gekozen werd voor twee veranderingen, ten eerste de poli zou worden bemand door verpleegkundigen met de arts-infectieziekte als aanwezige achterwacht. Ten tweede de poli zal een SENSE-poli worden. Een SENSE-poli is een poli die niet alleen antwoord geeft op soa-vragen maar tevens op vragen met betrekking tot seksuele gezondheid. SENSE is geen afkorting maar staat als woord op zichzelf.
Het SENSE-project is een initiatief van het ministerie van VWS om seksuele gezondheid in Nederland te bevorderen. GGD'en konden met een 'SENSE-plan' inschrijven op het door ZonMW gecoördineerde project en een subsidie verwerven om het SENSE-plan verder uit te werken en te implementeren in de GGD-regio. Het is GGD Flevoland gelukt om, samen met GGD Zuidelijk Zuid-Holland en GGD Midden Holland in aanmerking te komen voor een opstartsubsidie. Dit heeft geleid tot de prille stappen naar een SENSE-poli. Het plan van GGD Flevoland is in samenwerking met o.a. de lokale huisartsen, gynaecologen en seksuologen gevormd. Het houdt in dat een laagdrempelige ingang wordt geboden voor eenieder die een probleem ervaart op het gebied van seksuele gezondheid. Afhankelijk van het probleem zal een vraagverheldering worden geboden, adviezen worden gegeven, snelle doorverwijzing plaatsvinden of onderzoek en behandeling worden verricht. Het contact is anoniem en kosteloos en bedoeld voor die situaties waarbij de gang naar de huisarts niet mogelijk is of als ongewenst wordt ervaren. Voorbeelden hiervan kunnen zijn een vraag tot soa-onderzoek en behandeling, een tienerzwangerschap, een abortuswens, een voorlichtingsvraag betreffende anticonceptie of een hulpvraag bij problemen met het hebben van seks. Voor meer informatie verwijs ik u graag naar www.sense-flevoland.nl en www.sense.info.

O067

Public health and open sexually transmitted disease outpatient clinic in small city areas in the Netherlands

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The increase of sexually transmitted diseases (STD) is subject to constant awareness by local and national government health care authorities. In 2006 the national STD public health care policy was changed by the minister of health to which is intended to be a structural national network coordinated by the national centre for infectious disease control (CIb). The public health (PH) care to STD has been divided into eight regions. In each region STD-PH care is dedicated to a coordinating PH-department of a main community and various local PH-departments. The objective of this structural change is to create a national network of low barrier STD-care for high risk groups in order to monitor and reduce the incidence of STD nationwide. The new structure is meant to support both the so-called 'randstad' areas and the non-randstad areas with free of charge, complete STD-care.
Within this new policy of STD care laboratory investigations are restricted to Medical Microbiology laboratories (MML) contracted by the various PH-departments. The obliged cooperation of PH-departments with MMLs is meant to create maximal support for active tracing of STD causes i.e. *Chlamydia trachomatis* (including LGV), *Neisseria gonorrhoea*, syphilis, HBV and HIV, and subsequent treatment. The costs of treatment of newly found STD patients, with an estimated incidence of 8%, appears to be a matter of debate as STD-care has both a preventive (PH-care) and an individual care aspect. Ideally the two should be combined.
Acknowledging the benefits of combining PH-care with individual care, the PH-department of Kennemerland (400.000 inhabitants) and the department of internal medicine/infectious diseases in cooperation with the department of dermatology joined forces in 2004 to start an open STD outpatient clinic. Thus establishing a regional centre for guidance, prevention and treatment of STD close to individual patient care and to improve the expertise of each participant.
From the start of the combined open STD-outpatient clinic in 2004 and the ongoing years 2005 and 2006, the number of clients increased from 906 to 1175 and 1465 respectively. As in 2003 only 340 clients visited the PH-department, the almost 5 fold increase in two years exemplifies the regional demand and need for combined STD care.
The overall incidence of STD diagnoses from 2004 till 2006 was 14, 16 and 9% respectively. The annual number of clients which were included anonymous i.e. free of charge changed from 97 (11%) to 111 (10%) and 413 (29%) respectively. In 2004, 2005 and the first half of 2006 clients were offered free of charge STD care irrespective of their risk classification (according to the national STD risk guidelines). In the second half of 2006 the inclusion criteria for anonymous and free of charge care were changed to include only high risk patients., This led to an increase in the number of anonymous patients from 76 in the first half of 2006 to 337 in the second half. The overall incidence of STD in anonymous clients was 8% and 14% for the first and second half of 2006 respectively.

Finally, regarding the total amount of samples investigated for STD agents by the MML Kennemerland , only 10% originates from the open STD outpatient clinic. The majority of samples is still send in by family doctors (58%) and gynecologists (28%) of which 7% and 2% are STD positive respectively. However, an open STD outpatient clinic organized by joining a PH-department within the Hospital care and an adequate MML facility, fulfills the goals for national STD PH-care.

O070

News from the past: chlamydial symbionts of amoebae

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Chlamydiae belong to the most successful groups of obligate intracellular bacteria; chlamydial infections are widespread among animals where they cause a broad spectrum of diseases, and in humans they are the major cause of preventable blindness and sexually transmitted disease. In addition, chlamydiae are also widespread as endosymbionts of amoebae, and there is increasing evidence for the existence of an untold diversity of chlamydiae in the environment, whose pathogenic potential is still unclear. Genomic analysis of the obligate *Acanthamoeba* symbiont *Protochlamydia amoebophila* UWE25 suggested that *Chlamydiae* have lived within eukaryotic host cells for hundreds of millions of years, and that several virulence mechanisms used by modern pathogenic chlamydiae have already been developed at that time – long before humans roamed the earth. Early members of the chlamydial evolutionary lineage were thus major inventors of mechanisms for the exploitation of eukaryotic cells as an ecological niche. Today, the amoeba symbiont *P. amoebophila* exploits its host by using a set of five nucleotide transport proteins, which have recently been characterized in detail. The metabolic model inferred from these data illustrates a previously unseen, tight and apparently well balanced coupling of endosymbiont and host metabolism. The analysis of symbiotic chlamydiae and their interaction with their amoeba host cells will continue to contribute to our understanding of the evolution of chlamydiae and will thus help to understand the emergence of major bacterial pathogens of animals and humans.

O071

LIL-FISH in the big metagenomic pond

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Metagenomics is a culture-independent, explorative approach that allows for the discovery and appreciation of the genetic complexity of microbial ecosystems. Currently, a clear discrepancy exists between the relative ease with which a large metagenomic library can be constructed and the practical limitations of analyzing all clones from that library for genes or gene functions of interest. To close this gap, much effort is going into the development of methodologies that increase the rates of gene finding among large numbers of metagenomic library clones. I will present a novel method called LIL-FISH (large-insert library fluorescent in situ hybridization) which allows high-throughput screening of metagenomic libraries for inserts that contain rRNA genes. The method is based on the heterologous expression of such genes in *Escherichia coli* and subsequent detection by fluorescent in situ hybridization. In combination with fluorescence activated cell sorting, LIL-FISH has great tool potential for the quick recovery of metagenomic DNA fragments carrying rRNAs, which are extremely valuable in describing and understanding microbial communities by establishment of a link between phylogeny (rRNA) and function (as predicted from genes flanking this rRNA).

O072

Diversity and activity of sulfate reducing bacteria along a salinity gradient in soda lakes

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Soda lakes are a special type of saline lakes with pH values up to 10.5, which are kept constant by the high buffering capacity of the two major anions present in solution, i.e., carbonate and bicarbonate. It has been shown that the diversity of metabolic types decreases with salinity due to energetically reasons. Several studies investigated the influence of salinity on bacterial diversity in different environments, but the diversity along a salinity gradient in soda lakes was never studied before.
Since the sulfur cycle is one of the most active element cycles in these lakes we focused our study on the diversity and activity of sulfate reducing bacteria in four different soda lakes from the Kulunda Steppe (South East Siberia, Russia). For this purpose, a combination of culture-dependent and independent techniques was applied. The general bacterial and SRB diversity were analyzed by DGGE targeting the 16S rDNA gene, while RNA were used a template to infer active populations. Individual DGGE bands were sequenced and a phylogenetic analysis was performed. In addition, the overall activity of SRB was obtained by measuring the sulphate reduction rates (SRR) and their abundance was estimated by serial dilution.
A diverse microbial community was revealed by DGGE analysis and significant differences were observed

between DNA- and RNA-based profiles, indicating a difference between those that are present and those that are active at the moment of sampling. A decrease of the bacterial diversity was not observed with increasing salinity. The most dominant and active SRB detected in these soda lakes belong to the genera *Desulfovibrio*- and *Desulfomicrobium*, to the Gram-positive *Desulfotomaculum*, and to representatives of the genera *Desulfosarcina*-, *Desulfococcus*- and *Desulfonema*-. Members of the genera *Desulfobulbus*- and *Desulfobacter*- were only found in few a samples. No members of the *Desulfobacterium*-group were detected. The highest SRR were measured in the most saline lakes (200 g/L Na+). These results indicate that common ideas about a decrease of diversity and activity with salinity might not always be true for all functional groups and needs more careful consideration.

O073

RNA stable isotope probing direct identification of starch fermenting bacteria in the human colon

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Introduction: The human colonic microbiota comprises a complex microbial ecosystem vital for human gut health. An essential function of this community is the fermentation of dietary substrates, which escape digestion in the upper digestive tract. Such substrates, in particular dietary fibers, prebiotics and resistant starch (RS), are known to be important promoters of intestinal health, mainly through their interactions with the human colonic microbiota. However, these interactions are far from being fully understood. To create a link between distinct groups of microorganisms and the fermentation of dietary relevant carbohydrates in the human colon we used RNA-stable isotope probing (SIP).
Material and methods: The microbial community of an in-vitro model of the human colon (TIM-2) was initially supplemented with 1.0 g of [U-13C]-starch (IsoLife, Wageningen, the Netherlands) isolated from potatoes grown in presence of 13CO2. RNA was extracted from lumen samples taken at different time point (0 h, 2 h, 4 h and 8 h) and subsequently density resolved by means of ultra centrifugation in cesium trifluoracetate. Comparison of the 16S rRNA composition in heavy vs. light fractions was done using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting and cloning techniques. Phylogenetic analyses using the ARB software package determine the taxonomic affiliation of the starch consuming bacteria. The major products of bacterial fermentation were quantified by means of LC-MS analyses.
Results and discussion: After 4 and 8 h of incubation with [U-13C]-starch, isotopically labeled RNA could be isolated. 16S rRNA-based phylogenetic analyses revealed a microbial community consisting of four major groups: *Bifidobacterium*, *Bacteroidetes*, *Clostridium* cluster IV (*Clostriudium leptum* group), as well as *Clostridium* cluster XIVa (*Eubacterium*, *Dorea*, *Roseburia* and *Ruminococcus*). Terminal-restriction fragment length polymorphism analyses indicated that species closely related to *Ruminococcus bromii*, *Bacteroides-Prevotella* group and *Eubacterium rectale* were strongly involved in starch metabolism *in vitro*. The starch fermentation yield acetate (18 mM), butyrate (5mM) and propionate (1mM). Integrating molecular and metabolite data, it can be hypothesized that there is metabolic cross-feeding in the system, where species closely related to *Ruminococcus bromii* and the *Bacteroides-Prevotella* group ferment starch to acetate, which is subsequently utilized by *Eubacterium rectale* for the formation of butyrate. Further studies on this metabolic cross-feeding are underway to confirm our assumption.
In conclusion, RNA-SIP combined with metabolic analyses using 13C-labeled substrates proved instrumental for the identification of bacteria actually involved in colonic fermentations and link them to metabolic products in the course of in-vitro studies, opening promising avenues for unraveling functionality of the intestinal microbiota in-vivo.

O074

MLST – principles and applications for Gram-positive bacteria

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In recent years, the multilocus sequence typing (MLST) has become a method of choice for epidemiological typing of several Gram-positive bacterial species. The method is based on sequencing of defined regions in a few (usually 7) house-keeping genes, followed by a query of Internet-accessible database (such as www.mlst.net) to establish an allelic profile and resulting sequence type (ST) of an isolate. This approach allows for unambiguous identification of an isolate, accumulation of data for large numbers of isolates and easy data exchange and comparison. The most important Gram-positive bacteria, for which significant size databases exist include *Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis* and MLST has proven high utility in establishing and following the distribution of major antibiotic-resistant and virulent clones of these species. Examples of such studies will be presented.

O075

Molecular typing to unravel international food-borne virus transmission

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The food-borne viruses in Europe network is a collaboration among virologists and epidemiologists from 24 institutes in 13 countries in Europe funded by the European commission since 1999 (www.eufoodborneviruses.co.uk). The main aim is to understand the mechanisms of emergence of variant norovirus (NoV) strains, develop robust methods and criteria for linking cases in different outbreaks through combined molecular and epidemiological data collection, and provide estimates for the incidence of food-borne NoV infections.

Methods: We built a European surveillance structure for outbreaks of viral gastroenteritis. The first phase was designed to review existing surveillance systems for viral gastro-enteritis, design and agree a minimum dataset for collection, review and evaluate methods for detection and typing of NoV, and build a common database of epidemiological and virological data for use by all participants. Epidemiologic data-collection was based on Kaplan's criteria for patients and outbreaks although these criteria were not always met completely. Collection of sequence results was focused on region A of the genome (www.rivm.nl/bnwww), but allowing other entries (regions B, C, D) because of lack of standardization between cooperating laboratories. Variables that define a minimum dataset were defined as year and month of onset and setting. Outbreaks for which this information is available are grouped according to suspected mode of transmission.

Results: Since 2000, we have collected data on over 9000 norovirus outbreaks. Genogroup II.4 strains predominate, and are more frequently associated with person-to-person outbreaks than with food-borne outbreaks. Periodically new variants of GGII4 viruses emerge nearly simultaneously in the different countries of the FBVE network, which coincides with increased numbers of outbreaks. Molecular analysis shows that these emerging variants constitute drifted viruses, with mutations clustering primarily at the surface exposed P2 domain. Additional molecular characterisation of outbreak strains shows a high frequency of recombination. When comparing outbreaks reported as food-borne with those reported as person-to-person outbreaks, GGII4 viruses are detected significantly less frequently ($p<0.00001$), and a wider range of norovirus variants is found. This information can be used to trace outbreaks linked to a common source internationally This larger diversity in foodborne genotypes makes a link more probable when two identical sequences are found. A specific problem is that often contaminated food contains mixtures of viruses, increasing the risk of generation of new recombinant strains. A partial data analysis for the entries submitted between 2000 and 2003 showed that 14% of outbreak strains were recombinants. All recombinants identified sofar have a cross-over point located immediately upstream of the start-codon of the capsid gene.

Conclusions: Knowing the background information on diversity of noroviruses in the community is essential for correct use and interpretation of strain sequencing in the linking of outbreaks to a common source. Molecular epidemiological analysis shows a high rate of change through mutation and recombination of noroviruses. These changes have a clear impact on their epidemiology and the use of molecular data for strain typing.

O076

A novel and quick MLVA (Multi-Locus Variable Number of Tandem Repeat Analysis)-based typing system for *Staphylococcus aureus*

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Objectives: To date, much effort has been put into a simple, efficient and high-resolution molecular typing method for *Staphylococcus aureus* to rapidly detect outbreaks especially in hospital settings. Therefore, we developed and evaluated a Multiple Locus Variable-number tandem repeat Analysis (MLVA) system on human *S. aureus*.

Methods: Eighty-eight *S. aureus* isolated from hospital patients were subjected to the PCR amplification of six loci with repeat sequences (SIRU01, 05, 07, 13, 15 and 21). The repeat unit of each locus varies from 24 to 131 bp. The number of repeats was calculated for each SIRU and each unique combination of repeat numbers was assigned an MLVA-type (MT). In addition, 53 isolates belonging to six outbreaks (n=24), one time observations with a foreign link (n=7) and unexpected cases (n=22) were used for confirmation. Multi Locus Sequence Typing (MLST) was used as a reference method. All isolates have a known sequence type (ST) and they were well distributed within the population of S. aureus. In addition, the spa-type was determined by PCR and sequencing.

Results: Sixty-six isolates yielded a complete MLVA profile, while 22 isolates yielded no specific products in some loci and these loci were assigned an X. This lack of amplification products might be due to polymorphisms at the position of the primers or the absence of the locus. Variations in repeat number were observed in all loci.

Among thirty-two MLST types, 71 MLVA-types, 47 spa-types including 2 new spa-types were discerned. The Simpson

index of diversity and 95% confidence interval were 0.941 (0.922-0960), 0.989 (979-0.999), and 0.964 (0.947-0.982) for MLST, spa-typing and MLVA, respectively.
Preliminary analysis of the results for the 53 confirmation isolates showed general agreement between MLVA typing and previous assignments of the isolates to outbreaks, unexpected or one time observation with a foreign link. MLVA agreed for 3 outbreaks. In one outbreak MLVA excluded one isolate, but this isolate showed also a one band difference in PFGE. In a suspected outbreak of 5 isolates, 3 showed an identical MT and 2 showed a difference in a single SIRU. A similar result was obtained for another outbreak. Four isolates of a group of 6 isolates with the same spa-type/phage type were previously assigned to an outbreak. Three of these 4 isolates had the same MT, the fourth had a difference in one SIRU. Two isolates had the same MT as 3 outbreak isolates, but these 2 were from an outpatient and a member of the personnel coming from a different hospital. So, despite identical MT, spa-/phage type no obvious epidemiological link was found. All other unexpected isolates or one time observations with a foreign link had different MLVA-types, except 2 isolates which had an identical MT and also an identical spa-type.
Conclusions: The majority of S. aureus isolates can be typed by MLVA. The discriminatory power for MLVA is significantly higher than for MLST, and almost significantly higher than for spa-typing. The method is a rapid method for typing of MRSA for epidemiological purposes.

O077

Loco-regional outbreaks of *Salmonella typhimurium* in the Zaanstreek-Waterland area

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Introduction: In the autumn of 2006, a substantial increase of Salmonellosis was observed in the Zaanstreek-Waterland area, in particular involving *Salmonella typhimurium* (ST). Between September and December 2006, a total of 42 ST isolates were cultured compared to 12 ST isolates in 2005 in the same period. Nationally, an increase in ST was also observed, including an outbreak in the Twente area caused by ST phagetype 560 (ST560), associated with contaminated hard farmhouse cheese. Is the increase of ST in the Zaanstreek-Waterland area caused by this ST560 strain?
Methods: Phagetyping and multi locus variable number of tandem repeat analysis (MLVA) of 33 St isolates from faeces performed at the RIVM. An epidemiological survey was performed to identify the sources of the outbreaks.
Results: Two of the 33 ST isolates could not be cultured or the phagetyping failed. Of the remaining isolates, 14 isolates were ST560 (45%), 9 ST460 (29%), 4 ST296 (13%), 2 ST506 (6%), 1 ST507 (3%) and 1 ST510 (3%) The clear clustering of the different ST phagetypes in different regions was remarkable. 9/13 ST460 isolates were from Monnikendam/Volendam, 11/11 ST560 isolates from Purmerend/Edam and 4/4 ST296 isolates were from Zaandam. The Purmerend/Edam ST560 isolates were all identical when tested with MLVA, but slightly differed from the ST560 isolates from the Twente area in one of the five loci. Unfortunately, the epidemiological survey revealed no definite sources of the loco regional outbreaks.
Conclusion: Phagetyping showed that there were three separate outbreaks in the Zaanstreek-Waterland area. Furthermore, MLVA typing suggested that the increase of ST560 in Purmerend/Edam was not directly related to the ST560 from the Twente area.

O079

Testing for HIV drug resistance

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In most European countries, drug resistance testing has become part of routine clinical management of HIV infection, particularly at the time of antiretroviral therapy failure. Genotypic testing is performed most frequently because of its relatively low cost, technical convenience and virological benefit, shown in clinical trials.
However, a resistance test remains a snapshot of the current situation, which does not necessarily reflect all relevant mutations that have been selected in the past. Therefore, additional information such as treatment history, dosing, the patterns of viral suppression under previous regimens, prior resistance tests, toxicity, adherence and drug levels should be taken into account before therapy is changed.
For interpretation of the genotypic profile, knowledge on the effect of numerous mutations on drug-susceptibility and the relationship with clinical outcome is needed. The European as well the Dutch guidelines advice the use of an algorithm in the interpretation process since the effect of specific combinations of mutations might be rather complex. Use of an interpretation algorithm will indeed give a clinician a prediction of the susceptibility of all available antiretroviral drugs and an indication of which drugs to avoid in a new regimen.
However, the interpretation algorithm does not give direct insight into the susceptibility of a complete new regimen. Also the algorithm fails to provide information on the possible future evolution of the virus and its concomitant drug susceptibility. Knowledge of evolutionary pathways is essential to make an estimation of the 'genetic barrier' of

the proposed antiretroviral regimen which might facilitate selection of a regimen that would be more successful in the long-term.
For therapy-naïve individuals, resistance testing is recommended by European as well as Dutch guidelines in case of acute infection and in case of chronic infection depending on the overall prevalence of transmitted drug-resistant HIV. The prevalence of drug-resistance in naïve patients is influenced by a number of factors, such as the proportion of HIV-infected individuals on suppressive therapy, the rate of resistance in the HIV-infected population and the transmissibility and fitness of resistant viruses.
In the prospective, European Commission supported HIV drug resistance surveillance programme SPREAD, the majority of individuals infected with drug-resistant HIV carried virus with only one resistance-related mutation. Although preliminary studies have shown that transmitted resistance can persist for a long time after infection, clinical practice has shown that less extensive mutational patterns can easily revert over time. In the future more sensitive technologies might be able to detect minority species with more extensive resistance in these patients. Pending these techniques it is of utmost importance to preserve plasma for (future) genotypic resistance testing as soon as possible after HIV-diagnosis.

O080
Testing for HCV drug resistance

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Current treatment for hepatitis C is a combination regimen of pegylated interferon and ribavirin resulting in viral clearance in approximately 50 percent of patients. As genotype 1 and 4 exhibit intrinsic interferon resistance compared to other genotypes, consensus treatment duration for these genotypes is 48 weeks, compared to 24 weeks for the other genotypes.
A central domain in the NS5A gene has been linked to interferon resistance therefore designated Interferon Sensitivity Determining Region' (ISDR) as the number of mutations in this domain, compared to a HCV prototype, is correlated with a beneficial treatment response. However, 'mutant' viruses are rare in many parts of the world including the Netherlands, and predictive value of ISDR mutations for treatment outcome is low. Furthermore, whether the ISDR really determines treatment outcome is still fiercely debated. Therefore, apart from genotyping, there is no place for resistance testing for the treatment of chronic hepatitis C.
This may change in the nearby future with new antivirals as protease inhibitors and polymerase inhibitors now entering phase 2 and 3 clinical trials. Cell culture and patient data have shown that resistance occurs quickly, when these antivirals are given as monotherapy. The presentation will focus on resistance dynamics of these new antivirals Implications for the clinical virology laboratory will be discussed.

O082
Denaturing gradient electrophoresis (DGGE) to detect periodontal pathogens and to study mixed microbial infections

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Objectives: Bacteria play an important role in the initiation and progression of periodontal diseases and are part of an oral biofilm, which can contain over hundred different species. Denaturing Gradient Gel Electrophoresis (DGGE) is a promising tool to study such complex microbial communities. The objective of this study was to compare DGGE and culture results of subgingival plaque samples from adult patients with periodontitis.
Methods: From 25 periodontitis patients, the deepest pocket in each quadrant was sampled by paperpoints and pooled. Samples were analyzed for the presence of *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) and *Tannerella forsythensis* (Tf) by cultivation, species-specific PCR and DGGE. To obtain identity information from DGGE profiles, DNA from the DGGE gel was transferred to a nylon membrane. The membrane was subsequently hybridized with species-specific fluorescent probes to detect Aa, Pg, Pi and Tf.
Results: The sensitivity of DGGE compared to cultivation in the detection of *A. actinomycetemcomitans* (Aa), *P. gingivalis* (Pg), *P. intermedia* (Pi) and *T. forsythensis* (Tf) was 100, 100, 88 and 100%. The sensitivity of DGGE compared to PCR was 100, 90, 88 and 96%. With DGGE it was possible to distinguish between Aa serotypes a, d, e, f and b and c. Moreover, the identification could be extended with different probes as shown for *Treponema denticola*.
Conclusion: From these results it can be concluded that the clinically relevant subgingival microbiota is represented in DGGE profiles. Hybridization with species-specific probes of blotted DGGE gels is a useful tool to obtain identity information from DGGE profiles and to screen multiple samples for different pathogens at once. Furthermore, the sensitivity of DGGE seems to exceed cultivation

and PCR. This study shows that DGGE can be used to detect pathogens and to study the subgingival microbial population involved in periodontal diseases.

O083

Effect of *Veillonella parvula* on the protein expression of *Streptococcus mutans* grown in a biofilm

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Streptococcus mutans is considered the major pathogen involved in dental caries. In our previous studies, we found that *S. mutans* grown in a biofilm becomes less susceptible to various antimicrobials (chlorhexidine, CPC, erythromycin, amine chloride, zinc chloride) when *Veillonella parvula* is present. The aim of the current study was to identify changes in protein expression of *S. mutans* when grown with *V. parvula* , to find a possible cause for this decreased susceptibility. Single and dual-species biofilms of *S. mutans* and/or *V. parvula* were grown on polystyrene for 48 h. Protein extraction and two dimensional difference gel electrophoresis of the three types of biofilms were performed. Gels were analyzed using Decyder 6.5 software package (GE Healthcare). Differential expression of proteins was evaluated using specialized mathematical and statistical analysis. Significantly differentially expressed proteins were excised from the gels and analyzed with mass spectrometry. We found that 43 out of the 1402 proteins detected were significantly differentially expressed in dual-species biofilms compared to single-species biofilms ($p<0.05$). Of these 43 proteins, seventeen were identified. The expression of proteins responsible for protein synthesis, protein folding and protein breakdown was upregulated in *S. mutans* when grown with *V. parvula* . The majority of these proteins have shown to be related to bacterial adaptation to and survival of antimicrobial exposure previously, and are thus good candidates to explain the observed increase in resistance.

O084

Biomedical informatics in chronic infectious and inflammatory disease research: periodontitis as a case study

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Introduction: Common diseases, such as cardiovascular disease, cancer, metabolic imbalances, and chronic inflammatory illnesses impose a major drain on society. Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth. If left untreated, teeth will become mobile and migrate, and will eventually exfoliate. Chronic inflammatory diseases like periodontitis have a complex pathogenesis and a multifactorial etiology, involving complex interactions between multiple genetic loci, infectious agents and environmental factors such as diet and smoking habits. The general paradigm is that certain individuals are genetically more susceptible than others to the risk factors, and these subjects are more likely to succumb to the illness. Within the European network INFOBIOMED, periodontitis is designated as a case study for investigation into integration of bioinformatics and medical informatics to the new field of biomedical informatics (BMI).

Methods: Data has been collected from different sources and Periodontitis Data Warehouse (PDW) was built taking care of ontology and gene definition issues using BMI tools such as ontodatacleaning, anonymisation, connection with public web databases and data import of digitally analyzed dental images.

Included in the PDW are from periodontitis cases and non-periodontitis controls:

- Intermediate phenotype: age, gender, ethnicity, medical and dental history, education, weight, length, blood pressure, biochemical markers.
- Genomic data: 25 SNP's in 12 immune response genes.
- Microbial data: total anaerobic counts, proportions and prevalence of subgingival species *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Peptostreptococcus micros* and *Campylobacter rectus*.
- Environmental data: current and past smoking habits.
- Phenomic data: the extent and severity of periodontitis.

Data mining tools Decision Tree Learning (C4.5) and Association Rule Mining (HealthObs) were applied to the PDW.

Results: Periodontitis has served as a case study for the integration of bioinformatics and medical informatics. The PDW was constructed applying principles of both disciplines and is now fully functional. It is located on a dedicated server at the VUmc and is accessible from different research centres with appropriate clearance and anonymisation measures. For data analysis, designated researchers have access rights to build queries. The PDW contains currently cross-sectional data for over 800 patients and controls. The preliminary data mining results show that genomic information together with microbial data provides the best accuracy in distinguishing patients from controls. Genomic data alone is inferior to microbial data when analysed by Decision Tree Learning to distinguish patients from controls. On the basis of Association Rule

Mining, Caucasian non-smokers could be classified as periodontitis patients when the sum of the proportions of the 7 microbial species present in cultivable subgingival microflora exceeded 64%. In contrast, when the sum of the proportions of the same species was <3.6% subjects could be classified as periodontally healthy.

Conclusion: The Periodontitis Data Warehouse has been constructed and allows an integrative research approach. Available tools from BMI will contribute to new insights in peridontitis. Similarly the infrastructure and concepts are generally applicable to other chronic inflammatory and infectious diseases.

* Supported by the INFOBIOMED network, 6th R&D Framework, EC (IST 2002 507585).

O085

The presence of Barrett's epithelium is associated with a specific bacterial flora in the esophagus

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Background: Persistent mucosal bacterial colonization may lead to chronic inflammation, a condition which is thought to be associated with neoplasia development. Intestinal metaplasia of the esophagus, or Barrett's esophagus (BE), is a chronic inflammatory disorder and a precursor of esophageal adenocarcinoma. So far, little is known about the bacterial flora in the esophagus and its association with esophageal diseases like BE.

Aim: To determine whether BE is associated with a specific bacterial flora.

Material and methods: Biopsies of esophageal squamous epithelium (SQ), BE, and gastric corpus (GC) epithelium were collected in 66 patients (M:F 2:1, mean age 57 yrs, range 22-89) with BE (confirmed intestinal metaplasia by histology). Acridin Orange and modified Gram staining were performed to localize and identify Gram-negative and positive bacteria in the biopsies. A 16S rDNA micro-array analysis was performed to determine bacterial species per biopsy region.

Results: SQ predominantly contained Gram-negative cocci, closely associated with the epithelial surface. In contrast, BE was predominantly colonized with Gram-positive bacteria, located in the mucous layer and intestinal gland lumen. GC contained smaller numbers of the same bacteria at these locations. The semi-quantitative micro-array for 342 differential sequences indicated the presence of >114 different species in all biopsies, that primarily belonged to 5 large phyla: *Actinobacteria* (n=16), *Bacteroidetes* (n=16), *Firmicutes* (n=57), *Fusobacteria* (n=5), and *Proteobacteria* (n=14). The bacterial population within each region was stable, and showed no variation between different patients (p=0.57), while a significant difference between regions, i.e. SQ, BE, and GC, was seen (p=0.03). A significantly higher number of species was present (as mean percentage of total species per phylum) in SQ compared to BE for *Actinobacteria* (38% vs 26%; p=0.04) and *Bacteroidetes* (55% vs 45%; p=0.04), and BE versus GC showed borderline significance for *Bacteroidetes* (45% vs 53%; p=0.05). The total concentration of species was also generally higher in SQ compared to BE and GC. Strikingly, BE contained the highest concentration of *Bacteroides prevotella*, *Prevotella* and *Fusobacteria* species.

Conclusion: BE mucosa is invariably colonized by various bacterial. These include in particular *Bacteroides prevotella*, *Prevotella* and *Fusobacteria* species. The latter two are known mucosal pathogens (such as in chronic peridontitis), and may also play a role in the chronic inflammatory process of BE. This would implicate that antibiotics might be an option for BE treatment.

O086

Protein secretion in the third domain of life, the Archaea

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Archaea constitute the third domain of life and are similar to other prokaryotes in most aspects of cell organization but are unique in the lipid composition of the cytoplasmic membrane and the structure of the cell surface. Most archaeal cells are surrounded by a proteinaeous layer, the S-layer. Membranes of archaea are composed of glycerol-ether lipids instead of glycerol-ester lipids and are based on isoprenoid side chains. The unique cell surface of archaea requires distinct solutions to the problem of how proteins cross this barrier to be either secreted into the medium or assembled as appendages at the cell surface.

Sulfolobus solfataricus is a hyperthermophilic archaeon that thrives in extreme acidic environments. It belongs to the group of crenarchaeota, and is used as paradigm in genetic and molecular studies for hyperthermophilic archaea. We will discuss our current insights in the assembly of different and unique cell surface appendages such as flagella, UV induced pili and the bindosome, an extracellular substrate-binding protein complex.

O087

Protein secretion and secreted proteins in pathogenic *Neisseriaceaea*

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Secreted proteins of pathogenic bacteria are often essential virulence factors. They are involved, for example, in the

adherence of the bacteria to host cells or required to suppress the host's defence mechanisms. Until recently, the IgA1 protease was the only secreted protein that had been studied in considerable detail in the pathogenic *Neisseriaceae*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*. The availability of their genome sequences, however, revealed a much larger repertoire of secreted proteins and boosted research in this area. Here, a survey of the secretome of the pathogenic *Neisseriaceae* is presented, which is based upon these available genome sequences.

Of the six protein-secretion pathways widely disseminated among Gram-negative bacteria, three pathways were identified in the *Neisseriaceae*, i.e. the autotransporter-, the two-partner- and the type I-secretion pathways. Comparison of the predicted secretomes reveals a considerable flexibility. As compared with *N. meningitidis* and the non-pathogen *Neisseria lactamica*, *N. gonorrhoeae* appears to have a considerably degenerated secretome, which may reflect its altered niche occupancy. Furthermore, the flexibility of the secretome may be enhanced by the presence of partial ORFs in the genomes that potentially encode fragments of secreted proteins. We hypothesize that these ORFs may substitute for the corresponding fragments in the full-length genes through recombination, thereby changing the host-cell receptor specificity of the secreted protein.

Autotransporters constitute the most extensively studied neisserial transport system and I will present a short overview. Finally, I will address the expression of the neisserial two-partner secretion system. This system had only been identified at the genomic level, but our recent results show that it is expressed in *N. meningitidis*, most likely also during colonization of the human host.

O088

Limited tolerance towards folded elements during secretion of the autotransporter Hbp

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Many virulence factors secreted by pathogenic Gram-negative bacteria belong to the autotransporter (AT) family. ATs consist of a passenger domain, which is the actual secreted moiety, and a β-domain that facilitates the transfer of the passenger domain across the outer membrane. The traditional model describing the mechanism of autotransport predicts the outer membrane translocation of passenger domains in an unfolded, extended conformation via a monomeric β-domain that forms a β-barrel pore structure in the outer membrane. This view is currently under debate.

We have investigated folding of the AT passenger in the periplasm in relation to the maintenance of its translocation competence using *Escherichia coli* Haemoglobin protease (Hbp) as a model protein. Dual cysteine mutagenesis, instigated by the unique crystal structure of the Hbp passenger, resulted in intramolecular disulphide bond formation dependent on the periplasmic enzyme DsbA. A small loop tied off by a disulphide bond did not interfere with secretion of Hbp. In contrast, a bond between different domains of the Hbp passenger completely blocked secretion resulting in degradation by the periplasmic protease DegP. In the absence of DegP, a translocation intermediate accumulated in the outer membrane. A similar jammed intermediate was formed upon insertion of a calmodulin folding moiety into Hbp. The data suggest that Hbp can fold in the periplasm but must retain a certain degree of flexibility and/or modest width to allow translocation across the outer membrane.

O089

Bile acid resistance is essential for urease mediated gallstone formation by *Helicobacter* species

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Background: *Helicobacter* species colonize the gastrointestinal and hepatobiliary tract of many mammals, and often cause inflammation-associated diseases. Infection with *Helicobacter hepaticus* has been linked to the formation of cholesterol gallstones in mice, and we have recently shown that urease activity of *H. hepaticus* may contribute to this phenomenon by mediating precipitation of calcium. However, to be able to initiate gallstone formation, the bacteria must also survive the antibacterial components present in bile, including bile acids.

In this study we have investigated whether enterohepatic *Helicobacter* species are more resistant to bile than gastric *Helicobacter* species. Furthermore because bile acid resistance in bacteria is often mediated via specific efflux mechanisms, we have initiated the characterization of bile acid resistance and putative bile efflux systems of *H. hepaticus*.

Methods: Bile acid resistance was assessed in broth cultures of *H. hepaticus* ATCC51449 and *Helicobacter pylori* strain 26695, grown in serum-supplemented Brucella broth for 20 h at 37 degrees C. The effect of 6 different bile acids was assessed by comparison of the OD600 of the cultures grown with increasing concentrations of bile acids. Putative bile efflux systems were identified by screening the *H. hepaticus* and *H. pylori* genome sequences using the BLAST algorithm using sequences of known bile efflux systems of *Campylobacter jejuni*.

Results: *H. hepaticus* was 2 to 4 fold more resistant to cholic acid, taurocholic acid and taurodeoxycholic acid when compared to *H. pylori*, whereas their resistance to the other bile acids did not differ. The *H. hepaticus* and *H. pylori* genome both contain several putative TolC-like efflux mechanisms, including a system corresponding to the HefABC transporter. However, *H. hepaticus* contains an operon orthologous to the *C. jejuni* bile-efflux system CmeAB, whereas this system is not present in *H. pylori*.
Conclusion: *Helicobacter hepaticus* displays increased resistance to specific bile acids present in murine and human bile. This difference may be dependent on expression of the putative CmeAB bile efflux system, since this is absent in the bile-sensitive *Helicobacter* species *H. pylori*. Differences in bile acid-resistance may be important in determining whether *Helicobacter*species are capable of gastric or enterohepatic colonization.

O090
Convertase inhibition by *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* secretes several molecules to evade the human immune system. Staphylococcal Complement Inhibitor (SCIN) is a 10kD protein that inhibits C3 convertases of all complement pathways and thereby blocks all biological effects of complement. Database analyses revealed two SCIN homologs in *S. aureus*: SCIN-B and SCIN-C. These proteins are located on a putative immune evasion cluster that also encodes Extracellular Fibrinogen Binding protein (Efb), an Efb homolog (Efb-like) and alpha-haemolysin. Here we show the immune modulating properties of SCIN-B, SCIN-C, Efb and Efb-like.
Methods: Proteins were cloned and expressed in *Escherichia coli* and purified by Nickel affinity chromatography. Complement deposition and binding to complement components was studied by ELISA. C5a generation was analyzed by calcium mobilization of neutrophils. Effects on convertases were measured by immunoblotting or flow cytometry. Alternative pathway (AP) haemolysis assays were used to determine human specificity of the proteins.
Results: SCIN-B and SCIN-C have the exact same function as SCIN: they prevent complement activation by inhibition of C3 convertases. Efb and Efb-like act different from SCIN since they inhibit C3b deposition in the AP but not in other pathways. However, Efb and Efb-like efficiently block C5b-9 formation and C5a generation in all complement pathways. Efb and Efb-like do not stabilize convertases on bacterial surfaces, in contrast to SCIN and its homologs. Efb and Efb-like specifically bind C3 (fragments) via its C3d domain. The binding of Efb and Efb-like to C3b containing convertases results in convertase inactivation. Efb an Efb-like are not human specific.
Conclusions: 1) SCIN-B, SCIN-C, Efb and Efb-like are complement-evading molecules excreted by *S. aureus*. 2) SCIN-B and SCIN-C act similar to SCIN: they prevent complement activation by inhibition of C3 convertases. 3) Efb and Efb-like are unique complement inhibitors since they specifically inhibit C3b containing convertases. 4) Binding of Efb and Efb-like to C3b containing convertases results in inhibit of C3b deposition in the AP and C5b-C9 deposition in all complement pathways. 5) In contrast to SCIN and its homologs, Efb and Efb-like are not human.

O092
Mushrooms as agents of human disease

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Mushrooms, polypores, and corticioid fungi are common in the urban human environments. It is likely that humans inhale high numbers of airborne meiospores (basidiospores) while being in- or outdoors. Sputum or bronchial lavages often contain strains that are either determinable as filamentous basidiomycetes by their micromorphological characters, or are putative basidiomycetous *mycelia sterilia*. This suggests that filamentous basidiomycetes are frequent colonizers of the respiratory system. Their involvement in human disease, though suspected for decades, has only rarely been proven. In recent years, the number of mycoses caused by filamentous basidiomycetes as well as the number of species involved seems to increase. Most case reports refer to infections of the upper respiratory system of immunosuppressed patients, mainly caused by *Schizophyllum commune* and *Hormographiella* spp. (teleomorph *Psathyrellaceae*). In addition, cases of brain abscesses, endocarditis, eye infection, ulcerative lesions of the mucosa and onychomycosisis have been described.
In the first part of my presentation I will review the current state of knowledge regarding cultural characteristics, alternative diagnostic tools, pathogenicity, and *in vivo* and *in vitro* susceptibilities of filamentous basidiomycetes with proven or probable clinical significance. In the second part I will focus on the anamorphic genus *Hormographiella*. At this point of time, only two species, *Hormographiella aspergillata* and *Hormonographiella verticillata,* have been described from clinical sources. Sequence analyses of the ITS region of the nrDNA of clinical *Hormographiella* strains have revealed that additional species are involved in mycoses. The comparatively frequent evidence of Psathyrellaceae from inner surfaces of the human body might be due to the abundance of thermotolerant species

in this family. Thermotolerance may have evolved in adaptation to dispersal via the intestinal tract of herbivore mammals, or to the frequent occurrence of Psathyrellaceae in self-heating substrates such as compost and wood chips.

O094

Parallel phylogenies of *Pneumocystis* organisms and their mammalian hosts

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Pneumocystis is a group of organisms assigned to the Fungal Kingdom. The genus comprises pathogens dwelling in the lungs of terrestrial, aerial and aquatic mammals. Occasionally these organisms induce fatal pneumonia, particularly in immunocompromised patients. The highly ubiquitous occurrence and the marked pathogenic potential of *Pneumocystis* species, especially of the human-associated *Pneumocystis jirovecii*, has stimulated a growing interest in these peculiar fungi and pneumocystosis. On the basis of morphological, phylogenetic and experimental approaches we are now beginning to realize that *Pneumocystis* constitutes a highly diversified biological group, with numerous species that are host-specific and well adapted to live inside the lungs of a great diversity of mammalian species.

A first study in primates showed that large subunit of mitochondrial rDNA sequence divergence among *Pneumocystis* species was correlated with the phylogeny of their hosts. This observation, which could be extended to other mammals, suggested that cophylogeny can explain the current distribution of pathogens in their hosts. In order to test this hypothesis, aligned DNA sequences of three genes from *Pneumocystis* isolates originating from 20 primate species were subjected to separate phylogenetic analyses, and then combined in a single data set. At least 61% of the homologous nodes of the cladograms of hosts and pathogens may be interpreted as resulting from codivergence events.

The genetic diversity of *Pneumocystis* fungi was also examined in a single mammalian species. ITS and DHPS sequences have been extensively used to type *Pneumocystis jirovecii* and better understand the epidemiology of human pneumocystosis. We recently had the opportunity to examine many lung samples from common woodmice (*Apodemus sylvaticus*) originating from different regions in Europe. A very high variability among *Pneumocystis* sequences was demonstrated. The analysis of the genetic structure revealed two distinct groups. The first one comprised *Pneumocystis* from woodmice collected in continental Spain, France and Balearic islands. The second one included *Pneumocystis* from woodmice collected in continental Italy, Corsica and Sicily. These two genetic groups were in accordance with the two lineages currently described within *Apodemus sylvaticus* ('Western lineage' and 'Italo-balkanic' lineage). Similar analyses were conducted in order to examine the genetic structure of *Pneumocystis* from macaques and pigs. These studies confirmed that *Pneumocystis* diversity may be used as an original and powerful tool for a better understanding of mammalian systematics.

O095

Candida antibodies can precede invasive candidiasis in patients with hematological malignancies who have undergone multiple courses of chemotherapy

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Objectives: The kinetics of *Candida* antigen (Ag) and antibodies (Ab) were studied in patients with hematological malignancy using two commercially available systems (Platelia *Candida* EIA and Platelia *Candida* Ab/Ac/Ak, Marnes-La-Coquette, France, and Serion ELISA Antigen *Candida* and Serion ELISA Classic *Candida albicans* IgG/IgM/IgA, Institut Virion\Serion GmbH, Würzburg, Germany).

Methods: From 21 patients treated for a hematological malignancy or undergoing an allogenic hematopoietic stem-cell transplantation (HSCT) and with proven invasive candidiasis (IC), 242 serum or plasma samples were collected between first admission and 253 days after the candidemia. As controls served 135 samples collected from 30 patients from the same population but without evidence for IC. Ab and Ag were determined using 2 detection systems (Biorad and Serion). Reactivity was related to number of days of neutropenia.

Results: Ab and Ag were detected in more samples from patients with IC as compared to the control group for both systems ($p<0.05$). Both Ab and Ag were detected before positive blood culture. Ab was generally detected earlier than Ag with a median of 23 (Biorad) and 20 (Serion) days before microbiological confirmation of IC. For Ag detection these values were 1 and 11 days before microbiological confirmation in the BioRad and Serion assay, respectively. Ab were detected more frequently in patients with >10 days of neutropenia, which corresponded with multiple episodes of neutropenia, as

compared to those with <10 days of neutropenia ($p<0.05$). Analysis of the kinetics in individual patients showed that Ab were first detected following a previous episode of neutropenia, suggesting subclinical IC. The reactivity of the assays appeared not to be influenced by colonization or mucositis, since in the control group Ag and Ab were not detected in 91.1% and 93.3%, and in 93.3% and 97.6% of the samples with the Biorad and Serion test, respectively,

Conclusion: These data suggest that subclinical IC causes an Ab response, preceding a clinically manifest IC. Therefore, *Candida* Ab can be early markers of IC in hematology patients who have undergone multiple courses of chemotherapy.

O097

Tuberculosis diagnostics on respiratory samples: is culture always necessary? (pro)

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Introduction: In most laboratories, detection of *Mycobacterium tuberculosis* is based on the use of microscopy and culture. The last fifteen years commercially available amplifications methods were introduced in routine laboratories. Many studies have shown discrepancies between conventional culture and amplification methods.

Methods: Respiratory and non-respiratory samples derived from patients with possible tuberculosis were compared by using microscopy, culture and an amplification method over a period 10 years.

Results: On this moment only a small part of the evaluation can be shown. Out of 485 respiratory samples 82 samples (16,9%) gave a positive result in microscopy, culture and amplification alone of in combination in two or three methods. 54 samples (11.1%) were positive in microscopy and 46 (9.5%) were positive on solid media, and 66 (13.6%) were positive in liquid media. 54 samples (11.1%) were positive for *M. tuberculosis* complex in the amplification method.

Conclusion: Our data show the combination of microscopy, culture and amplification give a higher score then culture or amplification alone.

O098

Tuberculosis diagnostics on respiratory samples: is culture always necessary? (contra)

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Introduction: Detection of *Mycobacterium tuberculosis* complex can be performed by microscopy, culture and PCR. Many studies have focussed on the comparison of different (e.g. solid versus liquid) media and PCR techniques.

As a result of the highly variable and suboptimal sensitivity and specificity of PCR in these studies, culture has as yet not been replaced by PCR. Differences in sensitivity and specificity are explained by variances in PCR-assay and controls. Most studies were not stratified for volume of specimen or microscopic results. In the Netherlands, five laboratories developed a sensitive and specific real-time PCR for *M. tuberculosis* complex. All of them used the same decontamination procedure, the same liquid culture system and the same real-time PCR. This allowed these laboratories to compare the real-time PCR with microscopy and culture for the detection of *M. tuberculosis* complex in respiratory samples.

Methods: A total of 5008 respiratory samples from patients with possible tuberculosis was used for microscopy with Auramine and/or Ziehl-Neelsen staining, culture with liquid and solid media and real-time PCR, targeting the IS*6110*, as previously described [P.H.M. Savelkoul, et al]. All five laboratories used the NaLC/NaOH procedure for sample decontamination.

Results: Culture-positive were 189 samples (4%), 79 of which were microscopic-negative (42%), 96 microscopic-positive (51%) and 14 samples were not tested microscopically (7%). For 204 PCR-positive samples (4%), 86 of which were microscopic-negative (42%), 98 microscopic-positive (48%) and 20 samples were not tested microscopically (10%). Of the 85 samples positive for either culture or PCR, 50 were positive according to the culture and 35 samples were positive by real-time PCR.

Conclusion: Our data show that PCR and culture are equally sensitive for diagnosis of pulmonary TB. Despite this, the real-time PCR is more useful than culture because it is more rapid and may play a role in hospital hygiene, especially in microscopy-negative patients as long as multiple samples are used for each patient.

O099

Microbial consortium couples anaerobic methane oxidation to denitrification

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Modern agriculture has accelerated biological methane and nitrogen cycling on a global scale. Freshwater sediments often receive increased downward fluxes of nitrate and upward fluxes of methane generated by anaerobic decomposition. In theory prokaryotes could make use of nitrate to oxidize methane anaerobically but such organisms have never been observed in Nature nor isolated in the laboratory. Microbial oxidation of methane is thus believed to proceed only with oxygen or sulphate. Here we show that the direct, anaerobic oxidation of methane coupled to the denitrification of nitrate is possible indeed. A microbial consortium, enriched from anoxic sediments, was demonstrated to oxidize methane to carbon dioxide coupled to denitrification in the complete absence of oxygen. The consortium consisted of two microorganisms, a bacterium representing a phylum without any cultured species and an archaeon distantly related to marine methanotrophic archaea. Accordingly, labelled methane was incorporated into both archaeal and bacterial lipids. The detection of relatives of these prokaryotes in different freshwater ecosystems worldwide indicates that denitrification with methane may make a substantial contribution to the natural methane and nitrogen cycles.

O101

Freshwater Crenarchaeota, their ecology and ecophysiology with regard to climate change

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Understanding and predicting climate variability is a major scientific challenge, particularly with regard to climate-induced environmental change and its impact on human society. In cooperation with the Royal Netherlands Institute for Sea Research and the University of Utrecht proxies will be developed for surface temperature of lakes based on fossilized crenarchaeotal membrane lipids, and the fossils of chironomids and cladocera and the stable carbon and oxygen isotope composition of their chitin. Since essentially nothing is known on the functioning of pelagic freshwater Crenarchaeota, the aim of this project is to learn more about ecology and ecophysiology, especially in relation to the TEX86 temperature proxy. For this purpose, different molecular tools (e.g., qPCR, FISH) will be developed to study the diversity and spatio-temporal distribution of current Crenarchaeota. Subsequently, attempts will be made to enrich and isolate these microbes for physiological studies. In this way the TEX86 proxy will be validated for non-temperature interfering mechanisms inside or outside the crenarchaeotal cell (e.g. seasonality, conductivity, competition, light).

O102

Characterization of a benzene-degrading chlorate-reducing microbial community

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A stable anoxic enrichment culture was obtained that degraded benzene with chlorate as electron acceptor. Denaturing gradient gel electrophoresis of part of the 16S rRNA gene, cloning and sequencing showed that the culture had a stable composition after the seventh transfer. Five bacterial clones were further analyzed. Two clones corresponded to bacteria closely related to *Alicycliphilus denitrificans* K601. The three other clones corresponded to bacteria closely related to *Zoogloea resiniphila* PIV-3A2w, *Mesorhizobium* sp. WG and *Stenotrophomonas acidaminiphila*. DGGE analysis of cultures grown with different electron donors and acceptors indicated that the bacterium related to *A. denitrificans* K601 is able to degrade benzene coupled to chlorate reduction. The role of the other bacteria could not be conclusively determined. The bacterium related to *Mesorhizobium* sp. WG can be enriched with benzene and oxygen, but not with acetate and chlorate, while the bacterium related to *S. acidaminophila* grows with acetate and chlorate, but not with benzene and oxygen. As during chlorate reduction oxygen is produced, an aerobic pathway of benzene degradation is most likely.

O103

Multilocus sequence typing and the population and evolutionary biology of bacteria

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Multilocus sequence typing is widely used to unambiguously identify strains of bacterial pathogens for molecular epidemiological studies, but it also provides data that can be used to address the population and evolutionary biology of bacterial pathogens. For example, the relative contributions of recombination and mutation to the diversification of strains of different species can be measured, and the population structures of different species can be compared using programs such as eBURST. The concatenated sequences of the seven loci used in MLST can also be used to explore the patterns of clustering of the genotypes of populations assigned to closely related species, and to ask whether species exist and how they can be circumscribed, and to develop electronic taxonomic schemes. The wide availability of MLST data has stimulated the development

of population genetic models that can be used to simulate the evolution of bacterial populations. These models allow the estimation of recombination and mutation rates from MLST data and have been used to explore, by simulation, the conditions under which speciation does or does not occur in bacteria.

O104

Impact of nationwide vaccination against *Haemophilus influenzae* serotype b (Hib) on the composition of the circulating Hib population

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Introduction: Recently, there has been an increase in the Netherlands in the number of cases of invasive disease caused by *Haemophilus influenzae* serotype b (Hib) despite the fact that vaccination against Hib is included in the national vaccination program. This prompted us to assess a possible change in the properties of the currently circulating Hib population that could explain the rise in incidence.

Methods: A multiple-locus variable number tandem repeats analysis (MLVA) was developed to genotype *H. influenzae* isolates. The MLVA was used to genotype a collection of 520 Hib strains isolated from Dutch patients with invasive disease. The strains were collected from 1983 from 2002, covering a time period of 10 years before and 9 years after the introduction of the Hib vaccine in the Dutch national vaccination program. In addition, the DNA sequences of the capsule gene clusters Hib strains were assessed and the structure and level of expression of the capsular polysaccharide were determined.

Results: The MLVA enabled the differentiation of *H. influenzae* serotype b strains with higher discriminatory power than multilocus sequence typing (MLST). MLVA revealed a sharp increase in genetic diversity of Hib strains isolated from neonates to 4-year-old patients after 1993, when the Hib vaccine was introduced. Hib strains isolated from patients older than 4 years in age were genetically diverse, and no significant change in diversity was seen after the introduction of the vaccine.

Analysis of the DNA sequences of the capsule gene cluster of 9 Dutch Hib strains revealed two variants, designated type I and type II. The variants displayed considerable sequence divergence in the *hcsA* and *hcsB* genes, involved in export of capsular polysaccharide. Application of *hcsA* type specific PCRs on 667 Hib strains collected from Dutch patients with invasive Hib disease showed that 3% of the Hib strains were type II. No type II strains were isolated after 1995, 2 years after the introduction of Hib vaccination. All type II strains were isolated from 0-4 year old non-vaccinated children only. Analysis of a worldwide collection of Hib strains revealed significant geographic differences in the distribution of the type I and type II strains. NMR and immunological analysis of type I and type II capsule polysaccharides did not reveal structural differences. However, type I strains were shown to produce twice as much surface bound capsular polysaccharide.

Conclusion: The results obtained by MLVA suggest that after the introduction of the Hib vaccine young children no longer constitute the reservoir for Hib and that they are infected by adults carrying genetically diverse Hib strains.

The higher capsule expression of type I strains may have provided a selective advantage over type II strains in vaccinated children as type II strains may be less well equipped to resist vaccine induced immunity than type I strains. This may have resulted in the elimination of type II strains from the Dutch Hib population and may have contributed to the increased number of vaccine failures in the Netherlands.

O105

Emergence of ampicillin resistant *Enterococcus faecium* (AREfm) in the Netherlands

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Introduction: We recently reported an increase in the proportion of invasive enterococcal infections caused by AREfm (2% in 1994 to 32% in 2005) among enterococcal bloodstream infections in the UMC Utrecht (Top *et al.*, CMI in press). In our hospital all AREfm belonged to CC17 (*Enterococcus faecium*, a clonal complex associated with nosocomial outbreaks worldwide. A nationwide study was initiated to determine whether these ecological changes had also occurred in other hospitals in the Netherlands.

Methods: Sixty-six microbiology laboratories, serving all hospitals of the country, were asked to provide data on annual numbers of invasive ampicillin resistant (ampR) enterococcal isolates and to submit the first 30 enterococcal blood stream isolates per year (1 per patient) from 1994 until 2006. Multiplex PCR based on the (*ddl* gene was performed to distinguish (*E. faecium* and (*E. faecalis* and susceptibility to ampicillin was determined using a screen plate containing 16 mg/L ampicillin. All (*E. faecium* isolates were genotyped using multiple locus variable number tandem repeat analysis (MLVA).

Results: Twenty-five labs (38%) serving 28 hospitals provided data, and 9 (14%) labs provided isolates. The mean number of invasive ampR enterococcal isolates

per hospital increased from 5 in 1994 to 26 in 2005. The average number per year increased from 5 (1994) to 47 (2005) in university hospitals (n=5, including UMCU data from CMI study) and from 4 (1994) to 19 (2005) in non-university hospitals (n=22). Among enterococcal blood isolates proportions AREfm increased from 4% in 1994 to 27% in 2005 in university hospitals (p<0.001). No significant increase was observed in non-university hospitals (6% in 1999 and 12% in 2005, p=0.39). All *E. faecalis* isolates were ampicillin susceptible, while 78% of the *E. faecium* isolates were ampicillin resistant. MLVA typing of 303 *E. faecium* isolates revealed 61 MLVA types (MT). Four predominant types (MT-1, -5, -12 and -159) belonging to CC17, were found in ≥5 hospitals, including university and non-university hospitals. Although MT-1 was found in low numbers between 1994 and 1999 (range; 0-2 isolates), this type increased after 1999 to 21 isolates in 2005. The first MT-12 isolates emerged in 2002, but a strong increase together with MT-159 has been recently observed in 2006, 13 and 14 isolates respectively.

Conclusions: Invasive AREfm, belonging to CC17, have increased nationwide, especially in university hospitals and have partially replaced ampS *E. faecalis*. This rapid emergence has resulted from clonal spread of 4 MLVA types. The epidemiological difference between university and non-university hospitals probably reflects differences in patient population with haematology and transplant patients (at highest risk for AREfm bacteraemia) over represented in university hospitals.

O106

Molecular epidemiology of cutaneous *Leishmania* isolates from Dutch patients

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Cutaneous leishmaniasis is caused by different Leishmania species. *Leishmania* evolves clonally, with occasional phenomena of hybridization. This implies that Leishmania populations are stable in space and time and consequently have epidemiological relevance. We analyzed Leishmania genotypes found among >150 patients that presented at the Academic Medical Center during the past three years.

Leishmania DNA samples were obtained directly from biopsies or from cultured *Leishmania* isolates from patients. The mini exon repeat sequence was amplified by PCR and sequenced. Sequences were analyzed with CodonCode Aligner and MEGA. Genotype data were matched with patient data.

In the Old World (Asia, Africa and Mediterranean), *Leishmania major* genotypes accounted for most cases, due to an *L. major* outbreak by a single genotype among military personnel in Afghanistan in 2005. *L. mexicana* accounted for most cases from Central America, *Leishmania brasiliensis* and *Leishmania guyanensis* for most cases from South America. Two cases with different genotypes of *Leishmania naiffi* were detected in patients that visited Surinam. This species has been detected rarely in humans.

O107

Testing for HSV and CMV resistance

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For decades, bacterial resistance testing has been common practice to guide antibacterial therapy. The opposite is true for viral infections, with the exception of human immunodeficiency virus infections. However, in immunocompromised patients, the need for antiviral susceptibility testing of herpes simplex virus (HSV) types 1 and 2 and cytomegalovirus (CMV) has emerged in the past years.

Prophylaxis and (pre-emptive) antiviral treatment is frequently applied in immunocompromised patients. This treatment consists mostly of nucleotide analogues such as acyclovir (ACV) for HSV and ganciclovir (GCV) for CMV. If therapy with these first-line agents fails, cidofovir, a nucleotide analogue, and foscarnet, a pyrophosphate analogue, are available. In this population, prolonged or repeated HSV or CMV infections have raised the question whether the virus is still susceptible to the antiviral drug used. Several studies to date have shown varying prevalences of resistant HSV strains up to 25% in allogenic stem cell transplantation patients. As prolonged HSV or CMV infections might also be due to failure of cellular immunity or insufficient anti-viral drug levels, susceptibility testing can be helpful in this situation.

Susceptibility testing can be performed both phenotypically, testing the growth of a virus isolate in the presence of the antiviral agent, and genotypically, usually by sequence analysis of the genes that encode the target protein of the antiviral agent. Phenotypical resistance testing has traditionally been performed in the plaque reduction assay in which viral growth suppression due to antiviral agents is measured by counting viral cytopathic plaques in cell cultures in semisolid medium. Since plaque counting is a manual and thus inaccurate process, methods using more objective endpoints have been developed. Examples are antigen measurement using enzyme immune assays, vital dye exclusion and, more recently, DNA measurement using DNA-probes or PCR. Alternatively, genotypical resistance testing can be performed by direct sequencing of viral isolates. Both ACV and GCV are dependent on phosphor-

ylation by viral protein kinases for activation. Hence, the genes for these enzymes, HSV thymidine kinase and CMV UL97 respectively, are the sites for resistance-associated mutations. Phosphorylated ACV, GCV and cidofovir, and foscarnet all act on the viral DNA polymerase to cause inhibition of viral replication. Therefore, mutations in the viral DNA polymerase (named UL54 in CMV) can lead to resistance to all these types of anti-herpes virus medication.

The advantage of phenotypical resistance testing is in the direct observation of viral growth in the presence of antiviral medication. Disadvantages are the long duration of the assay and the possible selection of viruses with the best replicative capacity, most likely the wild type virus, from mixed isolates. Genotypical resistance testing is fast but requires the availability of specialised equipment. Also, for many sequence variations in viral genes the relation with resistance has not been established yet, especially in the case of HSV. Therefore, at present both methods are used complementarily.

Apart from the need for more knowledge on resistance-associated mutations and faster phenotypical techniques, further studies are required to evaluate the clinical applications of these assays. Such studies can investigate the prevalence of resistance as opposed to failing immune recovery in patients with prolonged viral infections and reactivations under therapy and assess the value of susceptibility testing as a clinical tool in therapeutic decision-making.

O108

Early prediction of response during high dose interferon induction therapy in difficult-to-treat chronic hepatitis C patients

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Background & aims: The aims of our study were to determine (i) if early viral kinetics could predict treatment outcome in 'difficult-to-treat' hepatitis C patients during high dose interferon induction treatment, (ii) if fast-responders (≥3 log drop in HCV RNA at week 4) could stop treatment at week 24.

Methods: We treated 100 hepatitis C patients (46 previous non-responders/relapsers (any genotype), 54 treatment-naive genotype 1 and 4) with triple antiviral therapy: Amantadine hydrochloride and ribavirin, combined with 6 weeks interferon alfa2b induction (week 1-2: 18 MU/day, week 3-4: 9 MU/day, week 5-6: 6 MU/day), thereafter combined with weekly peginterferon alfa-2b, for 24 or 48 weeks. Fast-responders (≥3 log drop in HCV RNA at week 4) were randomized to 24 or 48 weeks. Patients with <3 log drop in HCV RNA at week 4 (slow-responders) were treated for 48 weeks. Patients with HCV RNA detectable by PCR at week 24 stopped treatment.

Results: 36 patients achieved SVR: 19 fast-responders after 24 weeks of treatment, 9 fast-responders and 8 slow-responders after 48 weeks of treatment. 64 patients became non-SVR (27 non-response, 9 breakthrough, 15 relapse, 13 dropout). Predictive values of early viral kinetics were different for treatment naive patients and previous non-responders/relapsers. In treatment-naive patients, PPV for SVR was 100% if HCV RNA was <5 IU/mL at week 1 or 2; PPV for non-SVR was 100% if HCV RNA was ≥615 IU/mL at week 12, or ≥5 IU/mL at week 16. In previous non-responders/relapsers PPV for non-SVR was 100% if HCV RNA was ≥615 IU/mL at week 4, or ≥5 IU/mL at week 8. Relapse rates among fast-responders treated for 24 or 48 weeks were 27% and 20%, respectively (p=NS). SVR in fast-responders treated for 24 or 48 weeks was independent of baseline HCV RNA ≥or <800.000 IU/mL.

Conclusion: With high dose interferon induction therapy: (i) early viral kinetics can predict SVR and NR in treatment naive patients and previous non-responders/relapsers, (ii) SVR is independent of baseline HCV RNA.

O109

Antiviral susceptibility of influenza viruses in the Netherlands

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Introduction: In addition to vaccines, antivirals could play a major role as a defence against influenza virus infections when the vaccine fails or during vaccine shortage in case of large epidemics or a pandemic. However, during large-scale use of antivirals emergence of resistance can be anticipated. Therefore, surveillance for the emergence and spread of resistant influenza viruses is needed. In this study, the neuraminidase inhibitor (NI) and adamantane M2 inhibitor susceptibility of Dutch influenza viruses isolated during the 2005-2006 flu epidemic were assessed.

Methods: Throat and nasal swabs from patients with influenza-like illness (ILI) were obtained from the national network of sentinel general practitioners, representing the Dutch population, that performs ILI surveillance. Oseltamivir and zanamivir susceptibility of isolated viruses [39 influenza A(H3N2) and 48 influenza B] were determined using a fluorescence based neuraminidase (NA)-inhibition assay. Susceptibility was determined as the amount of NI needed to inhibit the NA activity with 50% (IC_{50}). Potential outliers were identified with the use of box and whisker plots, showing the median, lower quartile and upper quartile. IC_{50}-values 1.5 or 3 times the interquartile range (IQR) outside the IQR were defined as mild or

extreme outliers, respectively. Adamantane resistance was determined using sequencing of the relevant part of the M2 gene of the influenza A(H3N2) viruses.

Results: First, outliers for NI susceptibility were identified. One influenza A virus (2.6%) had reduced susceptibility to oseltamivir, IC_{50} 1.1 nM. Of the influenza B viruses, two (4.2%) had enhanced susceptibility to zanamivir (IC_{50} 1.5 and 1.7 nM), one (2.1%) had enhanced susceptibility to zanamivir (IC_{50} 2.9 nM) and reduced susceptibility to oseltamivir (IC_{50} 23.6 nM) and two (4.2%) had reduced susceptibility to both NIs (IC_{50} 114 and 46.4 nM oseltamivir and 102 and 50.4 nM zanamivir, respectively).

Next, with the remaining data the baseline NI susceptibility for the 2005-2006 season was determined. For influenza A viruses baseline susceptibility IC_{50}-values were 0.24±0.06 nM (mean ±SD) oseltamivir and 0.83±0.18 nM zanamivir. For influenza B viruses, baseline IC_{50}-values were 13±2.7 nM oseltamivir and 6.9±1.3 nM zanamivir. In Europe, recent mean IC_{50}-values for influenza A(H3N2) virus were 0.6 nM oseltamivir and 0.7 nM zanamivir and for influenza B virus 42 nM oseltamivir and 7 nM zanamivir.

Preliminary sequencing data of 22 of the 39 influenza A(H3N2) viruses indicate that 77% of the viruses have the adamantane resistant S31N mutation in the M2 protein. This observation is in line with recent adamantane resistance data from many other countries in the world.

Conclusion: Baseline zanamivir susceptibility of Dutch influenza viruses is similar to that for the whole of Europe. However, for oseltamivir, the baseline susceptibility of Dutch influenza viruses is twofold higher compared to influenza viruses from the rest of Europe. Outliers and a subset of baseline influenza viruses are currently being sequenced to find the origin of altered NI susceptibility. The adamantane resistance data indicate that the M2 inhibitors are no option any longer for influenza A(H3N2) treatment.

O110

Evaluation of the NA-Star® kit for determination of oseltamivir susceptibility of influenza viruses

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Introduction: Influenza antivirals are particularly useful to treat influenza in unvaccinated patients, in case of failure or shortage of the seasonal vaccine and of vaccine shortage during a pandemic. Due to the increased used of antivirals resistant viruses might emerge. The fluorescent assay is considered the gold standard to measure susceptibility of influenza viruses for neuraminidase inhibitors. The new experimental NA-Star® kit, which uses a chemiluminescent substrate, claims to offer a more sensitive and standardised alternative which does not require pretitration of the virus.

Methods: We compared the NA-Star® kit with our in-house fluorescent assay for analysing oseltamivir susceptibility. Nine A(H3N2) and nine B sentinel influenza virus isolates of the 2005/2006 season, a reference A(H3N2) influenza virus isolated before the introduction of oseltamivir in the Netherlands and an A(H3N2) influenza virus with know resistance mutation E119V in the neuraminidase protein were included in the study. Fifty percent inhibitory concentrations (IC50) were calculated using GraphPad Prism 4 software. Potential resistant viruses (outliers) were identified using box and whisker graph analysis. The NA-Star® kit was kindly provided by Applied Biosytems.

Results: Time required to carry out the NA-Star® assay (~1 hour 20 min) was about 3x less compared with the fluorescent assay (~4 hours).

Baseline influenza A viruses had similar IC50s in the NA-Star® assay (mean 0.28 nM, SD 0.05) compared to the fluorescent assay (mean 0.26 nM, SD 0.07). The baseline influenza B viruses had a mean 4.5x lower IC50 in the NA-Star® assay (mean 2.7 nM, SD 0.6) than in the fluorescent assay (mean 12.2 nM, SD 3.8). Outlier viruses had lower IC50s in the NA-Star® assay than in the fluorescent assay, and this difference became wider with increasing IC50s, e.g. 0.42 and 1.12 respectively for a less resistant A(H3N2) virus compared to 2.8 nM and 49.2 nM respectively for the E119V variant. Of the five influenza viruses (2 A and 3 B) identified as outliers in the fluorescent assay, one influenza A and one influenza B virus could not be identified as outliers in the NA-Star® assay.

In the NA-Star® assay, baseline influenza A and B viruses had an up to 2x lower IC50 and outlier influenza A and B viruses an up to 2x higher IC50 when a higher dilution of virus was used. In the fluorescent assay a higher dilution resulted in an up to 2x lower IC50 for both virus types.

Conclusions: The NA-Star® assay is rapid, mainly because no pretitration of virus is needed. However, pretitration would also not be required for the fluorescent assay, as it shows with diluted virus similar IC50 variation than the NA-Star® assay. Although the analytical sensitivity of the NA-Star® assay is higher, the difference in IC50 between outlier and baseline viruses is less than in the fluorescent assay. This might result in losing sensitivity to detect potential resistant variants. As the number of viruses was low, additional studies are needed to confirm these observations.

O115

DNA repair mechanisms in *Campylobacter jejuni*

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The most common cause of bacterial gastro-enteritis in humans is *Campylobacter jejuni*. Genotypic analysis identified the *C. jejuni* population as genetically diverse with a few clonal lineages. MLST data suggest that horizontal gene transfer plays a major role in generating genetic diversity, but the contribution of mutational-based processes (DNA alterations) to this diversity is still largely unknown. DNA alterations may arise during DNA replication, and after exposure to UV-light, mutagenic agents or oxygen. The contribution of these alterations to genetic diversity is affected by the activity of DNA repair mechanisms. These mechanisms are designed to repair DNA damage, thereby ensuring bacterial viability and, depending on the mechanism, maintenance of genetic stability or generation of genetic diversity. More insight into the function of DNA repair mechanisms in *C. jejuni* may contribute to the understanding of how genetic diversity is generated in the *C. jejuni* population. The aim of this study was to investigate the presence and functionality of a number of DNA repair mechanisms in *C. jejuni*.

In silico analysis suggested the presence of the genes *mutS*, *uvrB*, *recA*, and *ung* in *C. jejuni*. In other bacterial species these genes are involved in DNA repair mechanisms, namely methyl-directed mismatch repair (MMR), nucleotide excision repair (NER), recombinational DNA repair and base excision repair (BER), respectively. To asses the function of *mutS*, *uvrB*, *recA*, and *ung*, these genes were inactivated in three *C. jejuni* strains by insertion of a chloramphenicol resistance cassette. Subsequently, the resulting mutants were analysed with regard to their spontaneous mutation rate, UV-light resistance, and the ability for homologous recombination.

Preliminary results suggest that at least two of the investigated DNA repair mechanisms are functional in *C. jejuni*. UvrB is involved in repair of UV-light induced structural changes, and RecA plays a role in recombinational DNA repair.

Concerning the contribution to genetic diversity, the NER pathway (UvrB) may limit the generation of diversity by repair of structural DNA changes induced by UV-light. The recombinational DNA repair pathway (RecA) could be involved in the generation of genetic diversity through insertion of DNA originating from related strains.

O116

Expression profiling of udder tissue in response to acute clinical *Streptococcus uberis* infection

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Streptococcus uberis (SUB) causes clinical and subclinical mastitis in cattle. Bovine mastitis is economically the most important disease in the dairy industry. To control SUB infections, more insight into the process of pathogenesis is required. Therefore, early host responses of mammary gland tissue after acute SUB infection were studied using microarray analysis and qRT-PCR.

Four lactating heifers were infected intramammary with SUB. The animals were sacrificed as soon as clinical symptoms of mastitis were observed, between 1.5 and 3 days post infection. Tissue samples were collected from different locations in the udder and both snap frozen for mRNA isolation and formalin fixed for histopathological analysis. The selection of tissue samples used for mRNA isolation was based upon histopathological observations. Both samples in which clear signs of infection were present (infected sample) as well as samples in which signs of infection were absent (uninfected control) were selected.

qRT-PCR analysis was used to study the expression of defensins and toll like receptors (TLR) 2 and 4. In accordance to data presented previously, beta defensin expression was strongly induced in SUB infected udder tissue. TLR 2 and 4 were upregulated as well but to a much lesser extent. These data indicate that the innate immune system is upregulated early in the process of SUB pathogenesis.

For microarray analysis a bovine 20K cDNA array was used. Each slide contained duplicates of each spot and a set of control genes. In each microarray experiment an uninfected control sample was compared to a SUB infected sample from the same animal. Samples from different locations in the udder were tested, and all experiments were dye swapped. The data were analyzed using BlueFuse software, and normalized using either R, or the normalization procedure included in BlueFuse. 246 genes were regulated in their expression with a false discovery rate lower than 5% when identified by the infected vs. uninfected comparison. Most of the affected genes were upregulated (197); 49 of the genes were repressed. Homology searches showed that most of the induced genes were associated with the innate immune response. Interestingly, a considerable number of differentially expressed genes found were also identified after analysis of LPS-induced mastitis in a mouse model. This clearly indicates the importance of these genes in the early onset of mastitis in general. In the future, a more detailed analysis of the affected genes may result in more insight into the molecular pathways underlying the pathogenesis of SUB mastitis.

O117

Staphylococcal superantigen-like protein 5 inhibits chemokine-induced cell activation

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Background: *Staphylococcus aureus* secretes several virulence factors that interfere with host-cell functions.

Staphylococcal superantigen-like proteins (SSLs) are a family of 11 newly identified exotoxins encoded on pathogenicity island SaPI2 present in all sequenced strains. SSLs show structural homology to superantigens, but do not exhibit superantigenic activity. Recently, we demonstrated that SSL5 binds to P-selectin glycoprotein ligand 1 (PSGL-1) dependently of sialyl lewis X (sLex) and inhibits P-selectin-dependent neutrophil rolling. Here we describe interference of chemokine-induced cell stimulation by SSL5.

Methods: A calcium mobilization assay was performed to investigate the influence of SSL5 on chemokine signaling in human neutrophils and monocytes. Binding experiments with SSL5 and interleukin-8 (IL-8)-biotin were performed on U937 cells. Human embryonic kidney (HEK) cells were transfected with CXCR1 or CXCR2. ELISA experiments were performed with IL-8-coated plates and PSGL-1/Ig. Importance of sialic acid residues was examined by treating neutrophils with neuraminidase or preincubating SSL5 with sLex.

Results: SSL5 inhibited calcium mobilization in neutrophils and monocytes induced by CC, CXC and CXC3C chemokines, but not by classical chemoattractants. SSL5 increased IL-8-biotin binding to cells, which was reduced upon treatment with neuraminidase. Transfection experiments demonstrated that SSL5 does not bind CXCR1 or CXCR2. However, SSL5 induced binding of PSGL-1 to IL-8. Treatment of cells with neuraminidase abolished SSL5 binding to cells and eliminated its inhibitory effect on chemokine cell activation. The same results were observed when SSL5 was loaded with sLex.

Conclusions: 1) In addition to binding to PSGL-1, SSL5 inhibits cell activation by chemokines. 2) SSL5 effect is dependent of sLex presented by molecules as PSGL-1 on the cell surface. 3) SSL5 is an important immunomodulatory protein of *Staphylococcus aureus*.

O118

Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain-Barr and Miller Fisher syndromes

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Introduction: Gastro-enteritis caused by *Campylobacter jejuni* is the most common infection preceding the Guillain-Barré syndrome (GBS), an acute immune-mediated neuropathy. Molecular mimicry of *C. jejuni* lipo-oligosaccharides (LOS) with gangliosides in peripheral nerves is considered to induce a cross-reactive immune response leading to clinical symptoms. The development of new mass spectrometry methods combined with serotyping and preliminary genetic knowledge to deduce LOS structures allows quick screening of many strains. The aim of this study was to determine the exact LOS outer core structures of our collection of neuropathy-associated *C. jejuni* strains and to relate these structures to clinical symptoms.

Methods: LOS outer core structures of 26 *C. jejuni* strains associated with GBS and its variant the Miller Fisher syndrome (MFS) were determined by capillary-electrophoresis coupled with electrospray ionization mass spectrometry (CE-ESI-MS), combined with DNA sequence analysis to determine the alleles of relevant genes.

Results: Sixteen out of 22 (73%) GBS-associated and all 4 (100%) MFS-associated strains expressed LOS with ganglioside mimics. GM1a was the most prevalent ganglioside mimic in GBS-associated strains (10/22, 45%) and in 8 of these strains GM1a was found in combination with GD1a mimics. All 7 strains isolated from patients with ophthalmoplegia (GBS or MFS) expressed disialylated (GD3 or GD1c) mimics. Three out of 22 GBS-associated strains (14%) did not express sialylated ganglioside mimics because their LOS locus lacked the genes necessary for sialylation. Three other strains (14%) did not express ganglioside mimicks because of frame-shift mutations in either the *cstII* sialyltransferase gene or in the *cgtB* galactosyltransferase gene.

Conclusion: This is the first report in which mass spectrometry combined with DNA sequence data was used to deduce the LOS outer core structures of a large number of neuropathy-associated *C. jejuni* strains. The majority of neuropathy-associated strains expressed single or multiple ganglioside mimics in their LOS. The structure of the ganglioside mimic determined clinical symptoms. We conclude that molecular mimicry between gangliosides and *C. jejuni* LOS is the presumable pathogenic mechanism in most cases of *C. jejuni*-related GBS. However, our findings suggest that in some cases other mechanisms may play a role. Further examination of the disease etiology in these patients is mandatory.

O119

The high pathogenicity island of an outbreak *Enterobacter cloacae* strain contributes to virulence

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Background: A nationwide multicenter outbreak of a multidrug-resistant *Enterobacter cloacae* strain occurred. Bacterial factors enhancing epidemicity were investigated and a large conjugative plasmid encoding multidrug

resistance appeared to contribute. The aim of this study was to determine the presence and function of the High Pathogenicity Island (HPI) in the outbreak strain. The (HPI) was investigated because it is described in *E. cloacae* and shown to increase virulence of *Escherichi coli* and *Yersinia* species. The core of the HPI that comprises 11 genes, which are nearly identical in all species, encodes for an iron uptake and regulation system. The siderophore yersiniabactin is produced during times of iron starvation to facilitate iron uptake by the bacteria. Iron in patients is mostly bounded to hemin, transferrin, and lactoferrin, which makes the ability to extract iron crucial for bacterial surviving in the host. However, lactoferrin has a role in host defence as well by three mechanisms: 1) During infection lactoferrin production by PMN is upregulated thereby reducing the availability of free iron for the bacteria. 2) Lactoferrin acts as an activator of polymorphonuclear leukocytes (PMN) via IL-8 and 3) lactoferrin containing iron is released into the phagocytic vacuoles of PMNs. Presumably, the iron then acts synergistically with H_2O_2 to form the highly toxic OH· in the Fenton reaction. Subsequently, the produced radicals and peroxides kill the pathogen. We hypothesized that yersiniabactin produced by bacteria limits the availability of iron to PMN by competitive binding and consequently inhibits the innate immune system.

Methods: The presence and function of the HPI was determined by PCR, Southern blot, and several protein expression experiments. To determine the effects of yersiniabactin a knock-out was created with the Targetron Knock-out system (Sigma). Subsequently, growth experiments with different iron sources were performed with the knock-out and the wild-type strain. Next, the iron uptake of, and the reactive oxygen species (ROS) response of PMNs were measured when challenged with supernatants of the wild-type and the knock-out strains.

Results: The HPI was present in 95% (137/144) of the outbreak isolates compared to only 7% (10-151) of non-outbreak *E. cloacae* isolates. The wildtype growth was faster than the knockout in media where lactoferrin was the main iron source indicating that the HPI favoured growth This difference was not shown when hemin or transferrin were present as the only iron source, indicating that *E. cloacae* could use iron from hemin and transferrin independent of HPI. When PMNs were challenged with supernatant of the knockout, iron uptake and the production of ROS by PMNs were significantly higher than when PMN were challenged with supernatant of the wildtype.

Conclusion: In the outbreak strain the virulence enhancing HPI was detected and expressed. Potentially, HPI-positive isolates withstand innate immune responses better than HPI-negative isolates. Iron uptake was decreased in the PMN, probably because less iron is provided by lactoferrin, when yersiniabactin is present. This was associated with a reduced ROS-response which likely result in a less efficient killing of HPI-positive bacteria by PMN. Thus, HPI contributes to virulence.

O120

Biosynthesis and immunosuppressive activity of mannose-capped mycobacterial lipoarabinomannan

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The C-type lectin DC-SIGN on dendritic cells (DC) recognizes mannose-capped lipoarabinomannan (manLAM), a major glycolipid in the cell wall of *Mycobacterium tuberculosis*. Purified manLAM and not capless LAM (araLAM) impairs maturation of dendritic cells and induces production of anti-inflammatory cytokine IL-10. Therefore, a role for the cap in mycobacterial persistence *in vivo* has been hypothesized. To test this, mannose cap lacking mutants in *Mycobacterium marinum* (*Mm*) were isolated in a screen of a transposon mutant library with a cap-specific monoclonal antibody. Two mutants which appeared negative in the screening were isolated. In one mutant the gene MM2439 (*capA*), a homolog of Rv1635c, was disrupted. This gene encodes a mannosyltransferase. Mutant *Mm 'capA* did not show mannosyltransferase activity in an enzyme assay and loss of the mannose cap of its LAM was confirmed by structure analysis. Parent *Mm* in SDS-PAGE/immunoblot bound to DC-SIGN-Fc, but *Mm 'capA* did not. In murine macrophages or fish leucocytes, *Mm 'capA* showed increased localization to phagolysosomes, but no differences in intracellular survival were found. *In vivo* in zebra fish, *Mm 'capA* did not survive less in liver and spleen. The homologous gene in *M. bovis* BCG was also mutated. In a SDS-PAGE/immunoblot, capless BCG did not bind to DC-SIGN in contrast to wildtype BCG. Surprisingly, no difference was seen in binding to DC-SIGN in an immunoblot on which whole colonies were probed. Furthermore, capless BCG did not bind less to human DC, did not differ from wildtype BCG in effect on DC maturation, did not induce less IL-10 and did not survive less in human THP-1 cells or murine macrophages. Capless BCG did not survive less in mice (lung, spleen, liver), and identical concentrations of cyto/chemokines

were induced *in vivo*. In the second cap-negative *Mm* mutant found in the screening, the transposon was inserted in a gene with unknown function (*capB*). In *Mm 'capB*, mannosyltransferase activity was still present and other phenotypic alterations, not present in *Mm 'capA*, were detected: in liquid culture, growth impairment, changes in pigmentation and bacterial aggregation, which was also observed with fluorescence microscopy. However, ConA (a mannose-binding lectin) in a SDS-PAGE/immunoblot did not reveal any changes in mannosylation of glycoproteins and glycolipids other than manLAM; thin layer chromatography showed a wildtype lipid profile for the *Mm 'capB* and no changes were detected in protein profile by Coomassie staining of SDS-PAGE.

Conclusions: 1) Screening of a transposon mutant library with specific antibodies was a useful tool to isolate capless mutants. A high number of transposants could be screened within a relatively short period of time to discover genes involved in mannose-capping of LAM. 2) At least two genes play a role in mannose-capping of LAM. 3) The role of the mannose cap in immunomodulation is limited. Binding to DC of the capless BCG in FACS could still be inhibited by mannan and therefore mannose-containing ligands other than the cap of manLAM are likely to determine the interaction with the host.

O122

Phylogeny of the *Exophiala spinifera* clade in search of new virulence factors

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Progress of virulence research in black yeasts is strongly dependent on adequate diagnostics. To confirm species delimitations in the *Exophiala spinifera* clade, four independent genes were analyzed and phylogenetic trees were reconstructed using different algorithms. Reproductive isolation within the clade and reproductive modes of species involved were also determined, in order to explore specific borderlines with Genealogical Concordance Phylogenetic Species Recognition (GCPSR). Sequences of the Internal Transcribed Spacer (ITS) region of ribosomal DNA gene (rDNA), partial Elongation Factor 1-α (EF 1-α), β-tubulin (β-TUB) and actin (ACT) genes were analyzed for a set of 156 strains belonging to the *E. spinifera* clade.

Though the topologies were not completely identical when different algorithms were used, the trees of the combined multilocus dataset did not provide more phylogenetic information than those of separate datasets. The phylogenetic relationship of species in the clade was also nearly identical to that based on 18S of rDNA sequences in previous studies. Most *Exophiala* species analyzed in this study were clonal. ITS is basically reliable for phylogenetic reconstruction and identification for species in this clade. Numerous black yeast-like fungi show a dual ecology, combining opportunism on humans and (cold-blooded) animals with assimilation of toxic alkylbenzenes. We identified a large number of environmental strains by enrichment using monoaromatic compounds. These compounds present only in trace amounts in nature, but may accumulate from animal sources. Their possible role in triggering virulence is considered.

O123

Mucormycosis; a new emerging disease?

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The class Zygomycetes is divided into two orders: Mucorales and Entomopthorales. Members of the Mucorales are the aetiological agents of mucormycosis. Fungi belonging to the class of Zygomycetes can be divided in six families of which the Mucoraceae represent the most frequent cause of mucormycosis. The family of Mucoraceae has 10 genera: *Mucor, Apophysomyces, Rhizomucor, Absidia, Rhizopus, Cunninghamella, Mortierella, Syncephalastrum, Cokeromyces* and *Saksenaea*. The mucorales growth very rapid on selective and aselective media and cover the entire plate in a few days. Identification of the species is based on microscopic morphological criteria and maximum growth temperature. Mucormycosis occurs mainly in patients with diabetes mellitus and ketoacidosis, in patients with haematological malignancies, in solid organ transplants, in patients receiving high dosis of corticosteroids and in patients treated with deferoxamine. Mucormycosis may also develop in patients with aspiration pneumonia after near-drowning. Mucormycosis manifests most commonly as sinusitis progressing to rhino-cerebral disease, pneumonia, cutaneous disease, osteomyelitis, brain abscesses, gastrointestinal disease, kidney disease or disseminated disease. The mortality is high, mainly due to a delayed diagnosis and severe underlying disease. Disseminated mucormycosis has a nearly 100% mortality rate, whereas rhinocerebral mucormycosis, pulmonary mucormycosis and gastrointestinal disease are associated with mortality rates of 50%-70%, 60% and 95%, respectively. The diagnosis is difficult and deep tissue samples are required. The presence of wide, non-septate hyphae is very suspicious for mucormycosis. Diagnosis only by histopathological findings is insufficient. Discrepancies between positive microscopy and negative cultures are reported in 10%, presumably due to the presence of fragile hyphae. Another possibility is that these diseases are

associated with yet unidentified non-culturable fungi, resembling mucormycosis. Diagnosis by imaging is very difficult and non-specific, whereas antigen serum tests are under development. The cornerstones for treatment of mucormycosis consist of correction of predisposing factors, surgical resection, and appropriate antifungal therapy. There is no role for hyperbaric oxigen. Liposomal amphotericin B, posaconazole and ravuconazole are considered as effective agents. Currently, an increase of mucormycosis is observed in institutions with haematological patients. Mucormycosis is the second most common invasive mycosis and occurs mainly in the lungs and sinuses. Extensive use of broad-spectrum antifungal therapy with voriconazol is considered as a predisposing factor, but exact data are difficult to obtain. In Leiden, only 6 patients with mucormycosis were diagnosed in a four years period (1-2-2003 to 1-2-2007). This is still a low incidence rate in comparison to 40 patients with definite or probable invasive aspergillosis. In the same period, 2 patients were diagnosed with Scedosporiosis. Of the patients with mucormycosis, 1 patient had gastrointestinal mucormycosis, 1 patient had an intracardial thrombus from which moulds were cultured, 1 patient had disseminated mucormycosis, and three patients had pulmonary mucormycosis. Remarkably, the three patients with pulmonary mucormycosis all had also pulmonary aspergillosis. Three patients died; one patient with pulmonary mucormycosis after near-drowning survived, and from two patients follow-up is not yet available.

O124

Detection of *Exophiala dermatitidis*, an emerging brain pathogen

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The origin of fatal, cerebral pathology of the black yeast *Exophiala dermatitidis* is still a mystery. Cases by black yeasts in young, otherwise perfectly healthy individuals are diagnosed nearly each year in East Asia, while this clinical syndrome is unknown in the Western world. Large-scale selective isolation of *E. dermatitidis* has shown that the fungus is present in the natural environment in association with frugivorous animals in the tropical rain forest. It is also very common in Turkish steam baths, and in polluted environments rich in alkanes and alkylbenzenes in the tropics.

Two preponderant genotypes are known, recognizable by anonymous ITS markers, which seem to have a different ecological preferences and predilection: nearly all (14) systemic, non-pulmonary cases were ITS genotype A, while genotype B was relatively more common in the natural environment. In order to reveal the route of transmission and infection of *E. dermatitidis*, rapid screening using RFLP was developed, for which digestion with *Taq* was sufficient. Subsequent AFLP typing generally revealed a large degree of homogeneity at a single positive site, showing that reaching a site that allows growth and reproduction is a relatively rare event. This underlines the uniqueness of contaminated sites, despite their apparent differences.

O125

Emerging *Aspergillus* species resistant to current antifungals

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Invasive aspergillosis is a lethal infectious disease which occurs mainly in immunocompromised patients. In 2002 voriconazole was shown to be more efficacious than amfotericin B for the primary treatment of invasive aspergillosis, and since then the drug is commonly used for this indication. Since 2002, we observed an increase of the number of *Aspergillus fumigatus* isolates with elevated MICs of voriconazole (2 to >16 mg/l), itraconazole (>16 mg/l), the investigational azole ravuconazole (4 to >16 mg/l) and posaconazole (0.5 to 1 mg/l). Thirteen isolates were cultured from 9 patients from six hospitals in the Netherlands. Four patients were azole-naïve and developed primary invasive aspergillosis, while in five patients breakthrough invasive aspergillosis was diagnosed during azole prophylaxis or therapy. A novel mechanism of resistance to azole drugs, consisting of a *Cyp*51A amino acid substitution at codon 98 (L98H) together with a tandem repeat in the gene promoter, was found to be responsible for the azole cross-resistant phenotype. This resistance mechanism was present in 12 of 13 isolates. Genotyping of the isolates showed no evidence for clonal spread of a single resistant *A. fumigatus* genotype. The prevalence of resistance was compared with a previously conducted nation-wide survey of 170 *A. fumigatus* isolates collected from 114 patients from 21 Dutch hospitals between 1945 and 1998. In this period no patients with azole cross-resistant isolates were found as compared to 10 of 81 patients in the period since 2002 (p=0.0001). Although the emergence of this new resistance mechanism coincides with the approval of voriconazole, the factors that may explain this phenomenon remain unclear. Our observation underscores the need to make an etiologic diagnosis of invasive mould infection and to determine antifungal drug activity in clinically relevant *A. fumigatus* isolates. Furthermore, international surveillance programs are warranted to investigate the spread of resistance in *A. fumigatus*.

O126

The antifungal effect of amphotericin B, itraconazole, voriconazole and caspofungin on conidia and hyphae of *Aspergillus fumigatus*

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Introduction: For filamentous fungi the predictive significance of *in vitro* antifungal susceptibility testing for *in vivo* antifungal efficacy remains unclear. In *in vitro* tests a conidial suspension is used as fungal inoculum. Exposure of a hyphal suspension might better reflect the fungal status in infected tissues. The application of the quantitative XTT assay measuring an inhibition of fungal metabolic activity allows susceptibility testing of fungal hyphal suspensions.
Methods: In the present study the comparative antifungal effect of amphotericin B (AMB), itraconazole (ITZ), voriconazole (VZ) and caspofungin (CAS) towards a panel of clinically-relevant *Aspergillus fumigatus* isolates was determined. *A. fumigatus* in the conidial form or hyphal form (homogeneous suspension at low density versus inhomogeneous suspension at high density) was exposed to the antifungal agents. Decrease in metabolic activity was assessed by the XTT assay, killing of fungi was assessed by subculture onto solid media.
Results: There appeared to be striking differences in the antifungal potential of the various antifungal agents. AMB showed a sharp concentration-dependent inhibition of metabolic activity for the conidial and both hyphal forms. The inhibitory concentration (IC) for the conidia and the homogeneous hyphae were in agreement for 92.3% of the isolates. The inhomogeneous hyphal suspension was also inhibited in metabolic activity, however at higher concentrations of AMB for all isolates. AMB killed both the conidial and the homogeneous hyphal suspension at one to two steps higher concentrations while the inhomogeneous hyphal suspension was not killed at all. For both azoles a more gradual inhibition of fungal metabolic activity was observed. Again the ICs obtained for the homogeneous hyphal suspension were comparable to those obtained for the conidial suspension. Both azoles were not able to inhibit the metabolic activity of the inhomogeneous hyphal suspension. The concentration needed for fungal killing differed for both azoles, but were in all cases much higher than the ICs. ITZ had a higher killing potency against the conidia while VZ had a higher killing potency against the homogenous hyphae. Both azoles were not able to kill the inhomogeneous hyphal suspension. CAS inhibited *A. fumigatus* in a unique manner, since the inhibition was not gradual concentration-dependent. For conidial suspensions of each isolate a concentration-dependent decrease of metabolic activity was observed, however a further increase in concentration resulted in an increase in metabolic activity, whereas at very high concentrations the metabolism dropped to non-detectable levels. The activity of CAS towards hyphae was inconsistent. Fungal killing capacity by CAS was never observed.
Conclusion: AMB, ITZ, VZ and CAS inhibit *A. fumigatus* in distinct manners. Whereas both AMB and the azoles had antifungal activity against *A. fumigatus* conidia as well as hyphae in homogeneous suspension, AMB was unique in being the only agent that inhibited the inhomogeneous suspension of hyphae at high density. The lack of antifungal activity of the azoles and CAS towards the inhomogeneous hyphal suspension may indicate that these agents are less able to penetrate into a hyphal clumb.

O127

Markers specific for zygomycetes detected in BAL fluid of patients with suspected invasive fungal infection

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Objectives: The incidence of invasive zygomycosis (IZ) appears to be increasing, especially in patients treated for haematological malignancy. At present, no biological markers exsist that facilitate early diagnosis, and one has to rely on conventional diagnostic methods, such as culture, which lack sensitivity. We investigated the presence of zygomycete-antigen (zAg) and zygomycete DNA in bronchoalveolar lavage fluid (BALF) of patients with documented IZ and of those at risk for this disease.
Methods: BALF of 18 neutropaenic patients, who underwent a bronchoscopy on suspicion of invasive fungal infection (IFI), was investigated for the presence of water-soluble somatic zAg by immunoblotting with a commercially available monoclonal antibody (anti-Rhizomucor, Dakocytomation, Denmark). Five of 18 patients had proven IZ. BALF from 10 non-haematology patients was used as control. BALF samples were also investigated for presence of zygomycete DNA by PCR, using 18S primers.
Results: The BALF of the 5 patients with proven IZ were all positive for zAg. In the remaining 13 neutropaenic patients, zAg was detected in BALF of 7. None of these patients had previous culture results suggestive of zygomycosis. The number of zAg positive BALF from neutropaenic patients with suspected IFI was higher than in the control group (7 of 13 versus 1 of 10, $p<0.002$). Zygomycete DNA was detected in 11 of 12 BALF samples positive for zAG, and all BALF samples negative for zAg were also negative for zygomycete DNA by PCR.

Conclusion: zAg and zygomycete DNA are present in BALF of patients with IZ, and might be a useful tool for early diagnosis. The presence of both markers in BALF samples from high-risk patients without a clinically manifest disease might indicate the presence of colonisation or subclinical infection.

O128

Comparison of interferon-gamma assays and TST results and the use in the routine laboratory diagnosis of latent or clinical tuberculosis

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Background: Recently, two commercial alternatives for the classical Mantoux test came available for diagnosing (latent) tuberculosis (TB). We compared the QuantiFERON-TB GOLD (QFT-G) and T spot-TB (SPOT) assays with TST results and determined the value of the QFT-G in the laboratory diagnostic process after one and a half year of routine use.

Methods: 157 patients were included in the comparison of GFT-G and SPOT. For SPOT, 8 ml of blood was drawn, peripheral blood mononuclear cells were separated and interferon-gamma spot forming cells were counted after T-cell stimulation with ESAT-6 and CFP-10. For QFT-G, 2 ml of blood was drawn and after stimulation of T cells in whole blood, interferon-gamma release was detected in the plasma by EIA. Both tests were compared for 98 patients in an additional comparison with TST test results in different categories according to cut off points and BCG vaccination status. So far, 398 QFT-G assays have been carried out routinely.

Results: QFT-G and SPOT tests were concordant in 144 out of 157 cases with 34 positive and 110 negative cases (94% agreement, kappa 0,87) whereas SPOT assay was the only positive test in 8 cases and QFT-G in 5. In 23 of 27 TST negative cases and in 45 TST positive cases both assays were negative, while the overall agreement was 46% (kappa 0.13). In 398 routinely performed QFT tests, 79 showed a positive result (19.8%). Remarkably, 17 subjects showed a QFT result just below the cut-off of 0.35 but much higher than the average negative test value. An in depth analysis of these subjects will be shown.

Conclusion: 1) The overall agreement between the results of QFT-G and SPOT assays is good. 2) A poor overall agreement was seen in the results of the comparison of the QFT-G and SPOT assays separately as well as combined with TST results according to the Dutch guidelines. 3) The results of the gamma interferon tests were not influenced by the BCG vaccination status in our study population. 4) The QuantiFERON-TB GOLD and T spot-TB assays were negative in a surprisingly large proportion of participants within the subset of subjects with a TST ≥15 mm. 5) A large proportion of subjects with QFT values just below the cut off had clinical or historical indications of TB or *Mycobacterium kansasii* infection, suggesting that the manufacturers cut-off value may be fixed to high.

O129

Interferon-γ release assays versus tuberculin skin test for detection of latent TB

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The tuberculin skin test (TST) has been used for the detection of *Mycobacterium tuberculosis* (MTB) infections for the past century. An important limitation of the TST is its low specificity due to cross-reactive immune responses to BCG or environmental mycobacteria. The new interferon-gamma release assays (IGRA) have been specifically designed to overcome this problem by measuring the immune response to MTB-specific antigens. Two such antigens, ESAT-6 and CFP-10, were found to be sensitive as well as specific for the diagnosis of TB disease. The commercially available IGRA, QuantiFERON-TB Gold (QFT-G) and the T-SPOT™ TB, are based on these antigens. Recent tuberculosis guidelines from the CDC recommend that QFT-G may be used in all circumstances in which the TST is currently used, and both tests are included in the UK (NICE) guidelines. However, evaluation and comparison of IGRA for detection of latent MTB infections is complicated by the lack of a gold standard and the level of agreement between TST and IGRA varied widely between studies. In contact investigations in low-endemic regions, IGRA correlated better to the level of exposure, as a surrogate marker for infection, than did TST. Discrepancies between the assays were mostly attributed to prior BCG vaccination. Among BCG-unvaccinated, close contacts a good agreement was observed between QFT-G and TST. Other studies from high-endemic regions show somewhat more contradictory results. In India, a good agreement between TST and QFT was reported despite high BCG prevalence. A study in the Gambia even indicated that TST correlated better to exposure than ELISPOT. Our data, from a large contact investigation and a study among persons with remote exposure to TB, show that a substantial group of BCG-unvaccinated persons with TST of ≥15 mm have negative results in QFT and ELISPOT. The observed differences between studies are most likely related to differences in study populations, such as degree of BCG vaccination, prevalence of MTB and recent contacts

versus past infection. The optimal diagnostic test for latent MTB infection therefore depends on the clinical and epidemiological setting. Data from various studies, including our recent studies evaluating IGRA in different settings will be discussed.

O130

Paradoxical reactions in tuberculosis

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Clinicians should be alert to the possibility of transient clinical or radiographic worsening (paradoxical reactions), despite appropriate tuberculostatic therapy that would eventually result in cure. Examples of this include ongoing inflammation at sites of lymphadenitis, worsened abnormalities on chest radiographs after several months of treatment, or the new appearance of pleural effusions during therapy for pulmonary tuberculosis.
Symptoms and signs may include high fevers, lymphadenopathy, expanding central nervous system lesions, and worsening of chest radiographic findings.
When these symptoms occur at the end or after finishing the tuberculostatic therapy the most pertinent question to ask is whether the manifestations are a paradoxical reaction or active tuberculosis with, in the latter case, the possibility of an (acquired) resistance for tuberculostatic drugs.
Reconstitution syndrome (Immune Reconstitution Inflammatory Syndrome)
(HA)ART restores immune responses (usually only partly) to *Mycobacterium tuberculosis*. A group of ART-treated patients will exhibit paradoxical deterioration despite satisfactory control of viral replication and improvements in CD4 lymphocyte counts. This clinical deterioration, known as immune reconstitution inflammatory syndrome (IRIS) is a result of an exuberant inflammatory response towards incubating opportunistic pathogens.
The differential diagnosis includes progressive disease due to non-adherence with treatment, drug- and multi-drug-resistant mycobacterial infection, adverse drug reactions, intercurrent opportunistic infections and malignancy.
A variety of manifestations of IRIS have been described, here we will discuss the manifestations of MTB. IRIS is (by definition) only seen in patients with AIDS but the manifestations in patients with an immune reconstitution (for instance after stopping immunosuppressive therapy like Infliximab) are exactly the same.
It is important for clinicians to recognise paradoxical tuberculous reactions and IRIS as inflammatory responses to treatment, and to understand that they do not necessarily indicate drug resistance or an inadequate response to therapy. Anti-tuberculosis and antiretroviral drug regimens need not be altered or discontinued, although corticosteroids may be useful in reducing inflammation.
Several examples (paradoxical and IRIS) from the clinic will be discussed.

O131

Positive blood culture with *Plasmodium falciparum*

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Introduction: In travellers returning from the tropics with fever, the differential diagnosis should include malaria and typhoid fever. Blood cultures should be drawn, even if the diagnosis malaria has been confirmed, since concurrent malaria and typhoid fever may occur. In patients with *Plasmodium falciparum* infection, high parasitaemia may result in 'false' positive blood cultures, as we show in this case.
Methods: Case report including microscopic pictures.
Results: A 53-year-old man presented with fever, malaise, and headache after travelling to Sierra Leone without taking malaria prophylaxis. Laboratory results showed slight anemia (hemoglobulin 8•0 mmol/L), leucopenia (white blood cell count 3•2 x 109/L with 2% atypical lymphocytes), severe thrombocytopenia (platelet count 23 x 109/L), unconjugated hyperbilirubinemia, and mildly elevated LDH, ASAT and ALAT (348 U/L, 41 U/L and 50 U/L, respectively). One blood culture set was drawn on admission and thin blood smears were performed for detection of malaria. The latter showed many young trophozoites of *P. falciparum*, with parasitaemia being 10%. Severe malaria tropica was diagnosed and antimalarial therapy with quinine intravenously (10mg/kg TD) and doxycycline orally (100mg BD) was initiated. After 24 h of incubation the BacT/Alert blood culture system flagged the anaerobic blood culture positive. A Gram stained smear of the anaerobic bottle showed no bacteria, but round intra-erythrocytic structures, some with yellow-brown pigment. A Giemsa stained smear demonstrated numerous pigmented mature trophozoites and few young trophozoites of *P. falciparum*. Aerobic and anaerobic subcultures of the anaerobic bottle showed no growth. The triggering of the blood culture system is most likely explained by an increase in CO_2 due to growth of *P. falciparum* in the blood culture. After 3 days of quinine and doxycycline, treatment was continued with atovaquone/proguanil orally (1000/400mg OD) for 3 days. The patient was discharged in good clinical condition 7 days after admission.
Conclusion: We describe a case in which high parasitaemia with *P. falciparum* resulted in a 'false' positive blood culture.

O132

Thoracic actinomycosis, presenting as a subcutaneous abscess

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Introduction: Actinomyces is a human commensal that is found in the oropharynx, colon and vagina. Infection occurs after disruption of the mucosal barrier, mostly secondary to dental procedures and/or surgery. Actinomyces israelii is the most frequently encountered species and gives rise to slowly growing polymicrobial abscesses typically progressing across tissue boundaries. Thoracic actinomycosis is a rare complication, usually secondary to aspiration of Actinomyces spp. It is characterized by formation of abscess and fibrosis in pulmonary or pleural tissue, mediastinum or chest wall.
Case: A 27-year-old woman, without further medical history, was referred because of chest pain and progressive parasternal swelling. She had noted the swelling 2-3 months ago, and it had since then slowly increased in size. She had no pulmonary complaints, fever, chills or weight loss. Two months before start of symptoms, she was treated by the dental hygienist for gingivitis and underwent an extensive dental cleaning procedure. One month before presentation she had received a short antibiotic course of flucloxacillin, which did not reduce symptoms. Physical examination showed a firm, painful, slightly fluctuating, left parasternal mass involving 3 ribs. There was no fever, no lymphadenopathy, and physical examination was otherwise normal. Laboratory findings revealed elevated inflammatory parameters: ESR 121 mm, CRP 381mg/L, leukocytosis (15.6 x 109/L), normocytic anaemia (Hb 5.8 mmol/L) and thrombocytosis (896 x 109/L). CT-scan of the thorax showed a large soft tissue mass around the third costo-sternal joint with destruction of 3 ribs, and extension into the anterior mediastinum, subcutaneous fat, and into the pleura and parenchym of the left upper lobe. Differential diagnosis included infection and malignancy. Fine needle aspiration of the subcutaneous swelling yielded purulent fluid. Gram staining showed many filamentous gram-positive, branched rods. Under the suspicion of actinomycosis, antibiotic therapy was started with penicillin 18MU/day intravenously. Culture yielded Fusobacterium necrophorum, Haemophilus aphrophilus, and a poorly growing, anaerobic gram-positive branched rod suspected for Actinomyces. Species determination was initially hampered by its poor growth, but was finally established as Actinomyces israelii, which was confirmed by sequence analysis of the 16S-RNA gene. Therapy was adjusted to amoxicillin 12gr/day IV to include treatment of all accompanying pathogens. Within days clinical and radiologic improvement was observed and inflammatory parameters normalized. Antibiotic therapy was continued intravenously for a total of 6 weeks, followed by oral treatment with doxycycline 2x100mg/day, planned for a total of 3 months. Patient is still under outpatient control and doing well during 2 months of follow up.
Conclusion: This case illustrates a typical thoracic infection with Actinomyces israelii, which requires prolonged antibiotic treatment. Diagnostic delay can be significant because symptoms can be unspecific, and may mimic malignancy. Awareness of this rare infection is essential to establish early diagnosis in order to start adequate antibiotic therapy as soon as possible.

O133

Retrospective analysis of 13 cases of extraintestinal *Campylobacter* disease and literature review

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Introduction: *Campylobacter* species are one of the most frequently isolated micro-organisms from the feces of patients with diarrhea, but are rarely isolated from extra-intestinal sites. The aim of this study was to analyse clinical and bacteriologic features of extraintestinal *Campylobacter* disease.
Methods: Cases of culture-confirmed extraintestinal *Campylobacter* disease at the University Medical Center Groningen from 1997 to 2007 were analysed retrospectively. A survey of the English language literature was undertaken to identify reports about the extraintestinal *Campylobacter* diseases found in our patients.
Results: Thirteen patients with culture-confirmed extra-intestinal *Campylobacter* disease were identified in our hospital. *Campylobacter jejuni* was the predominant species isolated. Seven patients had *Campylobacter* bacteremia. The other six patients had rare extraintestinal *Campylobacter* diseases; one patient had cholecystitis with *C. jejuni*, one patient had an intraorbital abscess with *C. showae*, two patients had brain abscesses with respectively *C. concisus* and *C. gracilis*, one patient had a vertebral abscess with *C. rectus*, and one patient had *C. jejuni* peritonitis. The most common underlying medical condition was hematologic disease. Four patients died with a cause directly related to their extraintestinal *Campylobacter* disease.
A variety of clinical extraintestinal *Campylobacter* diseases was reviewed from the literature. Twelve published cases of *Campylobacter* cholecystitis were found. No case-reports of *Campylobacter* orbital abscesses were detected. One of the reviewed published case reports of a *Campylobacter* brain abscess concerned a patient with a post-neurosurgical status, like one of our two cases with brain abscesses. All

three reviewed cases of *Campylobacter* spondylodiscitis and/ or vertebral osteomyelitis were caused by *C. fetus*, unlike our case.
Conclusion: We report 13 cases of uncommon extraintestinal *Campylobacter* disease, among them never published cases.

O134

A multidisciplinary approach to control an outbreak of MRSA in a long-term care facility for mentally disabled persons

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Introduction: A 30-year old woman with diabetes mellitus was admitted to a hospital because of a burn wound. During her stay, an outbreak of MRSA type 15 in the hospital was detected, and MRSA was isolated from her wound. After discharge, she returned to her home; a 6-persons' unit that was part of a long-term care facility for mentally disabled persons.
Methods: In the unit, at first hygienic interventions like hand hygiene and contact isolation were limited mainly to procedures with regard to care for the burn wound. Post-exposure screening cultures for MRSA were obtained from healthcare workers and from inhabitants of the unit who frequented hospitals as patients. After dissemination of MRSA had been noticed, an outbreak management team was formed with members of the nursing and medical staff of the institute, an infection control practitioner, a clinical microbiologist, the occupational health physician, and the municipal health service. Specific infection control interventions were formulated; MRSA screening was extended to all inhabitants and staff members of the unit of the index-patient as well as to contacts among staff and inhabitants of other units of the institute. MRSA-positive inhabitants and staff were temporarily not allowed to visit other units of the institute. All inhabitants and staff in the unit of the index-patient, whether or not they were found positive, were subjected to a simultaneous 5 days' MRSA decolonization therapy with mupirocine nasal ointment and chlorhexidine body and hair washing. The last day of therapy the unit was subjected to a thorough disinfection procedure, while the inhabitants enjoyed an excursion in the countryside. Afterwards, 3 sets of follow-up cultures were obtained from all persons.
Results: 15 health care workers and 6 co-inhabitants of the unit were screened for MRSA-carriage, of whom 3 (20%) and 3 (50%) were found MRSA-positive, respectively. Subsequently, 109 additional contacts (67 healthcare workers and 42 inhabitants of other units of the institute) were screened for MRSA-carriage and they were all found negative. The formerly mentioned 21 persons were subjected to decolonization therapy and all of them were found negative after finishing therapy and in follow-up cultures. In the meantime, the wound of the index-patient was healed and her MRSA-carriage was treated successfully with the above-mentioned MRSA decolonization therapy supplemented with trimethoprim-sulphamethoxazole and rifampicine.
Conclusion: Outbreaks of MRSA are not only observed in hospitals; they may also take place in chronic care facilities and disturb daily practice of inhabitants and staff. Within this institute, dissemination of MRSA was restricted to the unit where the index-patient lived despite frequent exchange of staff and contacts with inhabitants from other units. In a setting where both knowledge of infection control among staff members and compliance of hygienic interventions among inhabitants are not optimal, a multidisciplinary approach maybe successful in the fight against MRSA.

O135

A rare cause of prosthetic hip infection with unexpected consequences

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Case 1: A 76-year-old man presented with signs of loosening of the prosthesis one year after total hip replacement for symptomatic coxarthrosis. At surgical revision intraoperative frozen-section analysis of periprosthetic tissue demonstrated chronic infection and necrotizing granulomas. The prosthesis was removed and a gentamicin-impregnated cement spacer was inserted. Gram stain and microscopy for acid-fast bacilli (AFBs) of surgical specimens were negative as well as routine microbiology cultures. *Mycobacterium tuberculosis* (MTB) was isolated after 3 weeks incubation. Chest radiography did not show any signs of past or active pulmonary tuberculosis. Treatment with anti-tuberculosis drugs was started.
At the time of primary prosthesis surgery this patient had no history of tuberculosis or BCG vaccination. With a negative history of tuberculosis, and negative serology for HIV, HBV, HCV, HTLV-1, and syphilis, the patient fulfilled the criteria for donation of the resected femoral head.
Case 2: The femoral head of patient 1 was used as a bone-graft in a 20-year-old woman with juvenile rheumatoid arthritis who underwent total hip replacement. She already had received two knee prosthesis and a hip prosthesis on

the right side. Two months post-operatively, there was evidence of infection of the left hip prosthesis with pain, poor function, and a persistent wound or possibly a fistula. Because of the patient's condition, the infection was initially treated conservatively with levofloxacin and rifampin. However, after 10 days of treatment there was progression of infection with fever and purulent discharge from the wound. Antimicrobials were stopped and surgical debridement followed with insertion of gentamicin beads. Routine cultures from surgical specimens remained negative. Finally, 4 months after the total hip replacement, the prosthesis was removed because of persistent pain, fever, and dislocation of the cup resulting in a Girdlestone resection arthroplasty. Histology from the synovial membrane showed chronic active infection with granuloma formation but no AFBs. Coagulase-negative staphylococci were cultured from multiple specimens and the patient was treated for 3 months with teicoplanin and doxycyclin. When, 4 months after removal of the prosthesis, mycobacterial culture results from patient 1 became available there was a satisfactory situation following the Girdlestone procedure with no signs of infection. No archived material was available for culture. Additional histology sections did not show AFBs but PCR for MTB was positive on formalin-fixed paraffin-embedded sections and treatment for tuberculosis was initiated.

Conclusion: We present a case of a prosthetic hip infection caused by MTB. The femoral head resected from this patient at primary prosthesis surgery was used for donation. Transmission of MTB occurred via the bone allograft, which has not been described since 1953.

O136

Systemic cryptococcosis presenting as a solitary skin lesion in an organ transplant recipient on tacrolimus therapy

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Introduction: *Cryptococcus neoformans* is an opportunistic fungal pathogen that can give rise to meningo-encephalitis and disseminated infection in immunocompromised hosts. Cutaneous cryptococcosis may occur following traumatic inoculation, but may also be a sign of systemic cryptococcosis. Tacrolimus is an immunomodulatory drug which also has antifungal activity, and reaches high levels in CSF.

Methods: We describe an organ transplant recipient on tacrolimus therapy who presented with a solitary cutaneous cryptococcal lesion, but turned out to have systemic cryptococcosis.

Results: The patient, a 30 years old male, had received a liver-transplant 1 year prior to consultation because of auto-immune hepatitis and primary sclerosing cholangitis. He had been on tacrolimus (4 mg every 12 hours) and prednison (10 mg every 24 hours) since transplantation. In addition, he had a longstanding history of psoriasis vulgaris which was currently being treated with topical steroids, but was responding. He presented to the dermatologist because he had noted, since 2 months, a slowly growing, nodular lesion on the frontal side of his right upperleg. The patient had no recent history of trauma or puncture of the skin. Physical examination showed a purple-reddish, indurated, nodular lesion of about 6cm by 3cm. There was no local lymphadenopathy. The patient did not have any systemic symptoms and/or signs of infection, and had no fever. Laboratory examination showed: leucocytes 5.7x109/l, with normal differentation, CRP <5mg/l, CD4 count: 190x106/l, and normal liver and kidney function. Histopathologic examination of a puncture biopsy specimen showed deep cutaneous inflammation with abcess-formation. Staining using blancophore showed yeasts of irregular shape and size. Culture of biopsy specimens yielded Cryptococcus neoformans. Susceptibility testing showed MICs of: 0.031mg/l for itraconazole and voriconazole, 0.5mg/l for amphotericin B, and 4mg/l for fluconazole and flucytosine. Because the patient was on tacrolimus therapy, he was suspected for systemic cryptococcosis with a mitigated course. Blood and cerebrospinal fluid was cultured but yielded no cryptococcus. Cryptococcal antigen detection in blood showed a positive titer of 1:4, to which the patient was diagnosed with systemic cryptococcosis. Antifungal treatment was started with oral fluconazol (800mg every 24 hours) for 8 weeks, followed by 400mg every 24 hours. Fluconazol therapy was continued for a total period of 1 year. Follow-up investigation after 5 months of therapy showed a significant reduction of the lesion. No additional symptoms of systemic cryptococcosis had occurred.

Conclusion: Disseminated cryptococcosis in organ transplant recipients who receive tacrolimus therapy can follow a subclinical course without symptoms of meningitis. This mitigated course is due to the antifungal effect of tacrolimus. Clinicians should be aware of a mitigated course of systemic cryptococcosis in organ-transplant patients on tacrolimus therapy.

O137

High acquisition rates of ampicillin-resistant CC17 *E. faecium* (ARE) during hospitalization in a Dutch hospital

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Background: Enterococci are generally considered avirulent gut commensals. In the *Enterococcus faecium* population structure, based upon MLST, epidemic and most invasive isolates cluster in clonal complex-17 (CC17). In the last

decade infections (and colonization) with ARE increased in our hospital. We aimed to quantify admission and acquisition rates and to determine molecular epidemiology of ARE-colonization in 2 wards, where colonization was endemic.

Methods: From November '05 to February '06, all admissions on hematology (ward 1) and gastroenterology/ nephrology (ward 2) were screened for rectal ARE-carriage within 24 hrs after admission and within 72 hrs before discharge. Swabs, enriched in Enterococcal Broth, were cultured on Enterococcosel agar plates enriched with ampicillin (16 µg/ml). All ARE isolates were typed with Multi-Locus Variable-Number Tandem Repeat Analysis (MLVA).

Results: Of 358 admissions, 210 (59%) and 148 (71%) were screened on admission and before discharge, respectively. On weekdays 70% was included. Admission in weekends (n=32) on holidays (n=31) and length of stay of less than 3 days (n=61) were the main reasons of failure to screen on admission and therefore exclusion. ARE admission prevalence was 12% (16% and 10% in ward 1 and 2) and associated with 1 or more admissions in the past year (OR 5.0, 95% CI 1.8-14.0). Acquisition rate was 24.4%: 39.2% and 15.0% on wards 1 and 2. Five of 57 (9%) colonized patients developed invasive ARE-infection (with 3 bacteremias in 28 neutropenic patients). MLVA-typing revealed 12 different genotypes, all predicted to belong to CC17, with 2 (1 and 159) responsible for 94% of acquisitions. Because of epidemiological patient linkage and the absence of an ARE community-reservoir, exogenous transmission was considered the dominant acquisition route. In multivariate analysis β-lactam (OR 2.7, 95% CI 1.1'6.7) and quinolone (OR 3.1, 95% CI 1.1'8.2) use and hospital stay >7 days (OR 2.1, 95% CI 0.8'5.4) were risk factors for ARE-acquisition.

Conclusions: In the studied wards, ARE-epidemiology is characterized by high admission (12%) and acquisition (24%) rates of CC17 ARE. Exogenous transmission, readmission and antibiotic pressure (β-lactam and quinolone antibiotics) are the main forces of this hospital-wide outbreak. The infection attack-rate of 9% in immunocompromised patients challenges the opinion that enterococci are avirulent.

O138

Dissemination of *Bacillus cereus* in a pediatric intensive care unit traced to non-disposable ventilator air-flow sensors

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Introduction: Over a period of 2 weeks in November 2006, six pediatric intensive care unit (PICU) patients were treated with meropenem for suspected *Bacillus cereus* pneumonia. An investigation was initiated to reveal the cause of this unusually high incidence of nosocomial *Bacillus cereus* isolates.

Methods: In November en December 2006, surveillance cultures were obtained from respiratory tracts of all PICU patients and from their environment, including all airflow guiding mechanical ventilation equipment. Molecular typing of *B. cereus* isolates was performed using AFLP analysis. From 2003 through 2006, the incidence of *B. cereus* isolates from PICU patients was evaluated retrospectively.

Results: The retrospective analysis revealed an increasing incidence of *B. cereus* isolated from PICU patients from 2003 through 2006 with a peak incidence November 2006. Using AFLP, *B. cereus* strains from the 6 PICU patients in November 2006 were shown to be highly related, suggesting a common source of transmission. Upon subsequent surveillance *B. cereus* was detected in respiratory samples from 4 additional PICU patients, on non-disposable airflow sensors and in a 70% alcohol solution which was improperly used for on-site decontamination of the sensors. The AFLP patterns of these surveillance isolates were indistinguishable from those of the 6 patients. Control measures, including adequate cleaning and sterilization process for all non-disposable parts used for mechanical ventilation and the use of disposable airflow-sensors for each patient, resulted in a rapid termination of the outbreak.

Conclusion: 1) The cause of the *B. cereus* outbreak on the PICU was traced to contaminated non-disposable air-flow sensors and a 70% alcohol solution used for on-site decontamination of these sensors, 2) Cleaning and sterilization procedures for non-disposable air-flow guiding ventilation equipment should include the decontamination of spore forming microbes such as *B. cereus* species, particularly with respect to patients at high risk for opportunistic infections.

O139

Transmission of highly resistant *E. coli* (HR-EC) between patients in a Dutch hospital: does the WIP guideline for highly resistant micro-organisms work?

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Background: The Dutch Working Party on Infection Prevention (WIP) issued a guideline on the prevention of nosocomial transmission of highly resistant micro-organisms (HRMO). This guideline defines HR-EC and provides recommendations for isolation of patients, active surveillance and contact tracing.

Objective: The objective of this study was to determine the incidence of HR-EC and to determine how often transmission between patients occurred.
Method: Between 2005, January first and 2006 June 31 all HR-EC from hospitalized patients in the Amphiahospital were included. The definition for HR-EC according to the WIP (www.wip.nl) was used. From all patients at least one isolate was selected for genotyping using Amplified Fragment-Length Polymorphism (AFLP). When multiple isolates of HR-EC were found in one patient the selection was made based on the susceptibility pattern. All isolated with major (S<>R) differences in susceptibility for amoxicilline-clavulanic acid, cefalosporines, quinolones, meropenem, aminoglycosides or sulfa-trimethoprim were included. When the AFLP-patterns were at least 90% identical the isolates were considered genetically related. From each patient the following data were collected: admission date, discharge date and the wards were the patients had been during their stay. Epidemiological linkage was defined as two patients who had been in the same hospital ward with a maximum time window of 4 weeks. The number of bed days was collected from the hospital administration.
Results: Ninety-five HR-EC were found in 70 patients (incidence density (ID): 1.8 patients per 10,000 bed days), and 86 of those were available for typing. Eight HR-EC could not be typed. In the remaining 78 HR-EC, 58 different geno-types were found. From 10 patients multiple HR-EC, with differences in susceptibility, were included. In half of these patients the isolates were genetically related. There were 8 clusters with a total of 22 patients involved. Incorporating epidemiological linkage and AFLP analysis, there were 7 patients from 4 clusters in whom HR-EC was considered to be caused by nosocomial transmission (ID: 0.2 per 10.000 bed days).
Conclusion: From all HR-EC that were typable only 9% were caused by nosocomial transmission. The recommendations to prevent the spread of HR-EC in the hospital seem to work considering the very low ID of nosocomial transmission. The majority of the HR-EC are probably caused by selection of pre-existing resistant sub-populations under antimicrobial pressure or by the spread of mobile genetic elements. The relative contribution of these two warrants further investigations.

O140

Presence of highly resistant micro organisms (HRMOs) in long term care facilities in the Twente, eastern Achterhoek and Friesland regions

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Objective: to determine the presence of HRMOs in clinical material from patients in Long Term Care Facilities (LTCF).
Introduction: Recently a rise in the prevalence of HRMO's has been observed in the Dutch hospitals. A proportion of these patients is already infected or colonised by these HRMOs at admittance. The reservoir of these HRMOs remains unclear but it is postulated that Long Term Care Facilities might be a source for the spread of these organisms. This study was done to estimate the presence of these HRMOs in Long Term Care Facilities in Twente, the eastern Achterhoek and Friesland.
Methods: From July until november 2006 urine, feces, sputum samples and woundswabs obtained for standard bacteriological analysis offered to the laboraties in Enschede, Twente and Leeuwarden, Friesland from LTCF's were collected. These samples were screened for methicillin resistant *Staphylococcus Aureus* (MRSA) and *Vancomycin Resistant Enterococcus* (VRE), using isolation and enrichment broths and chromogenic media, following standard procedures according to Clinical Laboratory Standards Institute (CLSI) guidelines. Extended Spectrum Beta Lactamase (ESBL) producing enterobacteriaceae were screened for using a vancomycin (4 mg/l) and amoxicillin/clavulate acid (32/8 mg/l) containing enrichment broth and a 3mg/ml vancomycin containing CLED medium. Suspection for HRMOs was confirmed following CLSI standards.
Results: A total of 211 samples were analysed. Of these samples 2 (0,95%) contained an ESBL producing *Escherichia coli*, 2 (0,95%) contained a VRE, 1 (0,48%) contained a MRSA. One co-trimoxazole resistant *Stenotrophomonas maltophilia* was found.
Conclusion: Based on our observations, only a small number of samples obtained from residents of LTCFs contained HRMOs. This study does not confirm recently expressed concerns about LTCFs serving as a reservoir for HRMOs. Its findings are in agreement with previous studies from the Netherlands but contrast with several more recently conducted European studies showing a marked increase of HRMOs in LTCFs.

O141

High prevalence of integron class 1 in highly resistant Enterobacteriaceae (HRE)

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Nowadays, hospitals have to cope with highly resistant micro-organisms (HRMO) more often. Although the Dutch strategy for combating the emergence and spread

of antibiotic resistance is effective, we can not protect our self from the importation of resistant pathogens through travel and trade.
In the Netherlands there are national guidelines to control the emergence and spread of antimicrobial resistance. These guidelines cover the use of antimicrobial agents and infection control measures. Essentially infection control measures focus on the prevention of clonal dissemination of HRMO. However, this is not including the spread of mobile DNA elements, such as integrons, which play an important role in the dissemination of antibiotic resistant genes among Gram-negative bacteria.
In this study, we investigated all HRE (n=154) isolated from patients admitted to the Amphiahospital, Breda, the Netherlands during 18 months in 2005 and 2006. Amplified fragment length polymorphism analysis revealed that almost all patients contained a unique HRE. Three strains (*Escherichia coli*) were found in more than 1 patient. Subsequently, PCR analysis showed that (62.5%) of the HRE contained the *intI1* gene of integron class 1. In addition, we amplified and characterized the gene cassettes of integron class 1 positive strains. This revealed 21 unique gene cassettes of which 2 cassettes were most prominently found. Interspecies transfer of integron class 1 (n=8) was found within as well as between patients showing that integrons play an important role in the dissemination of antibiotic resistance genes among *Enterobacteriaceae*.
This study shows that the strategy to prevent clonal dissemination of HRE is highly effective. However, this strategy does not prevent intra- and interspecies transfer of mobile DNA elements.

O143

IT: implications of a national electronic patient record (EPR) in the Netherlands

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Along with many healthcare organizations, the National IT Institute for Healthcare in the Netherlands (NICTIZ) is working on the realization of a national IT infrastructure for healthcare and a national electronic patient record (EPR). The underlying national architecture is designed to enable this EPR virtually, not a national database, nor on a patient's smartcard. The required secure infrastructure provides generic functions for healthcare applications: localization of medical data stored in healthcare information systems via a national switchboard (dutch acronym LSP), patient identification, authentication and authorization of healthcare professionals. The first national applications in the EPR program are currently being rolled out in an 'early adopters' program. These applications are: the electronic medication record for pharmacists and MD's, and the electronic locum record for GPs. Other national applications are in different stages of design and their rollout is awaiting results from the first pilots.

Implications of a virtual EPR approach

A national electronic patient record (EPR) implies that all information about a patient will be stored at one central location, but that is in fact not true for the Dutch approach. The Dutch national EPR is a virtual record: information remains stored at the location where it has originally been recorded electronically. Authorized healthcare professionals can request medical details from those records. This enables the integrity and currency of the data to be maintained and the responsibility for the data remains where it needs to be: at the source.
To be able to guarantee the necessary privacy, a model of trust has been formulated. This model is in line with the Medical Treatment Contracts Act (Dutch acronym: WGBO). Key of this model, apart from a number of essential preconditions is the so called 'chain of trust'.
The first step in this chain is identification of healthcare professionals and patients. The identification numbers for healthcare professionals (Dutch acronym: *UZI*) and patients (Citizen Service Number (Dutch acronym: *BSN*) are available and legislation on use is being prepared. The second step is authentication: is the person really who he or she claims to be? This is important for security. The Unique Healthcare Provider ID chip card holds the unique number of the healthcare professional, together with certificates to prove authenticity.
The third step is authorization: Is the person allowed to view the requested medical information. An authorization mechanism is in place which is currently based on national authorization guidelines per application. These are written and validated by healthcare professionals and patients.
This however still means that information and some processes need to be standardized. Standardization is an absolute necessity if medical information is changed on a national level. Several questions need to be answered: which information should to be shared between healthcare providers, which information are or will be captured and which vocabularies are to be used? These topics need to be addressed for most new national applications as the national EPR is designed, developed and implemented.

O144

E-labs: new ways for sharing patient information across health care organizations

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The interaction between microbiology specialists from clinical laboratories and clinicians has always been essential. The microbiology specialists cannot start a proper investigation without a detailed description of the patient's condition and the physician cannot start the right treatment without the input from the microbiology specialist.
Lab work continues to be largely manual, but the information for and about the samples (and the patient) can be significantly improved by the use of IT applications. Nowadays, when methods get more and more complex, a growing amount of data is produced. Laboratorium Information Systems (LIS) evolve into Laboratorium Information Management Systems (LIMS) supporting more and more the work processes in the labs. Since laboratory and microbiology consults are part of the Electronic Patient Record (EPR), and since laboratories need to share data, harmonization is necessary for the exchange of laboratory results.
The interaction between microbiological laboratories and the healthcare organizations can be improved by using standards for reporting. Many IT initiatives have been taken in the healthcare sector, but there still is a great deal of fragmentation. NICTIZ as well as IHE initiative emphasize the importance of the interoperability between organizations. NICTIZ, bridges the gap between organizations by bringing together all the parties involved. NICTIZ stimulates favourable developments and helps to remove obstacles which might prevent widespread application. IHE is a joint initiative from both healthcare professionals and the software industry to improve the way computer systems in healthcare share information. IHE promotes the coordinated use of established standards such as DICOM and HL7 to address specific clinical needs in support of optimal patient care. Systems developed in accordance with IHE communicate better with one another, are easier to implement, and enable care providers to use information more effectively. Physicians, medical specialists, lab staff, nurses, administrators and other care providers envision a day when vital information can be passed seamlessly from system to system within and across departments as well as health care enterprises, and made readily available at the point of care. IHE is designed to make their vision a reality by improving the state of systems integration and removing barriers to optimal patient care.
Both organizations have the objective of using standards. Together IHE and NICTIZ start the Dutch e-lab initiative, an infrastructure for exchanging laboratory results. The initiative is both a process and a forum – where user groups have the lead – for encouraging integration efforts. It defines a technical framework for the implementation of established messaging standards to achieve specific clinical goals. It includes a rigorous testing process for the implementation of this framework, organizes educational sessions, exhibits at major meetings of medical professionals to demonstrate the benefits of this framework and encourage its adoption by industry and users.
The next step is to organize the user forum. This forum has to point out together with the industry relevant business cases which lead to a better interoperability in the near future.

O146

The high pressure shock response in *Escherichia coli*

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Like temperature, pressure is an important thermodynamic parameter that varies greatly throughout the biosphere. Although atmospheric pressures are typically considered as standard conditions, the high pressures (0-100 MPa) occurring in the deep sea often constitute an essential feature for the resident microbial community. In industrial settings as well, microorganisms may become confronted with very high pressures (100-1000 MPa) as high pressure processing is starting to be used as a non-thermal alternative in food preservation.
Contrary to heat stress, however, the cellular impact of high pressure stress and the physiological response it evokes remain poorly characterized, although it might reveal interesting microbial behavior. Using a screen for high pressure induced promoters, we demonstrated that both the heat shock- and the SOS-response are induced in *Escherichia coli* after high pressure treatment, and that both responses strongly affect the bacterial behavior after high pressure treatment. Heat shock genes are most likely induced as a result of direct high pressure mediated protein denaturation. Moreover, heat shock proteins seem to contribute to high pressure resistance in *E. coli* since (i) heat shocked cells display a marked increase in pressure resistance, and (ii) high pressure resistant mutants of *E. coli* exhibit constitutive high levels of heat shock proteins.
Even though it is considered as a typical DNA damage response, the SOS regulon not only encodes DNA repair functions but also a plethora of mechanisms that result in the promotion of (random) mutagenesis and the lateral spread of mobile elements (often carrying virulence traits) to even the control of basic cellular physiology such as respiration and division. In line with our finding that high pressure induces SOS genes, we could demonstrate high pressure mediated induction of lambdoid prophages, including the virulent Shiga-toxin encoding prophages of *E. coli* O157:H7.
Since high pressure does not and, based on thermodynamic considerations, cannot induce direct DNA damage, we investigated the mechanism of SOS induction.

Interestingly, some other pathways of DNA damage independent SOS induction, e.g. by beta-lactam antibiotics, have been recently described. SOS induction by high pressure was shown to be dependent on RecB, indicating that DNA double strand breaks initiated the response. The high pressure SOS induction was shown to be dependent on RecB, indicating that DNA double strand breaks initiated the response, while a subsequent random genetic screen revealed that the cryptic endogenous restriction endonuclease Mrr is the effector of this DNA damage. An outstanding question, however, remains as to how the perception of high pressure stress results in the activation of this restriction enzyme.

O147

Why to be serious about *Bacillus cereus*

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Bacillus cereus is involved in the industrialized world in food-borne disease as well as in invasive infections such as fatal bacteremia and a form of pneumonia remarkable similar to inhalation anthrax. Changing life styles, increased life expectancy and new eating habitats (e.g. increasing consumption of so called convenience food, ready-to-eat and heat-to-eat foods) have combined to increase the incidence of *B. cereus* infections. The emetic type of the disease is attributed to the heat-stable depsipeptide cereulide, while different heat-labile enterotoxins are the causative agents for the diarrhoeal syndrome. The spectrum of potential *B. cereus* toxicity ranges from strains used as probiotics in animal feed to highly toxic strains already reported responsible for fatalities. While the genetic variability of environmental *B. cereus* isolates and isolates connected to periodontitis and other human infections have been studied in some detail, information on the population structure of food poisoning *B. cereus* was quite limited. Nevertheless, in order to understand epidemical processes, an assessment of the population structure of this food pathogen would be important. An in-depth polyphasic approach was therefore applied to elucidate the population structure of toxic *B. cereus* strains connected to foodborne illness. This analysis revealed a single monomorphic cluster of emetic *B. cereus* strains while diarrhoeal *B. cereus* strains showed a high degree of heterogeneity and were scattered over different clusters when different typing methods were applied. If emetic strains are – like their close relative *B. anthracis* – indeed clonal, two different population structures would be present within the single species *B. cereus*. Why such different population structures should have evolved is currently unknown. However, based on the results of the performed population analysis, it is tempting to speculate that emetic strains have originated only recently through acquisition of specific virulence factors such as the cereulide synthetase genes.

The true incidence of *B. cereus* food poisoning is unknown for a number of reasons, including misdiagnosis of the illness, which may be symptomatically similar to other types of food poisoning. E.g., the emetic type of foodborne disease, caused by cereulide producing *B. cereus,* mimics the symptoms provoked by *Staphylococcus aureus* enterotoxins. Recently, it has been shown that cereulide is produced by an unusual modular enzyme complex, named cereulide synthetase, which is encoded on a mega virulence plasmid related to *B. anthracis* toxin plasmid pXO1. The complete biosynthetic code for the non-ribosomal synthesis of cereulide was deciphered and the genetic information was subsequently used to develop rapid molecular identification and typing systems, which were successfully applied to identify the etiological agent of recent emetic food poisonings in Southern Germany[4].

O148

Germination of *Bacillus subtilis* spores; the effects of thermal preservation on spores

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Introduction: *Bacillus* spores are very widespread in the environment, and are part of the common microbiological contamination on fresh food products. Current food preservation methods are generally capable of killing the majority of the microbial population, but spores do frequently survive these treatments. After the preservation treatment, the spore germinates in the food product and multiplies, causing food spoilage or health risks.

Spores are dormant and resistant to many insults, but loose these characteristics when they germinate. Germination occurs when sensors in the spore notify the presence of certain nutrient molecules, and the spore considers the presence of these nutrients as a signal that environmental conditions do allow cell growth. Although a spore population normally responds relatively similar upon addition of nutrients, this is not the case when spores were first exposed to high temperatures, as being used during food preservation. We have investigated the effect of a thermal treatment on spore germination and viability.

Materials and Methods: Spores of *B. subtilis* 168 and *B. subtilis* A163 (a heat resistant natural isolate) were subjected to various heat treatments and viability was

monitored using a newly developed Q-PCR based RNA degradation assay and colony formation. Furthermore spore germination was assayed using flow cytometry.
Results: The subjection of spores to various heat treatments resulted in a generally delayed germination response of the spore population and an increase in heterogeneity. Furthermore RNA degradation in the spore as a result of a heat treatment correlates with the spore's viability.
Conclusion: RNA degradation in the spore correlates with the spore's viability and can be used to rapidly assess the effectiveness of spore inactivation by thermal food preservation treatments.

O149

Identification of genetic polymorphisms involved in survival of *Campylobacter jejuni* in the poultry meat chain

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Introduction: This project aims at identifying genetic factors that are involved in survival of *Campylobacter jejuni* in the poultry meat chain. Stress genes, or nucleotide polymorphisms in relevant genes, can function as targets for molecular assays that detect *Campylobacters* with (enhanced) capacity for survival. Such assays would enable an early warning system for *Campylobacter* on poultry products, and can be used for risk assessments.
Methods: For the experiments, 36 *C. jejuni* strains were selected that had previously been isolated from one of three different niches (poultry farms, poultry retail products or clinical isolates). We assumed that these strains had experienced different levels of stress prior to isolation, with the retail group representing strains that survived the slaughter house. To investigate the capacity for survival, the strains were exposed to either oxygen or to chill (10 °C) stress in liquid cultures, and survival was followed by plate counting. In order to reveal genetic differences between these strains, six genes were selected for which a role in temperature and/or oxygen stress response has been proposed in the literature. These genes (*rpoD, rpoN, spoT, lytB, htrA*, and *fliA*) were amplified from the strains and (partly) sequenced. The resulting sequences and the survival experiments were analysed for correlations between polymorphisms and the capacity for survival.
Results: The stress survival experiments indicated a high diversity in the capacity of *C. jejuni* strains to survive oxygen and chill stress which seemed independent of their source of isolation.This suggests that survival of these types of stress is not niche-related. No single strain was resistant to both oxygen and chill stress suggesting that survival of these stresses requires different mechanisms.
Two niche-related polymorphisms could be identified in the *spoT* gene leading to amino acid changes in the encoded protein. These mutations were over-represented in the clinical isolates and were never observed in the poultry farm isolates. We found no relation between these mutations and oxygen or chill stress survival. However, it is possible that these mutations play a role in other survival strategies or in pathogenicity. Additional polymorphisms were identified in *rpoD* and *rpoN* of which some mutations in *rpoN* were shared by all chill stress survivors. The *lytB* sequence was highly conserved among all strains analysed displaying only diversity at 2 positions in the LytB protein sequence that were shared by only three strains .
Conclusions: High diversity in capacity of *C. jejuni* strains to survive chill or oxygen stress. No relation between capacity to survive chill or oxygen stress and isolation source of *C. jejuni*. Genetic polymorphisms were identified in the *spoT* gene that appear niche-specific, and several polymorphisms in *rpoN* are shared by all chill survivors but are not limited to chill survivors.

O150

Community-acquired methicillin-resistant *Staphylococcus aureus*

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Until the 1990s methicillin-resistant *Staphylococcus aureus* (MRSA) caused mainly nosocomial infections. Outside the hospital, MRSA were only found in discharged patients or close family members. From the 1990s on MRSA are increasingly found in the community, in particular in (healthy) individuals who had no direct or indirect link with healthcare settings. Main risk groups are persons in relatively poor hygienic conditions: homeless, IV-drug users, American Indians, Inuit, Aboriginals, Pacific Islanders, African Americans, military, athletes (contact sports), jail inmates, and children. In addition, compromised skin integrity is a risk factor. These so-called community-associated (CA) MRSA are causative agents of skin and soft tissue infections, but life-threatening infections and occasionally infections at uncommonly infected body sites are described. Patients with CA-MRSA seem to have more severe illness than those with other MRSA or MSSA strains.
Based on Multi-Locus Sequence Typing (MLST) studies most hospital-associated (HA)-MRSA belong to 5 clonal complexes and predominantly contain one of the SCC*mec* types I, II, or III. Community-associated (CA)-MRSA are genetically much more diverse thus belong to a wide variety of clonal complexes and MLST sequence types (ST). A striking difference with

HA-MRSA is that CA-MRSA do not contain SCC*mec* I-III but SCC*mec* type IV or V. Despite their genetic heterogeneity particular CA-MRSA clones dominate in some geographic regions. In the USA the USA300 clone is dominant, while in Europe ST80, in Taiwan ST59, and in the Austral-Pacific region the SWP clone and Queensland clone predominate. Sometimes isolates with the typing characteristics of HA-MRSA are described as CA-MRSA, but in most cases this appears to be due to the definition used for CA-MRSA. Isolates with the typing characteristics of CA-MRSA are now increasingly spreading and well established in hospitals and consequently may be called HA-MRSA. As a result the definitions of CA- and HA-MRSA are becoming increasingly indefinite. The most important differences between isolates with HA- and CA-MRSA are that CA-MRSA are generally able to spread in the community, carry SCC*mec* IV or V, and until now are less resistant to antimicrobial agents. Furthermore, PVL is more common among CA-MRSA isolates than hospital-associated (HA) MRSA and has therefore also been suggested to play a major role in the pathogenesis of skin and soft tissue infections. This, however, is only based on the association between PVL and skin and soft tissue infections. Most common among the severe infections is necrotizing pneumonia. The most important virulence factor for this type of infection is considered to be Panton-Valentine Leukocidin (PVL). It should be noted that more than 90% CA-MRSA in the USA are PVL-positive isolates in contrast to European isolates, where approximately half carry the *pvl* genes.

In the last decade MRSA are also increasingly isolated from companion animals, bovines and especially pigs (pig-associated or PA-MRSA). Screening suggests that up to 40% of the Dutch pigs carry PA-MRSA. PA-MRSA has also been demonstrated in pigs in France. These MRSA can cause infections in pigs and may be spilled-over to humans. PA-MRSA belong to ST398. However, three different SCC*mec* types have been found within this ST. These isolates have also been found among pig farmers and their household members and it has been demonstrated that ST398 can cause endocarditis.

The reason for the success of CA- and PA-MRSA is not understood. Knowledge about the strains and genetic mechanisms involved will be needed to develop adequate and cost-effective counter-measures to control the emergence of CA- and PA-MRSA.

O151

Mapping the secretome of *Staphylococcus aureus*

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Staphylococcus aureus is a dangerous pathogen that is becoming an increasing threat for human health, because it has an amazing capacity to develop resistance to antibiotics. To date, relatively little is known about mechanisms for protein export in this organism. As most virulence factors are localized in the cell envelope or secreted, a detailed characterization of transport pathways for proteins involved in staphylococcal virulence is important. Therefore, our recent studies were aimed at mapping the 'secretome' of *S. aureus*. By definition, the secretome includes both the machinery for protein export from the cytoplasm and the exported proteins. Our first approach was to predict the protein transport machinery as well as the secreted and cell wall-associated proteins using genome data from six sequenced strains of *S. aureus*. The results indicate that *S. aureus* has the potential to export 78-93 proteins with signal peptides that can be cleaved by type I signal peptidases, and 49-54 lipoproteins that can be cleaved by type II signal peptidases. The sequenced *S. aureus* strains contain up to 21 genes, encoding proteins with a signal for covalent attachment to the cell wall by sortases. Three proteins contain pseudopilin-like signal peptides, which are most likely cleaved by the pseudopilin signal peptidase ComC. Depending on the *S. aureus* strain investigated, 4-5 proteins could be exported *via* the Tat-pathway. Interestingly, compared to the total numbers of genes encoded by the respective genomes, *S. aureus* strains contain a higher percentage of predicted exported proteins than *Bacillus subtilis*. This is remarkable as *B. subtilis* is well known for its high secretion capacity and high number of exported proteins. This finding underscores the view that protein export is of prime importance for the fitness of *S. aureus*. Results from ongoing studies aimed at the verification of our *S. aureus* secretome predictions, using both sequenced strains and clinical isolates, will be presented.

O152

The discovery of a staphylococcal Fc gamma receptor inhibitory protein

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Introduction: Fc gamma receptors (FcgR) are receptors for immunoglobulin G (IgG) and have an important role in the defence against bacterial infections. Phagocytic cells, like neutrophils, monocytes and macrophages express members of three classes of FcgR, FcgRI, FcgRII, and FcgRIII. These FcgR share considerable structural and functional homology and recognize similar residues within

the Fc tail of IgG. Interaction with IgG-opsonized bacteria activates FcgR, evoking different cellular responses, like respiratory burst and phagocytosis.
Bacteria have evolved different mechanisms to escape from these FcgR-mediated host immune responses. One of these is the production of IgG binding proteins. Staphylococci, for example, express surface protein A, which by binding IgG leads to a dysfunctional IgG-FcgR interaction and protection from lethal phagocytosis. However, bacterial excretion products binding to cellular FcgR and in this way inhibiting the interaction with IgG-opsonized targets have not been found.
In this study we describe the discovery of a FcgR inhibitory protein, produced by *Staphylococcus aureus*. We identify this protein and characterize its immunomodulatory capacities.
Methods: The FcgR inhibitory protein was found by screening several bacterial supernatants for their inhibition of cellular FcgR detection by monoclonal antibodies in FACS. The protein was purified from the inhibitory bacterial supernatant by chromatography and identified by mass spectrometry. Binding of the recombinant inhibitory protein to the different FcgR subclasses and isotypes was characterized in FACS, ELISA, and BiaCore experiments. Influence of the recombinant inhibitory protein on FcgR mediated functions was determined in phagocytosis and respiratory burst, by neutrophils and other cell types.
Results: By screening a panel of different bacterial supernatants we found several strains of *S. aureus* to produce a very potent FcgR blocking protein. After purification of the staphylococcal supernatant by gel filtration and ligand affinity chromatography we identified a 12.3 kD protein, as the formylated peptide receptor like 1 (FPRL-1) inhibitory protein, FLIPr. FPRL-1 is the low affinity fMLP receptor and is expressed on a great variety of cells. It is a receptor for several chemoattractants.
With recombinant FLIPr we characterized binding to the different FcgR. FLIPr appeared capable of binding to all three classes of FcgR, although with different affinities. By testing FLIPr functionally in experiments like phagocytosis and respiratory burst, we could demonstrate its very potent inhibitory effect on these FcgR mediated functions.
Conclusions: Here we demonstrate the discovery of a bacterial FcgR inhibitory protein. This protein produced by *S. aureus* was identified as FLIPr, recently described as the FPRL-1 inhibitory protein. FLIPr binds to all classes of FcgR and is very potent in inhibiting FcgR mediated functions like phagocytosis and respiratory burst. Next to this new insight into the immune escape mechanisms of *S. aureus*, this discovery might lead to the development of novel therapeutic agents in FcgR mediated diseases, like allergy and autoimmunity.

O153

Methicillin-resistant *Staphylococcus aureus* strains isolated from pigs on different kinds of pig farms

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The aims of the present pilot study were to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthy pigs at different kinds of pig farms and to examine if the usage of antimicrobial drugs is a risk factor for MRSA carriage. Twelve farrowing farms, 11 finishing farms, 6 farrow-to finish farms, one rearing farm and one centre for artificial insemination were included. Samples were taken from the nares of 10 pigs on each farm. The farmer was asked if the pigs had been treated with antimicrobial drugs, what kind of antimicrobial drugs and at what moment. MRSA isolates were further analysed by PFGE, spa-typing, SCC*mec* typing, and multilocus sequence typing (MLST). Screening of 310 pigs from 31 farms in the Netherlands showed that 43 pigs (14%) carried MRSA in their nares. On 8 of the 31 (26%) investigated farms colonized pigs were found, including 3 finishing farms, 4 farrowing farms and one farrow-to-finish farm. At one of these farms the pigs were MRSA negative during the first screening, but 8 out of ten pigs became MRSA positive after they had been treated with oxytetracycline. The use of standard antimicrobial medication of the pigs was a risk factor for MRSA carriage ($p<0.01$). In the second part of our study, we sampled pigs at 7 farms supplying pigs for the MRSA positive farms. Screening of the pigs on these farms revealed that the pigs on all but one farm were MRSA positive. Genotyping revealed that all MRSA strains were non-typeable by PFGE using the *Sma*I restriction enzyme. However, PFGE using restriction enzyme *Xma*I was a useful tool in discriminating the isolates. All MRSA had MLST ST 398. Different spa-types were found including type t011, t108, t567, t899, but the spa-types on epidemiologically related farms were identical. Two SCC*mec* types were found: type IV and type V. On one farm MRSA isolates with ST 398, spa-type t011 but with different SCC*mec* types and different resistance patterns were found, suggesting that different SCC*mec* elements have been inserted into MSSA of the same genotype. All MRSA strains were resistant to tetracycline, but additional resistances to erythromycin, lincomycin, kanamycin and gentamicin were common. In conclusion, 26% of the pig farms were MRSA positive and 6 out of 7 farms supplying pigs for the positive farms were also MRSA positive, indicating that finishing farms and farrowing farms are colonized by MRSA through the

purchase of colonized pigs from other farms. The use of antimicrobial drugs is a risk factor for detecting MRSA on pig farms.

O154

From maximal to optimal molecular diagnostics. Is there a road back?

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The implementation of molecular diagnostics using real-time detection systems and automated nucleic acid isolation, has become more and more common practice in routine diagnostic virology. More targets can be detected simultaneously, however we have not reached the practice of 'sample-in, result-out'. Methodologies are not uniform and robust enough to enable nucleic acids to be isolated from different matrices with a proven efficiency. On the other hand, technologies have emerged to enable the detection of multiple targets in a multiplex manner. The samples even have to be handled in an unsafe manner with an increased risk for carry-over of amplicons. A practice that laboratories tried to avoid by using real-time detection systems.
It is clear that technologies on the front-end as well at the detection-end are evolving unequally. Chip technology opens possibilities, but with too limited targets being detected at high sensitivity. But who is in control?
We want and need to detect more viruses (micro-organisms) in relation to more clinical questions. We want to know and understand whether they are clinical relevant, or not. The last decade has enabled us to implement this molecular diagnostic technology at a very high standard – we are even getting accredited-, with an increased knowledge of clinical relevance, with a high sensitivity, a high level of standardization, and a critical common sense approach. Even at an acceptable price. There is no way back, but we have to stay in control!

O156

Rapid and sensitive detection of 5 gastro-intestinal pathogens using 2 internally controlled multiplex Real-Time PCRs

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Objectives: Traditional methods to detect gastro-intestinal pathogens are slow, and/or lack sensitivity. Molecular detection of gastro-intestinal pathogens has proven to be rapid and sensitive. Stool screening requires a large throughput; however, the use of monoplex PCRs greatly limits the capacity. Therefore, a multiplex approach is mandatory.
Methods: Real-time PCR assays for *Salmonella enterica* (SE), *Campylobacter jejuni* (CJ), *Giardia lamblia* (GL), shiga toxin-producing *Escherichia coli* (STEC), and *Shigella* spp./enteroinvasive *E. coli* (SH/EIEC) were developed and subsequently multiplexed in 2 assays combining SE/CJ/GL and STEC/SH/EIEC. Both assays also incorporated an internal control (phocin herpes virus [PhHV]). Stool DNA was extracted with miniMAG or easyMAG (bioMérieux). Assays were validated with regard to selectivity (135 strains), analytical sensitivity (spiked fecal specimens), and clinical performance (851 stool specimens).
Results: Both assays showed 100% selectivity with the tested panel of strains. Analytical sensitivity was in the range of 10<H>2-4</H>CFU/g of stool in both mono- and multiplex approaches. In 281 of the 851 clinical stools, a pathogen targeted by 1 of the multiplex assays was detected by conventional methods (culture or microscopy). Overall, the multiplex assays showed 98.2% concordance in these 281 specimens. In the 570 stools negative for the targeted pathogens by conventional methods, an additional 83 positive results were detected. Furthermore, 13 double infections were detected by the multiplex assays, compared to only 3 by conventional methods. Inhibition of the multiplex PCRs was observed in only 4.85% and 5.43% for the SE/CJ/GL and STEC/SH/EIEC assays, respectively.
Conclusion: Multiplex real-time PCR offers a rapid and sensitive method for the detection of gastro-intestinal pathogens. Multiplexing does not harm the analytical sensitivity if the assay is set-up properly. These multiplex assays will introduce a whole new strategy in screening stool specimens for gastro-intestinal pathogens.

O157

Two internally controlled multiplex Real-Time PCRs for diagnosis of viral gastroenteritis

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Introduction: Norovirus, rotavirus group A, astrovirus and adenovirus, especially serotypes 40 and 41, are common causes of viral gastroenteritis. Conventional diagnosis of these infections is based on antigen detection and electron microscopy. To improve the diagnostic possibilities, two internally controlled multiplex real-time PCRs were developed.
Methods: Five individual real-time PCRs were developed for the specific detection of norovirus genogroup I, norovirus genogroup II, rotavirus group A, astrovirus and adenovirus. After optimization, the individual PCRs were combined to two multiplex reactions and optimized further. Subsequently, the assay sensitivities were compared to the sensitivities of the individual reactions and the specificity was determined. Validation of the assay was performed

using faeces samples that were submitted for antigen detection from April 2006 up to now.

Results: The individual real-time PCRs for the detection of norovirus genogroup I, rotavirus group A, astrovirus and the internal control equine arteritis virus were successfully combined to one multiplex PCR. No difference in sensitivity was observed for the multiplex PCR compared to the individual PCRs. Furthermore, the individual PCRs for adenovirus and norovirus genogroup II were combined to one multiplex PCR. Whereas the sensitivity for norovirus genogroup II was comparable between the monoplex and multiplex reaction, the sensitivity for adenovirus was approximately one log decreased in the multiplex PCR reaction. Application to the clinical samples is currently being performed.

Conclusion: Two multiplex real-time PCRs were developed for diagnosing patients with viral gastroenteritis. Although the adenovirus assay in multiplex was less sensitive in comparison to the individual assay, this is not expected to be a problem in clinical cases.

O159

Rapid tests for the diagnosis of leishmaniasis

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Leishmaniasis is considered to be one of the most important parasitic diseases with approximately 350 million people at risk of contracting the disease. Leishmaniasis has a world-wide distribution and is endemic in at least 88 countries. There are 3 major clinical forms of the disease: 1) visceral leishmaniasis (VL), affecting approximately 500,000 people per year, is a deadly disease if left untreated. Parasites colonise internal organs, in particular spleen, liver, bone marrow, lymph nodes; 2) cutaneous, 3) muco-cutaneous leishmaniasis (CL and MCL, respectively) affecting at least 1,500,000 people per year. In the case of CL parasites are confined to the skin causing one or more slow-healing sores. In the case of MCL, the infection spreads to the mucosal membranes, especially those of mouth and nose, where it may cause extensive damage. More than 20 recognised *Leishmania* species are responsible for these diseases, each species having distinct epidemiological patterns.

Early diagnosis of leishmaniasis is important in order to avoid severe damage or even death of the patient. Leishmaniasis routine diagnosis relies on either microscopical demonstration of *Leishmania amastigotes* in aspirates from lymphoid tissue or liver, in slit skin smears or in peripheral blood or culturing. However, sample retrieval is uncomfortable to the patient and the isolation of parasites by culturing is time-consuming, difficult and expensive. Because of these limitations, a number of serological methods have been developed. These methods can however only be employed for the diagnosis of VL, as an *Leishmania* infection causing CL in most cases results in a very moderate to no systemic antibody response.

The direct agglutination test (DAT) remains the first line diagnostic tool for visceral leishmaniasis (VL) in many developing countries. The DAT is a relatively simple test with high sensitivity and specificity (95% and >98%, respectively). The method uses whole, stained promastigotes either as a suspension or in a freeze-dried form. The latter antigen is heat stable, thus facilitating the use of the DAT in the field. A limitation of the DAT is the relative long incubation time of 18 hours and the need for serial dilutions of blood or serum. In order to circumvent this, a fast agglutination screening test (FAST) for the detection of anti-*Leismania* antibodies in serum samples has been developed. The test is based on the same freeze dried antigen as the DAT, making it very stable at ambient temperature, but combines a higher parasite antigen concentration with a smaller test volume. Furthermore, it requires only one serum dilution and the results can be read within 2-3 hours. The FAST was evaluated under laboratory and harsh field conditions in the Netherlands and Ethiopia and showed a high sensitivity and specificity comparable to the current DAT and may thus provide a suitable alternative in particular for screening purposes.

As an alternative, dipstick tests based on cloned antigen of a 39 amino acid repeat part of a 230 kDa protein encoded by a kinesin-like gene of *L. chagasi*, have been developed. Initial reports were very promising: rK39 based tests were very sensitive and specific for visceral leishmaniasis, could be used in HIV-positive patients and antibody levels against rK39 declined rapidly after successful treatment. However, recent reports describe that some dipstick test lacks sensitivity and specificity, in particular in East Africa, one of the most important foci of VL. Therefore, the need remains for development of an affordable diagnostic test for VL that is suitable for use in peripheral health services (simplicity) with high sensitivity/specificity.

Ultimately, molecular biology, in particular polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA) assays may prove to be the most sensitive diagnostic tests for all clinical forms of leishmaniasis. Several targets, like ribosomal RNA genes, kinetoplast DNA, mini-exon-derived RNA genes and genomic repeats can be exploited for diagnosis and, often in combination with restriction length polymorphism, also for species identification, which may be important for choice of treatment. In addition, NASBA can be used to monitor the efficacy of treatment.

O160

Rapid detection with microscopy, quick-tests and real-time PCR of *Cryptosporidium*

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Background: *Cryptosporidium* remains largely underdiagnosed in current routine diagnostic practice in the Netherlands. We compared different diagnostic methods for the detection of *Cryptosporidium* in faeces from children till 6 years, with both acute and chronic diarrhea send in for bacteriological or parasitological examination. Furthermore the existing literature on the rapid detection of *Cryptosporidium* was reviewed.
Methods: In 2005 and 2006 two comparative studies, including over 700 faeces were analysed for comparison of microscopic examination on stained faecal samples (IHK-stain), Crypto-strip™ (Coris Bioconcept), ELISA (Novitec Cryptosporidium ELISA), immunocard STAT!™ (Meridian) and real-time PCR for the detection of *Cryptosporium*. Using a PubMed Medline Query seven studies on the rapid detection of *Cryptosporidum* species were found.
Results: Using real-time PCR as a reference method the sensitivity of microscopy, Crypto-strip™, ELISA and immunocard STAT!™ were 37-64%, 63-78%, 71% and 93%, respectively while the specificity always exceeded 98%. Results from literature will be reviewed and compared with our results. Most published studies presented difficulties to define a gold standard because PCR confirmation or other testing to determine if there were any false-negative or false-positive results was not performed.
Conclusions: Microscopy is less sensitive for detection of *Cryptosporidium* in feces. ELISAs also have good sensitivity and can be acceptable alternatives for microscopy. Crypto-strip has comparible sensitivity to microscopy while Immunocard STAT approaches sensitivity of real time PCR. Real time PCR is a very sensitive and specific method for the detection of *Cryptosporidium*. The clinical value of the additional positive PCR results needs to be evaluated. Remarkably, the majority of the positive *Cryptosporidum* samples were not found in watery but in mushy stools. The majority of positive watery samples was not sent for parasitology but only for bacteriology and would otherwise not have been detected.

P001

Acute anuric renal failure in a hemodynamically stable patient with mitral valve endocarditis due to *Staphylococcus aureus* infection

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A 75 year old woman was referred for fever and abdominal pain. She was febrile (39.5 °C) but hemodynamically stable, on physical examination there were no localizing signs. Serum CRP was 278 mg/L, the leucocyte count was 15.4/nL. The urinary sediment contained 5-10 erythrocytes per view, protein was +. No abnormalities were seen on a chest X-ray and ultrasound and CT-scan of the abdomen.

Cefazolin and metronidazole were started for a presumed diagnosis of septicaemia. A blood culture grew *Staphylococcus aureus*, sensitive for oxacillin and gentamycin. Cultures during antibiotic treatment remained negative. Echocardiography showed mitral regurgitation grade II and valvular vegetations compatible with a diagnosis of bacterial endocarditis. Therapy was switched to flucloxacillin 9 grams daily by continuous infusion and gentamycin 240 mg once daily.

Three days later the trough serum concentration of gentamycine was 9.0 mg/L (therapeutic range <1.0). Gentamycin was discontinued. Serum creatinine had increased from 105 to 445 ◂mol/L and she was found to be anuric, necessitating hemodialysis. There were no periferal septic emboli and there was no skin rash. There was no eosinophilia; a urinary sediment could not be obtained. Complement levels C3 and C4 were normal, anti-MPO antibodies were dubiously positive.

Light microscopy of a renal biopsy showed a diffuse endocapillary proliferative glomerulonephritis with influx of polymorphnuclear cells in the glomerular capillaries. There were no crescents. The interstitium showed only minor changes and there was no inflammatory infiltrate. Immune staining showed C3 depositions in the glomerular capillary membranes and in the mesangium; IgG, IgM and C1q depositions could not be demonstrated.

Acute endocapillary glomerulonephritis is still a common disease in developing countries but has become rare in western Europe. Infections with group A *Streptococci* or *Staphyloccus aureus* usually precede or co-exist with the development of renal symptoms but many other micro-organisms may be involved. Hypertension, hematuria and proteinuria are common symptoms; oliguric renal failure is relatively rare in children but is described in about 50% of affected adults. Gentamycin toxicity may have contributed to anuria in this patient. Treatment is symptomatic, immunosuppressive agents are usually not effective. Cardiovascular mortality has been described in 25% of adult patients in the early stage of the disease. While children usually have a good prognosis, in adult patients a high degree of chronicity with progression to renal failure has been described especially in the presence of significant proteinuria. The renal prognosis of patients with prolonged oliguria is poor.

P002

Ecology of mixed cultures producing PHAs

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Polyhydroxyalkanoates (PHAs) are a class of polyesters which are biodegradable and can be produced from renewable resources. They have properties similar to petrochemical plastics. PHAs are synthesised and stored as energy and carbon substrate by many different groups of bacteria. The production of PHAs with pure culture has been commercialised in the past, but was not very successful due to the high price of substrates required. The merits of mixed culture processes are the lower costs due to the application of cheap mixed waste based substrates, but until now a shortcoming is the lower yield and productivity of PHAs.

An aerobic sequencing batch reactor fed with acetate has been used for establishing a stable PHAs producing mixed culture. The selection of PHAs producing bacteria from a nature inoculum is based on the capacity of PHAs producers to grow in absence of external substrate. In a sequencing batch process sequential uptake of external substrate and subsequent growth on the polymers stored, gives a competitive advantage over direct growth on an external substrate. In order to optimise the production of PHAs by mixed cultures, the influences of several parameters on PHAs production were investigated, such as carbon to nitrogen ratio, solid retention time, temperature and cycle length.

To investigate microbial communities in these PHAs producing bioreactors, denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was used to study the species composition of the reactor. Based on the progressing results, some various diversities have been observed from the sludge under different temperature, carbon to nitrogen ratio and solid

retention time. Fluorescence in situ hybridization (FISH) is going to be performed to determine the abundances of the particular populations in the reactors.
Besides diversity analysis, functional genes involved in PHAs production will be investigated also. More than 50 PHA synthase genes (phaC) have been identified. PhaA, phaB and phaZ genes play very significant role in synthesis and degradation of PHA. Some other proteins, such as the granule-associated proteins (e.g. phaP) and regulators (e.g. phaR) are related to PHAs synthesis.
Goal of this project is to obtain insight into the ecology of PHA-producing bacteria with the aim to improve the production of PHA. Different genomic tools will be used to study the diversity and expression of genes encoding key enzymes involved in PHA production.

P003

Successful combat against *Clostridium difficile* PCR ribotype 027 at a regional outbreak from 2003-2006 in the Netherlands

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Introduction: Since 2003 severe cases of *Clostridium difficile* (CD) associated diarrhoea (CDAD) has been reported in hospitals in North America and Europe caused by a hypervirulent strain CD ribotype 027, toxinotype III. This ribotype is also detected in the Netherlands causing several outbreaks i.e. in three Hospitals in Kennemerland and hospital related cases in nursing homes.
Methods: The incidence of CDAD was measured retrospectively and prospectively before and after the introduction of specific infection control measures in the region. A case of CDAD was defined as a patient with diarrhea and a positive toxin test on a feces sample (ICTAB, Meridian). Strains isolated from positive feces samples were further investigated by PCR ribotyping.
Results: The Public Health Laboratory Haarlem, the Netherlands, services an area with Nearly 500.000 inhabitants including nursing homes and three hospitals (A, B and C) with monthly admission rates of 1050, 1300 and 1600, respectively; overall admission rate 47.000 yearly. Retrospective evaluation of laboratory data showed short episodes of CDAD outbreaks in hospital A already in October 2002 (incidence 62/10.000 admissions) and June 2003 (121/10.000); in hospital C in December 2003 (50/10.000) and April 2004 (44/10.000). The highest CDAD increase was found in hospital B in August 2004 (>130/10.000). The latter episode was found to be the start of a long term increase of CDAD with incidence peaks of >100/10.000 admissions per month in all hospitals as well as one nursing home to which hospital patients were transferred to. In the 3rd quart of 2005, 67% of 30 CD strains from hospitalized patients belonged to the hyper virulent ribotype 027. Specific measures against CDAD were introduced in all hospitals and nursing homes: strict barrier precautions as private room, glove use, gowns and hand washing, environmental cleaning with bleach and in 2/3 hospitals restrictive use of fluoroquinolones. Also a laboratory algorithm was introduced to investigate all fecal samples from hospitalized patients with diarrhea. Subsequently, during the 4th part of 2005 until the 3rd part of 2006, CDAD incidence decreased to a mean of 21 (range 30 '13)/10.000 admissions, corresponding with the national incidence. The percentage type 027 strains decreased from 67% to 36 and 8% in the 3rd 05, 1st 06 and 3rd 06 period, respectively.
Conclusion: Specific hygienic measures in combination with restrictions of antibiotic prescriptions and intensified laboratory surveillance are successful to overcome CD 027 outbreaks.

P004

Low functional levels of mannose-binding lectin despite normal genotypes in *Legionella pneumophila* pneumonia

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Objectives: Deficiency of mannose-binding lectin (MBL) has been associated with an increased risk of infection, compatible with its role as a pivotal protein in early complement activation. In infections with intracellular pathogens this association is more ambiguous, as some pathogens use MBL to enter their host cell. To study this relation with the intracellular pathogen *Legionella pneumophila*, we determined MBL levels and genotypes in a well-described outbreak of legionellosis at a flower show in the Netherlands. Since this clonal outbreak has no pathogen variability, this patient cohort is very suitable to study genetic host factors.
Methods: In a retrospective patient-control study MBL levels were determined in a hemolytic assay in acute phase serum samples from 122 patients, 59 asymptomatic seroconversion controls and 447 blood bank donors. All patients and seroconversion controls attended the flower show. Serum levels were classified as deficient (<0.2 µg/ml) or normal (>0.2). If multiple serum samples were available from a subject, the highest measurement was used for classification.
Genotyping of MBL was performed with denaturing gradient gel electrophoresis (DGGE) of an amplicon harbouring the three polymorphic sites in exon 1 ('0'

vs wildtype 'A' allele) and a SNP-PCR determining the promotor X/Y polymorphism. Genotypes were classified as deficient (o/o, XA/o) or sufficient (YA/o, A/A). Whole blood DNA isolates were available from 77 patients, 53 seroconversion controls and 223 blood bank donors. Two different nested PCR-DGGE protocols were performed on serum DNA isolates from 112 patients. Serum genotyping results were only considered in analysis when both protocols had corresponding results (72 patients). No discrepancies were found between serum and whole blood genotyping of exon 1 (38 patients). In this way 111 patients could be genotyped reliably.

Results: Compared to 30% of patients only 2% of seroconversion controls and 3% of blood bank donors had MBL levels less than 0.2 ug/ml (X^2 $p<0.01$). Deficient genotypes were found in only 14% of patients compared to 7% and 17% of both control groups (X^2 $p>0.05$). Significantly more patients than seroconversion controls with sufficient genotypes had MBL levels below 0.2 µg/ml (20% vs 0%, X^2 $p<0.01$).

Conclusion: Patients had significantly lower MBL levels at the acute phase of legionellosis than both control groups. This difference was not found in genotypes. This discrepancy suggest either an exhaustion of MBL or an inability to produce MBL in legionellosis, even in patients with sufficient genotypes.

P005

Pathogen related association of mannose-binding lectin deficiency with community acquired pneumonia

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Objectives: Mannose-binding lectin (MBL) is an activating protein of the lectin complement pathway. As a pattern recognition receptor of innate immunity, it binds to a variety of microorganisms including respiratory pathogens like pneumococci and influenza A virus. The association of MBL deficiency and pneumococcal disease has been studied with varying outcome. Less is known about the association with other pathogens in pneumonia. We studied the role of MBL deficiency as a risk factor for community acquired pneumonia (CAP) in relation to the causative agent and clinical course of disease.

Methods: In a prospective case-control study 193 immunocompetent patients with confirmed CAP were included. Severity of disease at presentation and clinical endpoints (intubation, death) were scored. Blood cultures, respiratory specimen cultures for bacteria and viruses, urinary tests for pneumococcal and *Legionella* antigen, serology and PCR for atypical pathogens were performed. MBL genotypes were determined by denaturing gradient gel electrophoresis of an amplicon harbouring the three polymorphic sites in exon 1 ('o' versus wildtype 'A' allele) and a SNP-PCR determining the promotor X/Y polymorphism. Genotypes were classified deficient (o/o, XA/o) or sufficient (YA/o, A/A). 176 healthy adults served as a control population.

Results: *Streptococcus pneumoniae* was found in 60 patients, viruses in 16 and *Legionella* and *Mycoplasma* both in 9. Significantly more patients with viral pneumonia were MBL deficient compared to controls (40% vs 12%). In contrast, none of the atypical pneumonia patients showed MBL deficiency. Patients with pneumococcal pneumonia did not differ from controls in MBL deficiency (18% vs 12%). However, MBL deficiency was associated with more severe disease at presentation in this subgroup. More MBL deficiency was found in patients with concomitant viral and pneumococcal infections than with pneumococcal infections alone. No effect of MBL was seen on mortality of CAP, independent of the pathogen.

Conclusion: MBL deficiency is associated with susceptibility to community acquired viral pneumonia. No association was found with susceptibility to pneumococcal pneumonia, although MBL deficient patients presented with more severe disease. The absence of MBL deficiency in 18 patients with atypical pneumonia is intriguing, as some other intracellular pathogens use MBL to enter their host cell. A larger study is required to confirm this relationship.

P006

Low incidence of nosocomial influenza infection in a hospital setting in the 2005-2006 season

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Introduction: In the Netherlands, there are no recent data on the extent of nosocomial influenza infections in hospitals. During the winterseason 2005/2006 we prospectively studied the incidence of nosocomial influenza infection in the Hilversum hospital and evaluated the use of rapid diagnostic influenza tests for timely initiation of control activities, such as antiviral treatment where indicated.

Methods: Patients admitted between 1 December 2005 and 1 May 2006 on four selected wards (internal medicine, intensive care, geriatric, and lung diseases) were eligible for inclusion in case they developed an influenza like illness (ILI) after at least 48 hrs of admission. ILI was defined as fever > 38°C with at least one of the following

symptoms: cough, sore throat, cold, headache, chest or muscle pain. Nurses and doctors were requested to report eligible patients to the investigator (JvL), and received a monthly reminder of the study. From included patients, a nasopharyngeal aspirate and/or nasopharyngeal swab were collected for virological diagnosis. Presence of Influenza A or B was assessed by two rapid diagnostic influenza tests (QuickVue Influenza A+B and Becton-Dickinson Directigen Flu A+B) and polymerase chain reaction (PCR). For each case, medical history, risk factors and details of vaccination status were collected. To monitor the possibility of nosocomial transmission through health care workers (HCW) the vaccination status of HCW was established.

Results: From a total of 859 admitted patients, nine (1.0%) patients developed an ILI and all were included. No samples were influenza-positive by rapid testing, one sample was positive by PCR. The average age of the total population (56% females) was 71 yrs and of patients with an ILI 62 yrs. Patients were hospitalised for a mean of 10 days, the patients with ILI for 12 days. The incidence of ILI among admitted patients was 1,04 / 1000 days of hospitalization. Among five ILI patients with risk factors for complications of an influenza virus infection, two (40.0%) had received influenza vaccination. The vaccination rate among all 9 patients with an ILI was 33%. The vaccination rate of HCW (doctors and nurses) on the four preselected wards was 14.8%.

Conclusions: During the winterseason 2005/06, 1.0% of eligible patients developed an ILI during hospitalisation on four selected wards. Of these, one patient (11.1%), had an influenza virus infection confirmed by PCR, but not by rapid testing. Despite the low vaccination rate, both among ILI patients and HCW, nosocomial spread of influenza was low. The mild influenza activity during the season may have contributed to the limited spread of influenza in the hospital.

P007

The Swedish genetic variant of *Chlamydia trachomatis*: also circulating in the Netherlands?

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Introduction: Sweden reported recently that a proportion of urogenital Chlamydia trachomatis (CT) infections could not be detected using standard nucleic acid amplifications tests manufactured by Abbott and Roche1 2. Based on data of almost 80% of all reported Chlamydia cases in Sweden during the period from January 2003 to June 2006 a 10% decline in number of *Chlamydia* cases diagnosed with Roche or Abbott tests can be seen, compared with a 1% increases in cases detected with the Becton Dickinson test. Additional sequence analysis identified a deletion of 377 bp in the CT plasmid target used by Roche and Abbott.

State of the Art: Through the CIb/RIVM a Workinggroup of experts was composed to make an overview of actions needed to address the potential CT diagnostic problem in the Netherlands. The Roche COBAS Amplicor test (not detecting the variant), performed at the MHS Amsterdam, and the Probetec test (detecting the CT variant), at the department of microbiology of the OLVG are being compared. Furthermore at the VUmc a retrospective study is started to see whether among CT positive samples detected with the in-house developed and validated real-time TaqMan PCR (detecting the CT variant), used by 10 Microbiological departments in the Netherlands since the last 2 years, the Swedish variant could be identified. Also other initiatives are in progress in Limburg, Twente and Groningen.

Results: 1) Retrospective data from three Dutch microbiological laboratories (Amsterdam, Groningen and Enschede) showed no significant differences in the number of diagnosed CT cases from 2003 ' 2006 (Groningen/Enschede), while in Amsterdam a slight reduction was observed. The national data surveillance from STI clinics showed an increase in positivity-rate up till 2005. In 2006 it levelled off, which might be due to a new surveillance system. 2) At the VUmc a specific real time PCR is developed to detect only the CT variant. 3) Data from current studies will be available end of January 2007.

Recommendations: The Workinggroup has made the following recommendation:

Laboratories using Roche or Abbott which might miss the CT variant, can, when the clinical presentation and the risk profile of the patient does not correspond with a CT negative laboratory result, send, for the 5 first months of 2007, the patient sample to the VUmc . The samples will be tested on the presence of the CT variant, results will be available within a week (Reembursement is being investigated by the CIb/RIVM).

Conclusion: The Swedisch CT variant issue has been addressed quickly by the RIVM. End of January we will have insight if the Swedish variant is circulating in the Netherlands. If so, major diagnostic implementations are needed to address this issue. Roche is in progress addressing the CT variant issue. Currently, collaboration is initiated with the ECDC and the ESSTI, regarding the potential CT variant distribution in Europe.

The recommendations and information are available on: www.infectieziekten.info (Inf@ct messages) and all NVMM members have been informed.

P009

Multilocus variable number of tandem repeats analysis (MLVA) compared to pulse field gel electrophoresis (PFGE) as epidemiological typing method for amoxicillin resistant *Enterococcus faecium*

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Introduction: Amoxicillin resistant enterococci (ARE) are an increasing problem, especially on ICU and haematology wards where a lot of broad spectrum antibiotics are used. In 2005 we suspected transmission of ARE on an ICU ward and we used PFGE as an epidemiologically typing method to compare the strains. The interpretation and therefore the comparability of PFGE results can be difficult when strains are not tested in the same run. Therefore it is complicated to keep a database for a longer period of time. Another typing method is multi locus variable number of tandem repeats analysis (MLVA). This method is less time consuming and gives clear six numbered comparable result. In this study we compared PFGE with MLVA as an epidemiological typing method for *Enterococcus faecium*.
Methods: Forty-six first isolates of *E. faecium* from different patients mainly collected from two hospitals were analysed by PFGE from February 2005 till February 2006. Subsequently the same strains were analysed by MLVA. PFGE was performed according to Kooistra-Smid *et al.* (Burns 2004;30(1):27-33) with minor modifications. MLVA was performed according to Top *et al.* (J Clin Microbiol 2004;42(10):4503-11) with minor modifications. Data were analysed using BioNumerics v4.61 software and the MLVA website (http://www.mlva.umcutrecht.nl).
Results: Typing of the 46 clinical isolates revealed 9 distinct PFGE types (PTs) and 4 MLVA types (MTs). Most strains were distributed among 2 main PTs (23 PT1 strains and 14 PT2 strains) and 2 main MTs (28 MT1 strains and 15 MT159 strains). The other 7 PTs and 2 MTs included 1 or 2 strains only. The PTs and MTs were for 87% concordant with each other. The MT1 group was linked to the whole PT1 group together with four other PTs (n=5). The MT159 group was linked to the whole PT2 group together with one other PT (n=1). This means that PFGE analysis showed 6 strains (13%) which did have a different PT, but did not have a different MT.
Conclusion: PFGE as well as MLVA revealed two major types and these were mainly concordant with each other. PFGE is probably in some cases more discriminating than MLVA, because 5 more PTs were found. Overall MLVA discriminates well and has several advantages above PFGE. It is easy and reproducible and therefore a reliable method to build a database for comparing strains during a longer period of time and between different laboratories.

P010

Stability of hypervariable microsatellite markers in *Aspergillus fumigatus*

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Introduction: Microsatellites or short tandem repeats are increasingly popular tools to discriminate between individual bacterial and fungal isolates because of their high discriminatory power. However, little is known about the stability of these sequences in filamentous fungi. We investigated the stability of 10 highly polymorphic microsatellite markers in *Aspergillus fumigatus*.
Methods: Five random *A. fumigatus* isolates were clonally expanded up to 55 generations. DNA was extracted from every generation and all DNA samples were analyzed using a 10 marker microsatellite panel consisting of three di-, tri-, tetra- and one nonanucleotide repeats.
Results: In total, 262 generations were analyzed (2620 markergenerations). In six cases, a change in the number of repeat units was observed. All alterations involved a change in 1 repeat unit. Five times the number of repeats expanded by 1 unit, in a single case a reduction of one repeat unit was found. Mutations were only observed in the di- and trinucleotide repeats, especially in isolates with high repeat units.
Conclusion: Microsatellites are sufficiently stable to be used in longitudinal epidemiological analyses of *A. fumigatus*. The large number of different genotypes indicates an overwhelming biological diversity in *A. fumigatus*.

P012

Molecular epidemiology of the global and temporal diversity of *Candida parapsilosis*

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Background: Our objectives were to study the global epidemiology of *Candida parapsilosis* and assess the discriminatory capabilities of restriction fragment length polymorphism (RFLP) typing methodology, which was compared with a RAPD method.
Methods: We used EcoRI digestion of cellular DNA to generate RFLP and compared brightly staining bands. RAPD analysis was done on genomic DNA. Band profiles were used to distinguish isolates and place them into 3 major DNA groups.
Results: From 7 diverse geographical areas, 536 isolates obtained over 35 years were divided into 23 RFLP subgroups. One subtype, VII-1, appears to be the dominant subtype worldwide, (82.4% of 536 isolates). For statistical analysis we divided the isolates into 2 groups, VII-1(442) versus non VII-1(92). Temporal variations for the United States pre-1995 versus post-1995 ($p<0.0001$), and for the United States pre-1995 versus Europe pre-1995 ($p<0.0001$), were found. Differences in genotype distribution existed among the localities ($p<0.0001$) and Mexico was unique ($p<0.05$), due to the high proportion of non-VII-1 isolates found in Mexico. There appeared to be no differences in distribution of types comparing babies versus adults, or blood stream isolates versus colonizing or environmental isolates. RAPD typing showed three separate major profiles, but was less discriminatory than RFLP.
Conclusion: The prevalence of *C. parapsilosis* type VII-1 apparently has risen in the USA and current isolates show some variation in distribution of types in some non- USA locales.

P014
Mycoplasma genitalium in the Netherlands

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Introduction: *Mycoplasma genitalium* (*Mg*) has been recognized as the third-most important cause of urethritis, after *Chlamydia trachomatis* (*Ct*) and *Neisseria gonorrhoeae* (*Ng*), and may also play a role in cervicitis and pelvic-inflammatory disease (PID). It may account for 15% of non-gonococcal urethritis. In contrast to *Mycoplasma hominis*, the prevalence of *Mg* in healthy subjects is low. Routine testing for *Mg* is not common in the Netherlands and data on the prevalence in the Dutch population are scarce. The goal of this study is to determine the prevalence of *Mg* in the Netherlands.
Methods: Study population: patients that visit their general practitioner (GP) with suspected sexual transmitted disease (STD) in the Delft/Westland area. Diagnostic STD-samples (urethral swabs, urine and vaginal swabs) were anonymized for this study. Patients were divided in the following groups, according to the clinical data provided by their GP: (1) men with urethritis, (2) women with Pelvic inflammatory disease (PID) / cervicitis, (3) men and women without clinical data but with diagnostic samples sent for STD-testing, (4) control groups of men and women: midstream urine samples of hospitalized patients, without suspicion of STD, tested in pools of 5 samples each.
Testing methods: (*Mg*) was detected by real-time PCR based on conserved regions of the MgPa adhesin gene as described by Jensen et al. (JCM 2004 vol. 42 pg. 683-92). Internal controls and positive controls were kindly provided by JS Jensen. *Ct* was tested by PCR, *Ng* was tested by PCR and/or by culture.
Results: The test results are depicted in the table. The *Ct* and *Ng* test results are given for comparison.
Discussion: The prevalence of *Mg* in our patient groups (12/393, 3.1%) is lower than in published data from STD clinics. This could be due to a difference in patient selection, i.e. the proportion of 'just to make sure' tests. *Mg* was found in numbers higher than *Ng* (4/393, 1.0%) but lower than *Ct* (37/393, 9.4%). *Mg* was not found in control samples. Although it must be taken into account that the control samples are less suitable for the detection of low-grade STD, this is in agreement with the concept that *M. genitalium* is a sexually transmitted micro-organism and that it is a causative agent of STD.

P016
Recombinant immunoblot and seven commercial EIAs for the diagnosis of Lyme borreliosis

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Background: Prior to 2005, we used the Virotech IgM and IgG EIAs as a negative screening: immunoblot was done on EIA positive sera only, unless requested by the treating physician. In blots by request we sometimes found positive results when the EIAs were negative.
Aim: To find out the truth about those discrepant results; to find the sensitivities and specificities of the assays involved in the study.
Methods: On a panel of sera, consisting of 46 EIA negative, immunoblot positive sera, 60 sera from *Borrelia*-negative donors, 16 from neuroborreliosis, 11 from early infections, 11 from late infections, 10 from syphilis, 15 from CMV and 15 from EBV patients, we performed Mikrogen Recomblot immunoblot, IgG and IgM EIAs from Virotech, Dako, Dade-Behring, Immunetics, and again Mikrogen. An internal reference was constructed by Latent Class Analysis (LCA).
Results: IgM. Sensitivities against the LCA reference were 7.7, 21.8, 70.1, 1.9, 3.7, 45.5, 37.5, 30.4, 0, 62.3, 46.3, 87.6, 59.0, 86.4% for p100, VlsE, p41, p39, OspA, OspC, p41i/B. garinii, p41i/B. afzelii, p18, Virotech (old), Dako, Dade-Behring, Mikrogen RecomWell, Virotech (new).

Specificities for the same antigens and assays were 99.3, 99.0, 81.0, 100.0, 99.2, 80.1, 96.2, 98.7. 100.0, 97.6, 95.8, 91.5, 82.6, and 91.7%. Sensitivity and specificity for the immunoblot as a whole were 62.5 and 80.5%.The LCA reference indicated a positive results for 14 of 15 EBV sera. IgG Sensitivities against the LCA reference were 51.4, 85.7, 82.9, 34.3, 0, 22.9, 28.6, 40.0, 48.6, 91.4, 60.0, 99.9, 94.2, 88.6, 98.6, 84.3% for p100, VlsE, p41, p39, OspA, OspC, p41i/B. garinii, p41i/B. afzelii, p18, Virotech (old), Dako, Dade-Behring (new), Dade-Behring, C6 (Immunetics), Mikrogen RecomWell, Virotech (new). Specificities were 96.6, 95.9, 48.3, 97.3, 99,3, 96.6, 97.3, 98.0, 100, 95.6, 98.3, 97.3, 98.2, 94.6, 95.6, 84.4%. Sensitivity and specificity for the immunoblot as a whole were 91.7 and 94.2%. The LCA reference gave plausible results across the serum panel.

Discussion: It is customary to use clinical status as a reference in studies such as this. Because serology is often important in establishing clinical status, this tends to favour the serological methods previously in use in the laboratory. Latent Class analysis presents a way out of this circle. It postulates two (or more) groups in the data, in our case positive or negative sera, and constructs a maximum likelihood estimate of group prevalences and of test sensitivities and specificities. Despite the obvious drawback 'it does not look beyond the data' it is a useful method in studies such as this one. The presence of Borrelia-reactive antibodies in EBV sera has been described before and is to be expected. Recombinant immunoblot showed finite accuracy. The warning (from Wilske) against primary immunoblotting appears well-founded. A diagnostic algorithm that uses two different EIAs and reserves immunoblot for discrepant cases should be considered.

P017

Development of a reliable protocol to prolong the shelf life of a commercial qualitative HCV-RNA assay

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Introduction: The shelf life of Bayer's Versant® QL TMA HCV-assay is, after opening of the kit, restricted to 3 months.

Due to the fact that several laboratories were testing just a very limited number of tests, only a part of the kit was used within these 3 months.

In 1994 we faced the same problems with a similar Gen Probe assay called Amplified MTD, for which we developed and tested an alternative protocol. We published the findings of this study in 1995[1].

The Versant HCV assay is based on the same technology as the Amplified MTD assay, using the TMA-system of Gen-Probe.

We developed and tested a protocol for an extended shelf life of the Versant HCV-assay in almost the same way as we did with Amplified MTD assay.

Methods: The amplification-, enzyme- and probe-reagents were aliquoted in four equal parts at the expiration date of the kit. Three parts were frozen minus 80 C. The remaining part was used the same day.

The proficiency panel, consisting of 10 known HCV-RNA concentrations, was also aliquoted at the expiration date and deep frozen at minus 80 °C.

The negative and positive calibrators were frozen at minus 20 °C

The other kit components ' the wash solution, selection reagent, oil and buffer for deactivation fluid – were stored beyond their expiration date at room temperature and the target capture reagent was put in the refrigerator at 6 C after opening at the expiration date of the reagent, assuming that all these reagents were rather stable by itself.

The test, using the proficiency panel, was performed four times at the following intervals: 0, 52, 111 and 182 days after the expiration date.

Results: The first 2 test runs gave the equal results at a 'normal' level. The results of the third run, 111 days after the expiratory date showed RLU-values at a approximately 10% lower level than the former test runs. The last run, after 6 months, however gave again results at a normal level.

Conclusion: The prepared reagents and enzyme mixture kept at minus 80 °C for at least 180 days didn't show loss of activity, so the kit can be used reliable for half a year longer than the given expiration date.

Reference:

F. Vlaspolder, P. Singer and C. Roggeveen
Diagnostic Value of an Amplification Method (Gen-Probe) Compared with That of Culture for Diagnosis of Tuberculosis, Journal of Clinical Microbiology, Oct 1995, Vol 33, No. 10, p. 2699-2703

P018

Validation of the bioMrieux MiniVidas varicella zoster virus, mumps virus and measles virus immunoglobulin G antibody assays for EDTA plasma samples

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Background: The MiniVidas (bioM rieux) is an automated immunoassay system that uses an enzyme linked fluorescence (ELFA) technology. For the assessment of varicella zoster virus (VZV), mumps and measles immunoglobulin (Ig)G antibodies, only serum is approved for use in the MiniVidas. This makes the system less versatile for diagnostic purposes, as often only EDTA plasma

is available. In the present study we have validated the MiniVidas VZV, mumps virus and measles virus IgG antibody assays for EDTA plasma.

Methods: Serum and EDTA plasma pairs that were taken within one day were obtained from 141 women aged 18-41 years and 3 men aged 25-39 years. All sample pairs were tested for VZV IgG and a subset of 60 sample pairs was tested for mumps virus and measles virus IgG.

Results: When using a VZV IgG reference serum for determination of the cut-off TV for positive VZV IgG levels, one discrepant serum EDTA plasma pair was found (positive for serum, TV=0.90 and negative for EDTA plasma, TV=0.76). Analysis of VZV IgG data (bioMérieux test value; TV) yielded a line with an intercept of -0.29 (SE=0.06; $p<0.05$ for intercept=0.0) and a slope of 1.14 (SE=0.02; $p<0.05$ for slope =1.0). These data showed a statistically significant difference for TV serum and TV EDTA plasma in the VZV IgG assay. When a subset of samples with TV serum <2.0 for VZV IgG was compared with the corresponding EDTA samples (manufacturers' positive cut-off of TV ≥0.9; n=55), a regression line with an intercept of 0.045 (SE=0.04; $p>0.05$ for intercept=0.0) and a slope of 0.97 (SE=0.03; $p>0.05$ for slope =1.0). VZV IgG TV's for serum and EDTA plasma did not differ significantly for TV <0.2. One discrepant sample pair was observed for mumps virus IgG and measles virus IgG each: serum indeterminate and EDTA plasma negative (according to manufacturers' cutoff). Analysis of mumps virus IgG data yielded a regression line with an intercept of 0.1 (SE=0.25; $p>0.05$ for intercept 0.0) and a slope of 0.98 (SE=0.09; $p>0.05$ for slope=1.0). Analysis of measles virus IgG data yielded a regression line with an intercept of 0.0 (SE=0.2; $p>0.05$ for intercept=0.0) and a slope of 0.96 (SE=0.08; $p>0.05$ for slope 1.0). No statistically significant differences between serum and EDTA plasma TV's were observed for mumps virus and measles virus IgG.

Conclusion: It is concluded that both serum and EDTA plasma samples can be used reliably in the MiniVidas for the assessment of VZV, mumps virus and measles virus IgG in an adult population.

P019

Evaluation of a new automated cytomegalovirus chemiluminescence immunoglobulin (Ig)M antibody assay

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Background: The recently introduced Immulite 2000 cytomegalovirus (CMV) immunoglobulin (Ig)M automated chemiluminescence assay (Immulite; Siemens) is compared with the established MIniVidas (Vidas; bioMérieux) CMV IgM and Axsym (Abbott) CMV IgM assays in immunocompetent and solid organ transplant (Tx) patients.

Methods: Serum samples of immunocompetent patients, aged one year and older were assigned to different groups according to their Vidas CMV IgM and Axsym IgG results. Group1: IgM negative or indeterminate and IgG negative (n=57). Group 2: IgM positive, IgG positive (n=44). Group 3: IgM negative and IgG positive (n=55). Group 4: IgM positive and IgM negative (n=6). Group 5: samples positive for varicella zoster virus IgM, Toxoplasma gondii IgM, hepatitis A virus IgM and Epstein-Barr virus IgM (n=23). Serum samples of Tx patients were assigned to different groups according to their CMV IgM and IgG results in a home made enzyme immunoassay (home EIA). IgM negative or indeterminate and IgG negative (n=17). IgM positive and IgG negative or indeterminate (n=11). IgM positive and IgG positive (n=40). IgM negative or indeterminate and IgG positive (n=14). CMV IgG in serum of Tx patients was determined with Axsym and Immulite.

Results: The sensitivities of the Vidas, Axsym and Immulite CMV IgM assays in immunocompetent patients (groups 1-4) were 100%, 81% and 86%, respectively (2/3 assays positive considered as true positive; indeterminate considered as negative). The specificities of the Vidas, Axsym and Immulite CMV IgM assays in immunocompetent patients (groups 1-5) were 98%, 92% and 98%, respectively (2/3 assays negative considered as true negative; indeterminate considered as negative). The sensitivities of the home EIA, Vidas, Axsym and Immulite CMV IgM assays in Tx patients were 100%, 98%, 91% and 94%, respectively (3/4 assays positive considered as true positive; indeterminate considered as negative). The specificities of the home EIA, Vidas, Axsym and Immulite CMV IgM assays in Tx patients were 85%, 100%, 88%, and 100%, respectively (3/4 assays negative considered as true negative; indeterminate considered as negative). Sensitivities and specificities for the home EIA, Axsym and Immulite CMV IgG assays were 98%, 100%, 98% and 96%, 96%, 100%, respectively (true negative, true positive and indeterminate as defined above). Panels of 4-6 consecutive serum samples from 10 Tx patients (all positive for CMV pp65 antigen), showed that CMV IgM was detected earliest in the Axsym and CMV IgG in the home EIA.

Conclusions: The Immulite CMV IgM showed an acceptable sensitivity and an excellent specificity for immunocompetent patients as compared to the Vidas CMV IgM assay. Excellent CMV IgM sensitivity and specificity were observed for the Immulte as compared to the Vidas and Axsym CMV IgM assays in Tx patients. An explanation for the observed differences for CMV IgM sensitivities between groups might be the selection of samples, as no discrimination between primary infection and reactivation of CMV was made.

P020

Evaluation of the ABBOTT M2000 SP/RT real-time *Chlamydia trachomatis* and *Neisseria gonorrhoeae* PCR assays using uro-genital and throat swabs in 2-SP transport medium

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Introduction: *Chlamydia trachomatis* (Ctr) and *Neisseria gonorrhoeae* (Ngo) are the most common micro organisms diagnosed in sexually transmitted diseases. Nowadays laboratory diagnosis of Ctr and Ngo is mainly based on molecular methods. In a prospective study we recently evaluated the new ABBOTT M2000 sp/rt real-time Ctr/Ngo PCR assay in urine specimens, and made a comparison with the ROCHE COBAS AMPLICOR (CA) Ctr/Ngo PCR test (see: abstract ECCMID, Munich 2007). We concluded that the M2000sp/rt assays are sensitive and specific, with a very low inhibition rate. For Ngo the M2000 assay is clearly more specific than the Ngo CA assay. The ABBOTT M2000sp/rt system is very suitable for routine diagnostic laboratories because of the automation and the high throughput. According to the Ctr/Ngo M2000 test protocol uro-genital swab samples or urine samples should be collected in a special Abbott transport medium. The system is not validated for other transport media, such as 2-SP, or other types of samples. In the present study we retrospectively tested selected uro-genital tract swabs and throat swabs in 2-SP in the M2000 sp/rt real-time Ctr/Ngo assay.

Materials and methods: Seventy-four uro-genital tract swabs in 2-SP medium were selected on the basis of the CA Ctr/Ngo PCR results: 5 were repeatedly inhibited in the CA Ctr/Ngo test but CA Ctr/Ngo negative after MagNA Pure isolation, 27 were CA Ctr positive, 43 were CA Ngo positive of which 25 were confirmed by an 'in house' real-time Ngo PCR.

Fourty-two throat swabs in 2-SP medium were selected on the basis of the CA Ctr/Ngo PCR results: 5 were CA Ctr positive, 37 CA Ngo positive, of which 3 were confirmed by the real-time Ngo PCR.

Results: None of the tested samples was inhibited. Of the 27 uro-genital tract CA Ctr positive samples, 24 were Ctr positive with the M2000sp/rt. The other 3 were not confirmed when the sample was repeatedly tested with the CA Ctr assay, indicating that these samples possibly gave a false positive initial result with the CA Ctr assay. All 5 CA Ctr positive throat swabs were also positive in the M2000sp/rt test. Of the 25 confirmed CA Ngo positive uro-genital tract samples 22 were positive with the M2000sp/rt Ngo assay. The remaining 3 samples were repeatedly negative by the M2000sp/rt and, at retesting, also in the 'in house' Ngo real-time PCR. Probably the DNA was degenerated during storage. The 3 confirmed CA Ngo positive throat swabs were also Ngo positive with the M2000sp/rt assay. The 52 initial CA Ngo positive not-confirmed samples (18 urogenital and 34 throat) were all negative in the M2000sp/rt assay.

Conclusions: The M2000sp/rt real time Ctr/Ngo assay performs well with specimens collected in 2SP-transport medium. Also with throat swabs the assay shows a good sensitivity and specificity when compared with the CA Ctr/Ngo PCR assay. For the detection of Ngo the M2000sp/rt assay is much more specific than the CA Ctr/Ngo PCR.

P021

Modification of a hemolytic assay for the measurement of functional human mannose-binding lectin by classical pathway inhibition

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Objective: Low levels of functional mannose-binding lectin (MBL), impairing early complement activation via the lectin pathway, have been associated with disease. Therefore, diagnostic procedures for MBL levels and lectin pathway activity are widely performed. In a previously described assay, MBL levels are measured via bystander-hemolysis after binding of MBL to mannan on *Saccharomyces cerevisiae* (Kuipers et al, 2002). All down-stream complement components are provided by adding MBL-deficient serum. As both the ligand-binding and complement-activating properties of the molecule are assessed simultaneously without interference of down-stream deficiencies, this is the only assay to quantitatively measure the level of functional MBL. Interference by classical pathway activity has been postulated, for example by C1q-binding immune complex formation of antibodies against *S. cerevisiae* (ASCA). Here we describe the classical pathway interference in the hemolytic MBL assay and the modification of this assay to prevent this artifact.

Methods: To determine the minimal inhibitory concentration, classical pathway activity was inhibited in a CH50 assay by preincubation of three 1:10 diluted ASCA-positive test sera with 0-150 ug/ml anti-C1q monoclonal antibodies. To examine the interference of classical pathway activity in the functional MBL assay, hemolytic activity was assessed in the MBL assay in absence and presence of 50 ug/ml anti-C1q in the three ASCA-positive sera and in 35 selected sera of donors with known genotypes.

Results: Anti-C1q inhibited all classical pathway activity dose-dependently, with full inhibition at 50 ug/ml. In three selected ASCA-positive sera, anti-C1q decreased hemolysis in the MBL assay in two samples. Most of the 35 selected sera did not show a significant difference with or without anti-C1q. A striking effect was found in some sera from donors with deficient genotypes XA/0 and 0/0.

Conclusion: The hemolytic MBL assay is designed to measure functional MBL levels by its ability to bind its ligand and activating complement, without interference of down-stream deficiencies. However, in some samples classical pathway activity can interfere with this functional MBL measurement, either via ASCA or other C1q-binding immune complex formation. This interference can and should be inhibited by anti-C1q to prevent that MBL deficiency is overlooked and patients are misdiagnosed.

P022

Validation of Dade-Behring Enzygnost Syphilis EIA against Fujirebio TPPA and Innogenetics LIA

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In order to establish usability for routine diagnosis of syphilis antibodies in a typical Dutch setting, the Dade-Behring Enzygnost Syphilis EIA was used in nearly 10,000 serum samples, in addition to the usual protocol, consisting of screening with Fujirebio TPPA and confirmation by line immunoassay ('blot'). In discrepant samples, the LIA result was used as a reference. A total of 9790 samples were tested, 9488 of which were negative in both tests. Two were equivocal in the EIA and negative in TPPA; one was positive in the EIA and negative in the TPPA. Five were EIA negative, but TPPA positive; four of those were blot negative. Six were EIA equivocal, TPPA positive. 288 samples tested positive in both tests. When equivocal results were counted as positive, sensitivity of EIA against TPPA was 98.33%; specificity, 99.97%. Under the same conditions, sensitivity of TPPA against EIA was 98.99%, and specificity, 99.95%. Background information on discrepant samples will be given to put these results in perspective. We conclude that the Dade-Behring EIA is usable for practical diagnosis of syphilis.

P023

Causative micro-organisms in community acquired pneumonia; high incidence of atypical bacteria and viruses

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Introduction: Community acquired pneumonia (CAP) is an infection with significant morbidity and mortality and is the most common infectious disease requiring hospitalization. CAP is caused by different micro-organisms each characterized by a different clinical course and need of antibiotic treatment. The aim of this study was to identify the causative micro-organism of CAP in a teaching hospital in the Netherlands.

Methods: All patients, admitted to the first aid department during a 22-month period, meeting the criteria for CAP, were found eligible for this study. Criterium for CAP was a new infiltrate on chest X-ray in combination with at least two out of the following five findings: cough, sputum production, temperature >38 °C or <35 °C, leukocytosis, leucopenia or left-shift in the differentiation and C-reactive protein >15 mg/dl. Patients with congenital or acquired immune suppression (including the usage of prednisone >20 mg for >3 days) were excluded for further analysis.

Microbiologic analysis was done by culture of sputum and blood, urine antigen testing for *Streptococcus pneumoniae* and *Legionella pneumophila*, serologic analysis for respiratory viruses and atypical pathogens, polymerase chain reaction (PCR) for *L. pneumophila*, *Mycoplasma pneumoniae* and *Chlamydia ps.* and viral culture of the pharynx. In case of positive viral serology or culture a CAP was only called a viral pneumonia in the presence of negative cultures of sputum or blood and negative findings of PCR and atypical serology, otherwise the viral infection was defined as co-infection.

Results: In total 201 patients (62% males) were included in this study, containing 61 (30%) patients with a pulmonary history, mainly COPD. Mean age was 63 years (SD 17). 117 (58%) patients were in Fine class I, II or III, 84 in Fine class IV or V. In 128 (64%) of the patients the causative pathogen of CAP was identified: 60 (30% of total population)) *S. pneumoniae*, 21 (11%) atypical micro-organism (9 *L. pneumophila*, 9 *M. pneumoniae*, 2 others), 21 (11%) Gram-negative pneumonia (mainly *Haemophilus influenzae)*, 16 (8%) viral pneumonia (6 influenza) and 8 (4%) Gram-positive bacteria (mainly *Staphylococcus aureus*). Viral co-infection was found in 17 patients, mainly with pneumococcal pneumonia. Mortality was 5% (10 patients; all in Fine-class V), ICU-admittance 10% (21 patients; mainly pneumococcal pneumonia) and median stay in hospital 11 days (range 3-143).

Conclusion: CAP is a serious infectious disease requiring long-term hospitalization. By using an extensive microbiological protocol it was possible to identify the causative micro-organism in 64% patients. As expected, most common found bacterium was Str. pneumoniae, followed by atypical micro-organisms and Gram-negative rods (especially in patients with COPD) and viral pneumonia. The incidence of both atypical and viral pneumonia is higher than expected, and is the result of to the usage of PCR and serologic techniques. There should be awareness of these micro-organisms in patients with CAP with negative cultures of sputum and blood.

P024

Analytical validation of real-time NASBA for detection of highly pathogenic avian influenza A/H5N1 viruses in comparison to real-time PCR

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Introduction: Infections of humans with highly pathogenic avian influenza (HPAI) viruses have been recognized for almost a decade now. Since 2003, the virus has caused widespread infections in birds and poultry. In addition, frequent human infections have been reported, mainly in Asia. By the end of 2006, 261 human cases were reported and 157 patients died (mortality 60%). The only way to confirm an infection with influenza A/H5N1 viruses is by laboratory diagnosis. Because of restrictions in culturing these viruses, most laboratories will perform molecular assays as real-time PCR. Here the performance of real-time NASBA is evaluated.

Methods: Serial dilutions of influenza H5N1 RNA of different HPAI isolates were subjected to H5 and N1 real-time NASBA and H5 real-time PCR. For further validation, the QCMD 2006 Influenza haemagglutinin typing pilot proficiency panel was subjected to these amplification assays.

Results: Both amplification assays enabled sensitive detection of three different HPAI (H5N1) strains, isolated in 1997 in Hong Kong, 2004 in Cambodia and 2005 in Vietnam. The sensitivity of the H5 real-time PCR was slightly better than that of real-time NASBA, but the NASBA provided faster results. The QCMD panel confirmed these results and revealed a good specificity of both assays.

Conclusion: Although no actual clinical samples were available for testing, real-time NASBA appears a sensitive and specific assay for diagnosing current human influenza A/H5N1 infections.

P026

Efficient DNA extraction from clinical samples for detection of *Mycobacterium tuberculosis* with real time PCR

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Introduction: For sensitive and rapid detection of *Mycobacterium tuberculosis* in clinical samples by nucleic acid amplification techniques (NATs) it is necessary to liquefy viscous samples, extract DNA and perform a real time NAT with an internal control. To decrease hands-on time and to avoid cross contamination between samples during manual extraction, we tested the Nuclisens easyMAG extraction robot (Biomérieux) for its efficiency to extract mycobacterial DNA and compared the results with the Boom method and with the outcome of culture.

Methods: Since the start of *M. tuberculosis* detection by NATs, N-acetyl-L-cysteine-NaOH (NALC-NaOH) was used for decontamination and liquefaction, followed by a silica based DNA extraction method (Boom JCM 1990;28:495). Real time PCR was performed targeting IS6110 and *Mycobacterium smegmatis* 1008 as internal control (Savelkoul J Microbiol Methods 2006;66:177).

Results: Stored NALC/NaOH pellets (n=17) from *M. tuberculosis* culture positive sputum samples were extracted with the Boom method and with the EasyMAG. The mean Ct value after Boom extraction was 0.82 lower than after EasyMAG extraction. However, when 23 other culture positive samples were not pre-treated with NALC/NaOH but with dithiothreitol (DTT), followed by proteinase K (PK) and sodium dodecyl sulphate (SDS), EasyMAG extracts showed Ct values 0.5 to 2.0 lower than after Boom extraction.

We also compared the results between culture and easyMAG plus real time PCR of 758 smear negative respiratory and non respiratory samples. 744 (98.2%) samples were culture and PCR negative, 5 (0.7%) were culture and PCR positive, 3 (0.4%) were only positive by culture and 6 (0.8%) were only positive by real time PCR.

Conclusions: The efficiency of NATs is improved by replacing the NaOH/NALC pre-treatment of viscous samples with DTT-PK-SDS. Furthermore, for detection of *M. tuberculosis* DNA the Nuclisens easyMAG is suitable for application on both respiratory and non respiratory samples. Introduction of this machine improves the quality of NATs and decreases hands on time. With this new approach the sensitivity of NAT testing of smear negative samples is as least as sensitive as culturing.

P027

Comparison of a molecular screening method with traditional culture or the detection of *Salmonella* spp. and *Campylobacter jejuni* in feces

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Introduction: *Salmonella* spp. and *Campylobacter jejuni* are the major causes of bacterial gastro-enteritis in the Netherlands. Conventional diagnosis is based on detection of both species in feces by traditional culturing which usually takes several days. We developed a sensitive molecular screening method (MSM) for the detection of both species which decreases the turn-around time significantly. This

study describes the comparison of this real-time PCR based screening method with routine culture for the detection of *Salmonella* spp. and *C. jejuni* in feces.

Methods: A total number of 2067 stool samples were analyzed at our laboratory. Routine culture was performed on fecal samples and consisted of enrichment, selective culture and phenotypic identification. The molecular method consisted of a semi-automatic DNA extraction in combination with real-time PCR assays for *Salmonella* spp. and *C. jejuni*. PCR positive samples as well as samples which demonstrated PCR inhibition were cultured afterwards, consisting of the identical procedure as described for routine culture. Also, data regarding time to generate final results were collected for the MSM.

Results: A total number of 2055 samples were included for validation of *Salmonella* spp. The detection of *Salmonella* spp. improved by 15% with molecular screening; sensitivity was 100% and specificity 99%. For *C. jejuni* 2009 samples were included and detection improved by 25%; sensitivity was 97% and specificity 75%. PCR inhibition was observed in less than 1.9% of all samples. The time to generate final results was less then 24 hours for all PCR negative samples (with exception of the inhibited samples), and in comparison to traditional culture there was no delay observed in generating results of culture confirmed PCR positive samples.

Conclusion: 1) The MSM has a great potential for rapid detection of *Salmonella* spp. and *C. jejuni* in feces. 2) Time to generate final results for negative samples was reduced dramatically to less then 24 hours. 3) The detection of *Salmonella* spp. and *C. jejuni* will improve considerably with molecular screening. 4) Automation of the extraction and detection procedures will further speed up the process and improve standardization of the molecular screening procedure.

P028

Highly efficient extraction of pathogen DNA from stool using the NucliSENS easyMAG Specific A 1.0.2 protocol

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Objectives: The NucliSENS easyMAG is a fully automated system for extraction of nucleic acids from a wide variety of clinical specimens. However, the extraction of DNA from stool with the standard protocol (Generic) has been shown to be less efficient compared to other clinical specimens. Preliminary data showed that this problem was in part related to impaired elution of the DNA. To overcome this problem, a new protocol, Specific A (bioM rieux) has been developed and validated for use with DNA rich specimen types, such as stool.

Methods: Clinical stools (n=94), including 43 stools positive for 1 or more intestinal pathogens, were used to challenge both extraction protocols. Relative recovery of DNA was assessed by spiking a known amount of *Hin*dIII-digested phage lambda DNA, and comparing the recoveries after gel electrophoresis. Impaired elution for Generic was assessed by a secondary elution of the DNA from the retrieved magnetic silica. Downstream performance with the extracted DNA was assessed with 2 internally controlled (IC) multiplex real-time PCRs each targeting 2-3 intestinal pathogens.

Results: Of the 94 stools tested, 61 showed nearly identical DNA recoveries without impaired elution, although the DNA yield was slightly higher with Specific A in all specimens. For the remaining 33 samples, 19 showed low DNA recovery with Generic, whereas Specific A showed variable but improved recovery. The other 14 specimens could be classified in three groups based on the ratio between the primary (1st) and secondary (2nd) elution for Generic. This ratio was 1st >2nd, 1st<2nd, and 1st=2nd for 4, 4, and 6 specimens, respectively. Results of the real-time PCRs confirmed the DNA recovery results. Ct values for Specific A were on average 0.9 and 1.1 cycles lower for IC and pathogen DNA, respectively, compared to Generic. The distribution of all the paired Ct values also showed a significant difference (paired 2-sided student T-test, $p<0.00002$). When the Ct values were addressed to the DNA recovery, the 61 specimens with nearly identical recovery showed on average 0.61 and 0.62 cycles lower Ct values with Specific A for IC and pathogen DNA, respectively, whereas the other specimens showed on average 1.5 and 1.9 cycles lower Ct values. Inhibition was not significantly different between both protocols (6-7%).

Conclusion: Based on the presented data, Specific A, results in a significant improvement in the performance of the NucliSENS easyMAG with stool specimens.

P029

Multicenter study of Shiga Toxin-producing *E. coli* in the Netherlands using real-time PCR assays

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Introduction: Traditionally detection of STEC is limited to serogroup O157 by stool culture on (CT-)SMAC. In 2000, 88% of laboratories used these cultures and only 6% tested for other STEC serogroups. In several European countries, STEC non-O157 are increasingly associated with diarrhoea, HUS and outbreaks. To assess the relative importance of STEC non-O157 in the Netherlands, a multicenter study was performed from October 2005 to November 2006.
Methods: Real-time PCR-based assays targeting the stx1 and stx2 genes were developed for LichtCycler (LC) and TaqMan (TM). Both assays proved to be specific for stx1 and stx2, had a sensitivity (100% hit rate) of approximately 10,000 (TM) or 100,000 (LC) CFU/g stool, and were very reproducible. Stool samples that were submitted for investigation of enteropathogens/diarrhoeal patients were screened for STEC using RT-PCR (2 labs LC, 6 labs TM) if the stool contains blood on macroscopic examination, if the patient had a history of bloody diarrhoea or if the patient was below 6 years of age, independent of bloody aspect. If these clinical criteria could not be met, a random sample was added to reach the required 10 specimens per laboratory per week. Very rare HUS cases were included as well. Stools testing positive were forwarded to the RIVM for STEC isolation on SSI Enteric medium and typing (O-serotyping, testing for stx1-, stx2-, eae- and ehly- genes by PCR).
Results: In total, 4,292 stools were tested, yielding 71 positive results (1.7%). The prevalence varied from 0.6% to 2.5% between laboratories. Inhibition of the PCR reaction was observed for 228 (5.3%) stool samples. The apparently lower sensitivity of the LC platform was not reflected in the study; the yield was 2.1% versus 1.5% on the TM platform. Nineteen stools (3.1%) containing blood on macroscopic examination yielded a positive result, 22 stools (2.4%) of patients with a history of bloody diarrhoea, 19 stools (1.0%) from children aged under 6, and 15 stools (1.4%) of the random additional samples. For only 24 (33.8%) RT-PCR positive stools, an STEC could be isolated. Eleven different O-serogroups were observed, in addition to some O-non-typable STEC strains. Most common were O157 (n=4), O103 (n=3), O8 (n=3) and O26 (n=2). Preliminary analysis of the first 3,498 stools tested, with a 1.3% STEC positivity-rate, showed that between 48 and 62% of the stools were also cultured for Campylobacter (n=2,150), Salmonella (n=2,143), Shigella (n=2,103) and Yersinia (n=1,685). Of these, 8.6%, 4.6%, 0.3% and 0.1% yielded a positive result, respectively.
Conclusion: 1) STEC were found in about 1 in 60 gastroenteritis patients tested, and were more common in patients with (a history of) bloody diarrhea, but not in children. 2) Compared to other enteropathogens routinely tested in many laboratories, STEC were detected more often than Shigella and Yersinia, but less often than Salmonella and Campylobacter. 3) As serogroup O157 represented only a small proportion of the STEC isolates, laboratories should be encouraged to use techniques, such as the developed RT-PCR, enabling them to detect the non-O157 serogroups.

P030
Direct detection of bacterial DNA in clinical samples; evaluating a 4 year period of sample collection and analysis

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Objective: Four and a half years ago the department of Research and Development (R&D) of the Laboratory for Infectious Diseases (LvI) started to analyse direct clinical specimens on request for the presence of bacterial DNA. This study shows an evaluation of 4½ years of detecting bacterial DNA in direct clinical specimens.
Methods: During the last 4½ years 480 specimens (that contain no bacteria in healthy individuals) were send to the LvI with suspicion of bacterial involvement, where culturing bacteria was not possible or unlikely to yield results, e.g. because antibiotic treatment had already commenced.
Putative bacterial DNA was isolated from these samples by means of mechanical disruption of cells followed by a Boom extraction. If the specimen consisted of tissue it was first digested in order to form a cell homogenate. A PCR was performed on these samples to detect the presence of bacterial DNA coding for 16S ribosomal RNA. Positive PCR products were subsequently sequenced to determine which organism was present in the clinical specimen. (ref. Schuurman et al., JCM 2004;42(2):734).
Results: Of these 480 specimens, 101 were found to contain a single bacterial species. In 351 specimens no bacterial DNA was found. In the remaining 28 specimens a single bacterial micro-organism could not be identified either because of the presence of more than 1 strain in which case sequencing yields an uninterpretable signal, or the PCR remained negative due to inhibiting factors in the specimen.
Specimens had a wide variety of origins but 67% of requests pertained to only 4 biological locations: joints 25%, blood 19%, central nervous system 16% and lung 7%. From these organs came 62% of the positive results with a single bacterial strain. None of the specimen types from the different organs submitted for bacterial DNA detection produced only negative results.
Many different species were found: the identified strains belonged to 30 different known genera. Streptococci were found by far the most: Of the positive specimens 33% contained streptococci, whereas the next most frequently found genus was *Bacteroides* with only 7% of all positive results.

Conclusion: Even though the number of positives isolated from various biological sites differs, every organ could yield a positive result. Therefore in cases where bacterial infection of normally sterile biological sites is suspected, determining whether bacterial DNA is present provides useful information.

P031

HBV DNA extraction from serum using the NucliSENS easyMAG platform

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Background: The NucliSENS easyMAG platform (bioMérieux) is designed for nucleic acid extraction from a broad range of different sample specimens. However, for some specific applications additional pre-extraction procedures and/or a specific extraction protocols might be needed. Recently a new extraction protocol (Specific A) was introduced for whole blood specimens.

Objectives: The aim of the study was to measure the recovery efficiency of HBV DNA from serum using five different extraction protocols. Four of these protocols were performed in combination with the NucliSENS easyMAG platform and one other manual method was included as reference.

Methods: A panel of 12 samples, including 4 standards (obtained from VQC, Amsterdam), 7 HBV DNA positive serum samples and 1 negative control, were tested with five different extraction protocols. Identical input (200 ul) and output volumes (110 ul) were used for the different methods. Briefly, for the NucliSENS easyMAG the following procedures were tested; 1) no pre-incubation and Generic extraction protocol, 2) pre-incubation with proteinase K and Generic extraction protocol, 3) no pre-incubation and Specific A extraction protocol, and 4) pre-incubation with proteinase K and Specific A extraction protocol. The High Pure PCR Template Preparation kit (Roche diagnostics) was used as reference method. To all samples control virus (PhHV) was added to measure overall extraction performance. Extracted samples were analyzed by real time PCR. Results: PhHV DNA was detected in all samples. Mean Ct values were 29.6, 29.8, 29.5, 29.3, and 31.4 for protocol 1, 2, 3, 4, and 5, respectively. HBV DNA was not detected in the negative control samples. For the remaining samples HBV DNA was detected in 100% (protocol 1, 2, 4, and 5) and 91% (protocol 3). For the samples scored positive with all five methods the mean Ct values were 32.8, 31.0, 32.9, 30.5, and 31.3, respectively. Conclusion: Best results, for both HBV DNA and control PhHV DNA detection, were obtained in combination with protocol 4 that uses proteinase K pre-incubation, followed by extraction with the NucliSENS easyMAG platform using the Specific A protocol. The proteinase K pre-incubation resulted in on average '2.1 lower Ct values for HBV DNA detection, whereas for this application the Specific A protocol contributed minimal (on average -0.2 Ct) to the overall improvement measured.

P032

Presence of *Toxoplasma gondii* in BAL fluid samples detected by means of real-time PCR

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Introduction: *Toxoplasma gondii* is an obligate intracellular parasite with a ubiquitous distribution. After the acute, in immunocompetent hosts often-asymptomatic phase of infection, bradyzoites remain within the human body. In immunocompromised hosts it is known for its tendency to reactivate and to cause disseminated disease. Pulmonary infection with *T. gondii* is associated with a mortality exceeding 50% and routine analysis of all BAL fluid samples from immunocompromised patients for the presence of *T. gondii* has been opted. With this retrospective study, we intended to study a cohort of BAL fluid samples of immunocompromised patients for the presence of *T. gondii* by means of real-time PCR.

Materials and method: This study was conducted at the University Hospital Maastricht, a 750-bed hospital. All BAL fluid samples obtained in the period January 2000 until December 2005 BAL from immunocompromised patients (patients with HIV, hemato-oncological malignancy with and without leucopenia, and/or using immunosuppressive drugs suspected of infection), and immunocompetent patients (patients without the above, suspected of ventilator-associated pneumonia (VAP) and out-patients suspected of having non-infectious pulmonary disease) were retrieved from –80 °C storage. All samples were analyzed by quantitative real time polymerase chain reaction (PCR) targeting the B1-gene of *T. gondii*.

Results: A total of 425 BAL fluid samples were included, 294 from immunocompromised patients (HIV-positive: n=37, malignancies: n=145, immunosuppressive drugs: n=112), 98 from patients suspected of VAP and 33 BAL fluid samples from out-patients with a normal BAL fluid differential cell count and negative bacterial cultures.

T. gondii DNA was detected in only one sample. This sample belonged to a 38-year-old male patient of Ethiopian origin admitted to the hospital for weight-loss, fever, dyspnoea and coughing. He was diagnosed with Pneumocystis pneumonia and AIDS. His CD4 count was $4x10^6$/L and he

was consequently treated for three weeks with high dose cotrimoxazole and highly active anti-retroviral therapy was initiated. The BAL fluid sample had been reported positive for *Pneumocystis jiroveci* with a high Pneumocystis load. However, upon careful review, no *T. gondii* parasites were observed.

Conclusions: Although the presence of *T. gondii* in the population tested was found to be low, real-time PCR analysis for the detection of *T. gondii* could be considered in immunosuppressed patients.

P033

Validation of the NucliSens^{EasyMAG} for automated DNA extraction from clinical specimens for routine diagnostics of *Mycobacterium tuberculosis*

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Introduction: Real-time PCR has improved the speed for detection of *Mycobacterium tuberculosis* infections. In the procedure of PCR diagnostics in routine diagnostic laboratories, the automation of DNA extraction is an important issue. As DNA isolation from *M. tuberculosis* is notoriously difficult, each newly applied method for DNA isolation from this species needs careful evaluation. We have compared the NucliSens® EasyMAG with the routinely used manual BOOM® technology for its efficiency of DNA isolation from Mycobacterial DNA in clinical specimens.

Methods: The recovery was first assessed by testing recovery of serial dilutions of spiked DNA in Tris-EDTA with carrier DNA and spiked CFU in pooled sputum with the EasyMAG and the BOOM® technology. The efficiency of DNA recovery was measured with a real-time PCR targeting the IS6110 sequence of *M. tuberculosis* (Savelkoul et al., JMM, 2006). Serial dilutions of DNA and CFU ranged from 106 to 1 CFU-equivalent. Next, 78 clinical samples; sputa (n=20), BAL (n=19), lymph nodes (n=3), urines (n=9), liquor (n=4), synovial- and pleurafluid (n=4) were tested. DNA recovery was tested for all these samples with an internal amplification control.

Results: With the BOOM® the DNA recovery was 10 CFU-equivalent/ml and improved with the EasyMAG™ to 1 CFU-equivalent/ml. The recovery from spiked sputa varied for both methods between 10-100 CFU-equivalent/ml. Of the 78 clinical samples, 2 samples showed PCR inhibition after BOOM® extraction; no inhibition was observed with EasyMAG™ samples. With extraction of the clinical samples with the EasyMAG the crossingpoints (CP) of the internal control varied according the inter-assay variation of 1 CP. The CPs of positive M. tuberculosis samples were highly comparable in both methods.

Conclusion: The EasyMAG™ showed better reproducibility at low CFU concentrations. With both methods the extraction from sputum was less efficient but was still satisfactory. Due to its automation is the EasyMAG™ less prone to contamination and is technician-friendly.

P034

Clinical value of bacterial DNA load in blood for prediction of Gram-positive bacteraemia in critically ill patients

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Introduction: We evaluated the clinical value of determination of *Staphylococcus aureus* and *Enterococcus faecalis* bacterial DNA load (BDL) during bacteraemia in critically ill patients. BDL was related to clinical and laboratory variables, and to microbiological culture results.

Methods: Blood samples for PCR were obtained whenever blood was drawn for culture from patients admitted to the intensive care unit of our hospital. After extraction of DNA from 200µL blood with the QIAamp DNA Mini Kit, real-time PCR amplification was performed on a TaqMan 7000 System with specific primers and probe targeting the 16S rRNA gene of *S. aureus* and
E. faecalis. A quantitation curve was included to determine the BDL.

Standard 2x2 tables were calculated to evaluate performance of BDL compared to blood culture results. Receiver operating characteristic (ROC) curves were designed to evaluate predictive values of BDL, C-reactive protein (CRP) and leukocyte count for bacteraemia. Finally, logistic regression analysis was performed including BDL, CRP and leukocyte count to study dependence between these variables.

Results: Blood samples for determination of S. aureus BDL were included from 98 episodes in 80 patients and from 100 episodes in 80 patients for determination of E. faecalis BDL. Sensitivities of *S. aureus* and *E. faecalis* BDL as compared to blood culture results were 67% and 75%. In 8 and 5 of the episodes, BDL was positive while blood cultures remained negative, resulting in specificities of 90% and 94%. Blood samples of these episodes were obtained more frequently under antimicrobial therapy than those that were blood culture positive ($p<0.05$). In addition, local *S. aureus* infection was less likely to be present in those with only positive BDL results as compared to those with positive BDL and blood culture results ($p<0.01$), especially an intravascular source was less common ($p<0.05$).

The median (range) *S. aureus* BDL was 440 (3-3370) cfu equivalents/mL; median E. faecalis BDL was 130 (12-6582). *S. aureus* and *E. faecalis* BDL were significantly lower

during episodes that were only BDL positive as compared to blood culture positive episodes (p<0.01).
The area under the curve (AUC) for the ROC curve for prediction of bacteraemia was 0.81 (p<0.001) for *S. aureus* BDL and 0.85 (p<0.001) for *E. faecalis* BDL. The AUCs for the ROC curves for leukocyte count and CRP did not have discriminative power for prediction of *S. aureus* or *E. faecalis* bacteraemia except for the AUC for leukocyte count in predicting *S. aureus* bacteraemia (0.67; p=0.03). Regression analysis showed that *S. aureus* and *E. faecalis* BDL had independent predictive value for bacteraemia, but not CRP or leukocyte count.
Conclusion: 1) Determination of *S. aureus* and *E. faecalis* BDL can be used to quantify bacteraemia in ICU patients, even when blood samples are obtained under antimicrobial treatment or when there is no clear focus of infection. 2) BDL may be used as an independent alternative to CRP or leukocyte count for prediction of bacteraemia in critically ill patients.

P036

Antibiotic resistance of *Pseudomonas aeruginosa* and seasonal differences in positive cultures results from a laboratory surveillance study (ISIS) in the Netherlands: an overview of 2001-2005

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Background: *Pseudomonas aeruginosa* can cause severe invasive, mainly hospital related, infections in immuno-compromised patients. Worldwide there is an increase in the number of nosocomial infections caused by multidrug resistant *P. aeruginosa*. In this study seasonal difference and resistance of *P. aeruginosa* in the Netherlands were analysed from 2001-2005.
Methods: Data from nine laboratories participating in a sentinel laboratory surveillance project (ISIS) covering approximately 3.3 million inhabitants were used. A *P. aeruginosa* infection was considered present if a culture was positive for *P. aeruginosa*. In order to include only one isolate per disease episode, only the first positive sample per patient over a period of 6 months was considered. Seasonal variation and trend over time of the positive cultures were studied. Results were analysed separately for age (0-1, >1-10, >10-49 and ≥50), type of material sampled (blood, wound, respiratory, urine) and for care taker (general practitioner, hospital, outpatient clinic, nursing home). Resistance against beta-lactams (piperacilline/ piperacilline+tazobactam/ ceftazidim), carbapenems (imipenem/meropenem), fluoroquinolones (ofloxacine/norfloxacine/ciprofloxacine/levofloxacine), aminoglycosides (gentamicine/tobramycine/netilmycine/amikacine), polymixines (colistine/polymixine B) was evaluated. Multidrug resistance was studied as resistance against at least three of the following groups: Beta-lactams, carbapenems, aminoglycosides and/or fluoroquinolones.
Results: A total of 15884 cultures were tested positive for *P. aeruginosa* from 2001 to 2005. Of these 998 (6%) cultures were from patients tested positive more than once every 6 months. For the latter 14886 positive cultures a seasonal pattern was seen with highest numbers of positive cultures in the summer. An 18% increase in positive cultures was observed from 2001-2005 (2799 to 3298 positive cultures/per year). 56% of all patients were man.
The age distribution showed an increased number of patients below the age of 1 year old and above the age of 60 when compared to the Dutch population age distribution. The most frequent material per age group and gender was: age 0-9, purulence/wound (37%) for boys and urine (27%) for girls; age 10-49, purulence/wound (37%) for men and respiratory (36%) for women; and age ≥50, respiratory (35%) for men and urine (34%) for women.
The most frequent material per care taker was: general practitioner, urine (35%); nursing home, urine (48%); hospital, respiratory (34%); and out-patient clinic, purulence/wound (41%). The 5 hospital wards with the most positive cultures were: respiratory diseases (1209 cultures), ICU (856), surgery (763), internal medicine (655) and pediatrics (342).
Resistance in 2005: beta-lactams 4%, carbapenems 4%, fluoroquinolones 12%, aminoglycosides 8% and polymixines 7%. In 2005 multidrug resistance was 5% (105 out of 2053 tested). From 2001-2005 resistance followed the same pattern.
Conclusion: The number of positive *P. aeruginosa* cultures was highest in the summer and lowest in the winter. Increased number of *P. aeruginosa* infections was observed with an increasing age. Resistance and multidrug resistance remained low and stable over the last 5 years.

P037

Incidence density of highly resistant Gram-negative bacteria in a Dutch hospital

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Objective: The objective of this study was to determine the incidence density of highly resistant Gram-negative bacteria (HRGNB) in a Dutch hospital.

Method: Between 2005, January first and 2006 June 31 all HRGNB (*Enterobacteriaceae, Acinetobacter* spp and *Pseudomonas* spp) from hospitalized patients in the Amphia hospital were included. The definition for highly resistant micro-organisms according to the national guideline (www.wip.nl) was used. From all patients, one isolate of each species was selected. When multiple isolates from one species were found in one patient the selection was made based on the susceptibility pattern. All isolated with major (S<>R) differences in susceptibility for amoxicilline-clavulanic acid, cefalosporines, quinolones, meropenem, aminoglycosides or sulfa-trimethoprim were included. HRGNB isolated more than 72 hour after admission or less than 72 hour after admission but within 30 days after discharge were defined as nosocomial. Patients who were on the ICU or who had been on the ICU in a period of 30 days before culture were defined as ICU-related. The number of bed days was collected from the hospital administration.

Results: In a period of 1.5 years, 121 patients with 139 HRGNB were identified, including: *Escherichia coli*(95), *Klebsiella* spp (11), *Proteus* spp (9), *Enterobacter* spp (7), *Citrobacter* spp (5), *Pseudomonas aeruginosa* (5), *Acinetobacter* spp (3), *Morganella* spp (2), *Salmonella* spp (1) and *Serratia* spp (1). 106 HRGNB were nosocomial, of which 59 HRGNB were ICU related and 47 were non-ICU related. The ICU accounts for 15.427 bed days and the rest of the hospital for 383.070 in 1.5 years. The incidence density for nosocomial HRGNB on the ICU was 38,2 and in the rest of the hospital 1.2 per 10.000 bed days (RR: 31.1 95%CI 21.3-45.5).

Conclusion: Estimating the incidence density of HRMO according to well-defined criteria can be used as a benchmarking method. Considering the enormous difference between the ICU and the rest of the hospital most attention to control the development and spread of HRGNB should be given to the ICU.

P038
Comparison of several methods to detect ESBL-producing strains

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Introduction: Resistance genes like ESBL's are difficult to detect in diagnostic laboratories. Within our laboratory, *Escherichia coli* and *Klebsiella* spp are suspected to produce ESBL if ceftazidime is resistant or cefazoline is resistant in combination with tobramycine and/or cotrimoxazol resistance. Subsequently the presence of ESBL should be confirmed by phenotypical methods. The goal of this study is to compare several available phenotypical methods.

Methods: The following methods were used:

- The automated VITEK 2 Compact system (BioMérieux, Marcy-l'Etoile, France).
- The Disc Approximation Test (DAT): 3 disks containing third generation cephalosporines are placed in the vicinity of a clavulanic acid containing disk and ESBL production is shown by growth inhibition due to the synergism between the clavunanic acid and a third generation cephalosporine.
- B&D test: a disk diffusion test of susceptibility to CTX (30 µg), CAZ (30 µg), CTX plus CLA (10 µg), and CAZ plus CLA (10 µg) (Becton Dickinson, USA). ESBL production is confirmed if testing in the presence of CLA the diameter of the inhibition zone increases for these drugs by at least 5 mm (compared with results obtained with the cephalosporin alone).

Quality control strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853) were used by each new batch.

Results: A total of 233 isolates of *E. coli* and *Klebsiella* spp (meeting the inclusion criteria) were studied for ESBL production. The result of this study showed that for 140 strains all 3 tests are negative. For 65 strains all the 3 tests are positive for ESBL. 24 strains were ESBL positive by Vitek, but negative by the other 2 tests. Only 1 strain was tested negative by the Vitek, while the DAT and BD tests were positive. 3 strains were negative by the BD-test and positive by Vitek and DAT.

Conclusion: Out of 233 strains, 205 test (88%) results are in correspondence with each other. A substantial number of strains (10%) are tested positive by Vitek, but negative by the other tests. We should conclude that the Vitek gives a discrepant result in 24 times, but these isolates should be further characterized by molecular techniques. In our hands, both phenotypical disk tests work well as a confirmation for ESBL carriage.

P039
Development of a multiplex PCR to detect the presence of blaTEM, blaSHV, blaCTX-M and qnrA1 genes

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Introduction: Antibiotic resistance due to the production of extended-spectrum β-lactamase (ESBL) is a growing problem in the Netherlands. The molecular basis of cephalosporins resistance is divers. The four most prevalent hydrolysing enzymes are encoded by TEM, SHV and CTX-M genes in *Escherichia coli, Klebsiella pneumoniae* and other *Enterobacteriaceae* and by the OXA gene in *Pseudomonas aeruginosa*. For all four genes, many genetic variants have been described. Besides this cephalosporin-resistance, fluoroquinolone-resistance is emerging too. Recently, in

the Netherlands a plasmid was identified which contained both cephalosporine resistance (blaCTX-M-9) and quinolone resistance (qnrA1) genes. As a first step in the development of a molecular ESBL typing system, we developed a multiplex PCR which detects all variants of blaTEM, blaSHV and blaCTX-M genes and the qnrA1 gene.

Methods: Primers directed against blaTEM, blaSHV and qnrA1 were developed in this study. Primers directed against blaCTX-M were used as described in the literature (A.A.C. 47: 3724'3732). The multiplex PCR was optimized by using a step-by-step protocol (BioTechniques: 504-511). The resulting PCR was tested against isolates obtained from an ongoing ESBL/QNR screening in our hospital.

Results: After optimization, the multiplex PCR was able to detect simultaneously the presence of blaTEM, blaSHV and blaCTX-M genes and the presence of the qnrA1 gene. The multiplex PCR could be performed on a simple lysate from a resuspended bacterial colony in water. PCR products were analysed on agarose gels. The whole procedure could be performed within 3 hours.

Conclusion: We developed a multiplex PCR to detect the major genes that confer cephalosporin-resistance and the qnrA1 gene that codes for fluoroquinolone-resistance. This multiplex PCR is a first step in the development of a molecular ESBL typing system and the assay could already be used as a fast confirmation of phenotypically suspected ESBL isolates.

P040

Detection of DNA mutations associated with ethambutol resistance in *Mycobacterium tuberculosis* complex strains

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The increase in drug-resistant *Mycobacterium tuberculosis* is a worldwide health problem. Therefore, the molecular basis of the respective resistance mechanisms has been studied extensively. In contract not much is known about mutations underlying ethambutol (EMB) resistance in *M. tuberculosis* complex.

In 60-70% of all ethambutol-resistant *M. tuberculosis* strains mutations are present in the *emb* CAB-operon. This operon, with a total length of 10 kb, consists of the *emb*A-, *emb*B- and *emb*C-genes. Codon 306 of the *emb*B-gene is the most frequently mutated codon in ethambutol-resistant strains.

Methods: Detection of mutations in codon 306 of the *emb*B-gene was performed by the real-time method, using *M. tuberculosis* complex specific primers (Applied Biosystems), a high specific LNA probe (Eurogentec S.A.) and the ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

Sequence analysis by the Genetic Analyzer 3100-Avant (Applied Biosystems) of two fragments of the *emb*B-gene and three fragments of the *emb*C-gene showed that there were fewer mutated codons in other positions of the gene.

Results: In total 85 *M. tuberculosis* strains were tested: phenotypical EMB-resistant (56), sensitive (14) and intermediair-susceptible ones (15). Real-time PCR showed that 61% of the EMB-resistant strains and 33% of the EMB intermediair-susceptible strains were mutated in codon 306 of the *emb*B-gene. Sequence analysis showed, besides mutations in codon 306, mutations in codon 497 and a deletion of six codons (404 to 409) of the *emb*B-gene. Analysis of the amplified fragments of the *emb*C-gene revealed mutations in codons 207, 394, 738 and 981.

Conclusion: By performing the real-time PCR for the detection of mutations in codon 306 of the *emb*B-gene, 61% of the phenotypically EMB-resistant strains could be detected. Molecular analysis can distinguish between genotypic EMB-resistant and EMB-sensitive strains which can explain some phenotypic intermediair susceptible *M. tuberculosis* strains.

Sequence-analysis of fragments of the *emb*B- and *emb*C-gene resulted in detection of more mutations causing EMB resistance. The real-time method is suitable for routine use in diagnostic laboratories. The hands-on time for DNA sequence-analysis is much longer in comparison with real-time PCR, which makes it less suitable in diagnostic settings, especially because of the length of the *emb*CAB-operon.

P041

Detection of mutations in *kat*G 315, *inh*A/*mab*A regulatory region and *oxy*R/*ahp*C intergenic region for isoniazid-resistant *Mycobacterium tuberculosis* complex by real-time PCR

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Introduction: Rapid and reliable detection of isoniazid (INH) resistance in *Mycobacterium tuberculosis* is crucial for the control of tuberculosis. However, mutations at several chromosomal loci of *M. tuberculosis* are associated with INH resistance. From previous studies is known that, besides the *kat*G ser315thr mutation (57% of the INH-resistant strains), 19% of the INH-resistant strains contain the mutation '15 C T in the *inh*A/*mab*A (fabG1, Rv1483) regulatory region and 14% are mutated in the *oxy*R/*ahp*C intergenic region. Real-time PCR makes it possible to detect mutations associated with INH resistance within a few hours. This study was conducted to investigate the detection level of this approach, targeting three genomic mutation sites associated with INH-resistance.

Methods: Three *M. tuberculosis* complex specific real-time PCRs were developed, using the ABI 7500 fast real-time PCR system (Applied Biosystems): one for detection of the *kat*G ser315thr mutation and one for the '15 C T mutation in the *inh*A/*mab*A regulatory region by using two TaqMan probes. A third test detects mutations in the *oxy*R/*ahp*C intergenic region, between bp 4 and '17; '22 and '51, by using two TaqMan probes of the wild type sequence.

In total 207 *M. tuberculosis* complex strains were collected in the Netherlands by the RIVM, in Tanzania and Indonesia. Strains were tested phenotypically for INH resistance; 157 INH resistant and 50 INH susceptible.

Results: A total of 207 *M. tuberculosis* strains was tested for mutations in *kat*G 315, *inh*A/*mab*A regulatory region and *oxy*R/*ahp*C intergenic region; 157 strains were tested phenotypically as INH-resistant and 50 strains were tested phenotypically as INH-susceptible.

Of the 157 INH-resistant strains, 88 (56.1%) contained the *kat*G ser315thr mutation, 41 (26.1%) the *inh*A/*mab*A regulatory region '15 C T mutation and 11 (7.0%) contained mutations in the *oxy*R/*ahp*C intergenic region.

Six (4.5%) of the 134 mutated INH-resistant strains were mutated at multiple loci; two strains contained the mutation '15 C T in the *inh*A/*mab*A-regulatory region and *kat*G ser315thr, four strains contained a mutation in the *oxy*R/*ahp*C intergenic region in combination with the *kat*G ser315thr mutation.

The MIC value of strains with the mutation '15 C T in the *inh*A/*mab*A-regulatory region was between 0.5 and 2 μg/ml; for the isolates with a mutation in the *oxy*R/*ahp*C intergenic region or *kat*G ser315thr, it was between 1 and 20 μg/ml. None of the phenotypic INH-susceptible strains contained a mutation in one of the three genomic sites analyzed.

Conclusion: Three real-time PCRs can detect 85.5% of the INH-resistant strains.

Detection of '15 C T single mutation in the *inh*A/*mab*A-regulatory region of *M. tuberculosis* indicates a low-level INH resistance.

These real-time PCR tests can be optimized for detection in clinical material as already shown for the *kat*G ser315thr mutation. It will improve early detection of INH resistance and prevent the spread of INH resistance.

P043

EUREGIO MRSA-net Twente/Münsterland: cross-border molecular surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA)

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The increasing cross-border transfer of patients and health-care workers (HCW) within the EU causes enormous problems with regard to control the spread of multi-resistant pathogens. Especially within the Dutch-German border region, there are great discrepancies in the rate of methicillin resistant Staphylococcus aureus (MRSA) of all *Stapholococcus aureus* infections (the Netherlands: <1%; Germany: 5-30%). In addition, within the last two years an increasing number of community-acquired (CA)-MRSA could also be noted in the Netherlands, which represent a danger particularly for the population outside of hospitals. Differences in the regional epidemiology of MRSA were determined. In order to elucidate the molecular cross-border epidemiology of MRSA within the EUREGIO MRSA-net Twente/Münsterland, MRSA isolates of patients and HCWs were typed. The epidemiological backbone of the project is a typing network providing a common language based on S. aureus protein A (spa) gene sequencing. Since the beginning of the project in July 2005, more than 1300 isolates from in- and out-patients and nursing home inmates were typed. The spa types t004, t009, t024, t036, t045, t051 and t091 were determined in the German part of the Euregio, only. On the Dutch side, spa t026 was the most prevalent. However, the majority of spa types on the German side were in concordance to the German national epidemiological trend with spa t001, t002, t003, t004 and t032 as the most common spa types. spa type t044 ' often associated with CA-MRSA in central Europe ' was detected on both sides and was the second most frequent spa type on the Dutch side, on the contrary to the German side, where t044 counted for less than 2% of all spa types. Focusing on hospitalized patients, MRSA in blood cultures were mainly spa type t001, t003, t004, t008 and t032. In summary, the

use of spa typing demonstrated the cross-border spread of MRSA. The trend analysis showed a growing homogeneity of spa types only on the German side of the EUREGIO, indicating an epidemic transmission on the German side. Based on these experiences, new co-ordinated regional and cross-border strategies need to be developed in order to fight MRSA in the EUREGIO.

P044

Fighting methicillin-resistant *Staphylococcus aureus* internationally: a systematic comparison of protocols

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Introduction: This study provides a conceptual framework for the international comparison of methicillin-resistant *Staphylococcus aureus* (MRSA) protocols. Aims of the framework are to 1) provide a basis for user adapted protocols applicable in cross-border situations, and to 2) predict problems concerning the compliance with protocols in practice.

Methods: A framework for protocol development for processing industry was adapted to the health care setting. National MRSA protocols from Germany, the Netherlands, the United Kingdom, and the United States were compared (n=13). Interviews with Dutch and German protocol makers from the responsible national authorities were conducted to explain the differences shown by the protocol comparison (n=4).

Results: According to the framework, two different types of MRSA protocols seem to exist: First, a protocol emphasizing safety regulations by referring to law and including levels of evidence (Germany, the United Kingdom). Second, a protocol in which MRSA is considered as a management problem, containing user-centered elements such as decision trees, and referring to information and communication technology (the Netherlands, the United States).

Conclusion: A protocol integrating the two opposing visions on an MRSA protocol does not seem to exist yet, according to the framework developed in this study. This might lead to poor compliance with MRSA protocols in practice. The lack of one of the visions might cause feelings of insecurity with health care workers about how to act adequately, so they will start applying their own insights. This may lead to a lower level of compliance and increase the spread of MRSA. Whether this logic can be generalized to practice is currently studied by a test with real time protocol use and a survey in Germany and the Netherlands.

P045

Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: 2005-2006 compared to 1999-2000

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Introduction: The Dutch Search-and-Destroy policy to prevent transmission of MRSA is based on screening and isolation of patients with known risk factors for carriage of MRSA, as defined by the guidelines of the WIP (Werkgroep Infectie Preventie). Patients without risk factors for MRSA (WIP risk category 4) are not screened, so the prevalence of MRSA within this group of patients has to be studied on a regular basis. In 1999-2000 the prevalence was 0,03%. Our study measured the prevalence of MRSA carriage at hospital admission in 2005-2006.

Methods: Between October 2005 and November 2006, patients expected to be admitted for at least four days to one of five participating Dutch hospitals (Amphia Hospital Breda, Erasmus MC Rotterdam, VU Medical Center Amsterdam, University Medical Center Utrecht, Canisius-Wilhelmina Hospital Nijmegen) were screened for nasal carriage of *Staphylococcus aureus* within the first 24 hours of admission. Nose swabs were inoculated on blood agar plates and in a non-selective phenyl mannitol broth. After 48 hours, a loop of broth was subcultured onto a blood agar plate. Colonies morphologically suspected as being *S. aureus* were tested with an agglutination test (Slidex Plus, Biomérieux, France). Slidex-positive strains were tested for cefoxitin-susceptibility by disk diffusion. All strains suspected for methicillin-resistance were confirmed by a *S. aureus* specific DNA hybridization test (AccuProbe, Gen-Probe Inc., USA) and a PBP-2'-Latex agglutination test (MRSA-Screen, Denka Seiken Co., Japan).

Results: 4446 patients were screened for nasal carriage of *S. aureus*. Six patients (0.13% of all patients screened) were found to be MRSA-carriers. Compared to 1999-2000, MRSA carriage rate has increased significantly (p=0.02, Chi square-test). One of these patients had been identified as belonging to one of the risk categories for MRSA carriage. He was isolated and screened upon admission in accordance with the guidelines of the hospital. One patient was a pig farmer, and would now have been identified as belonging to risk category 2 of the WIP-guidelines. However, he was screened before the addition of pig farmers to this category of the guidelines and thus considered as belonging to category 4. The four remaining patients (0.09%) were all belonging to category 4 of the

WIP-guidelines as defined at this moment. Thus, compared to 1999-2000, the prevalence of MRSA in patients at admission without known risk factors as defined at this moment has increased from 0.03 to 0.09%. This increase is not significant (p=0.14, Chi square-test).

Conclusion: We found that the overall prevalence of MRSA-carriage among patients admitted to the hospital in 2005-2006 was 0.13%. Compared to 1999-2000, when the prevalence was 0.03%, MRSA-carriage rate at admission has increased significantly (p=0.02).

However, the prevalence of MRSA carriage at admission in patients not belonging to one of the risk categories of the WIP-guidelines as defined at this moment was 0.09%. Compared to 1999-2000, this increase is not statistically significant (p=0.14).

P046

Transmission of methicillin-resistant *Staphylococcus aureus* from pigs with exudative epidermitis to humans

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This case study is the first report on the culturing of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinically diseased pigs.

In a farrowing compartment of a breeding farm (farm A), a litter of 3-week-old piglets had clinical signs of exudative epidermitis. A sample was taken from the skin lesions of one piglet. Large numbers of *Staphylococcus aureus* (*S. aureus*) were cultured. Usually, the causative agent of exudative epidermitis in pigs is *Staphylococcus hyicus hyicus*. However, no *Staphylococcus hyicus hyicus* was found. Antimicrobial susceptibilities were determined by agar diffusion. The *S. aureus* strain was susceptible to enrofloxacin, trimethoprim/ sulfamethoxazole and fusidic acid, but resistant to beta-lactams, gentamicin, kanamycin, tetracycline, erythromycin, clindamycin and tylosin. The presence of the *mec*A gene was confirmed by PCR. Two weeks later samples were taken from 5 other piglets with exudative epidermitis, from one healthy sow, and from two veterinary students working with the pigs on the breeding farm. In addition, samples were taken on a multiplying farm (farm B) supplying gilts to farm A (samples from the nares of 12 healthy weaners, 10 healthy gilts and two farmers). MRSA was cultured from one student, one farmers, the sow, the skin of three piglets with exudative epidermitis, and 20 pigs on farm B. All MRSA isolates were susceptible to trimethoprim/sulfamethoxazole and enrofloxacin, and resistant to tetracycline, gentamicin, and kanamycin. Most isolates were susceptible to lincomycin and erythromycin, but some isolates were resistant to these two antimicrobials. All MRSA-isolates were genotyped by PFGE using *Sma*I as restriction enzyme, spa-typing and Multi Locus Sequence Typing (MLST). Typing of the Staphylococcal Cassette Chromosome (SCC*mec*) was performed by PCR. All isolates were non-typeable by PFGE, had spa-type t011, MLST ST 398 and SCC*mec* type IV.

In conclusion, MRSA was cultured from piglets with exudative epidermitis on a breeding farm and from healthy pigs on a multiplying-farm selling gilts to the first farm and two persons working on the farms. Genotyping showed that the MRSA strains were indistinguishable suggesting direct transmission between pigs and humans.

P047

Methicillin-resistant *Staphylococcus aureus* and veal-calf farming

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen, highly prevalent in many European countries and throughout the world. In the Netherlands, the prevalence of MRSA among clinical isolates is still among the lowest in Europe. However, reports about MRSA in pig farmers are increasing and a recent patient-control study by the National Institute for Public Health and Environment (RIVM) suggested that farmers with contact to veal calves are more frequently colonized with MRSA. This led to a change in MRSA policy in the hospitals where pig farmers are now admitted in MRSA isolation and farmers with veal calves are cultured for MRSA at admittance.

Case: In march 2006, we unexpectedly cultured MRSA from an infected ear eczema of a 48-year-old woman. The strain was also resistant to erythromycin, doxycycline, clindamycin, trimethoprim-sulfamethoxasole, and ciprofloxacin. Initial screening cultures from nose, throat and perineum were negative, however, after one month follow-up cultures were positive. The strain was non-typable by pulsed-field-gel-electrophoreses (PFGE) at the RIVM. Screening cultures from the woman's partner revealed MRSA with similar resistance pattern in the nose, but throat and perineum were negative. The couple is involved in raising veal calves on a farm. To investigate whether eradication of the MRSA carriership could be successful and to find the source of the MRSA, we decided to screen calves and co-workers of the farm.

Investigation: Swabs were taken from anterior nares and rectum of 19 calves, aging from one to 15 weeks. In addition, swabs were taken from the nose and throat of

two co-workers , two swabs from manure in calf-boxes and five from equipment that was used to prepare the milk for the calves. All samples were cultured for MRSA according to national guidelines for detection of MRSA in human samples. Isolates were compared by random PCR fingerprinting (ERIC) and Amplified Fragment Length Polymorphism (AFLP).
Results: MRSA was cultured from a 9-weeks-old calf and from manure of a box of calves that were 7 weeks old. The two co-workers were culture negative. Comparison of MRSA isolates from the calf, the manure, the index patient and her partner by random PCR fingerprinting and AFLP revealed identical genotypes.
Conclusion: This report shows that veal calves can be colonized with MRSA. The fact that the MRSA strain is non-typable by PFGE suggests that it might be related to the MRSA from pigs. Furthermore, our investigation clearly shows the occurrence of clonal spread and transmission of MRSA between humans and veal calves. Based on these findings, a similar MRSA policy for pig farmers and farmers with veal calves should be considered.

P048
Rapid detection of the mecA-gene in *Staphylococcus* spp by real-time PCR

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The rapid detection of the *mec*A-gene in clinical isolates that present as methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus intermedius* (MRSI) and coagulase negative staphylococci (MRCNS) is very important. The detection of the *mec*A-gene by conventional PCR is a time consuming procedure. Real-time-PCR can be used to detect the *mec*A-gene in pure cultures within one hour. At the Veterinary Medical Diagnostic Center, we use a LightCycler for the detection of the mecA gene in staphylococci. Template DNA was prepared by the whole bacteria cell boiled lysate method. The primers used are MecA1 5'-GTT GTA GTT GTC GGG TTT GG-3' and MecAC3 5'-CTT CCA CAT ACC ATC TTC TTT AAC-3'. The reaction mix consisted of 10 µl of TaKaRa SYBR®Premix Ex TaqTM (Cambrex, Bioscience, Verviers), 1.0 l of each primer (6.25 pmol/µl), 1.6 µl of 25 mM MgCl2, 4.4 µl aqua dest. and 2 µl template. The LightCycler 1.5 instrument (Roche Diagnostics, Mannheim, Germany) was programmed to make a hot start at 95 °C for 10 sec., followed by 35 cycles of annealing at 52 °C for 15 sec., elongation at 72 °C for 15 sec. and denaturation at 95 °C for 15 sec., followed by melting curve analysis; product melting point was 76 °C.
A total of 270 multidrug-resistant staphylococcal isolates were tested and 203 were *mec*A positive. The isolates were from horses, cattle, pigs, dogs and cats. MRSA ATCC 43300 was tested as positive control and MSSA ATCC 29213 and ATCC 25923 were tested as negative controls. All MRSA isolates were sent to the RIVM and all were confirmed as being MRSA. The MRSI isolates were sent to the Eijkman-Winkler Institute and confirmed as *mec*A positive. In conclusion, the detection of the *mec*A-gene in methicillin resistant staphylococci by real-time PCR is a rapid and reliable method.

P049
High methicillin-resistant *Staphylococcus aureus* (MRSA) carriership among healthcare workers and their family members during an outbreak as detected by repetitive molecular screening

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Introduction: During the last years, a MRSA was isolated on several occasions from wound cultures obtained from patients at a neurology ward of a teaching hospital. None of these patients had a risk factor for MRSA-carriage and they were not epidemiologically related. After each MRSA detection all healthcare workers (HCWs) were screened for MRSA carrier ship. In all cases, the MRSA isolates were typed based on the *Staphylococcus aureus* protein A (SPA) gene sequences and it always was SPA-type T-026. Only during the last episode 2 HCW's were colonized. Based on these observations it was hypothesized that a HCW might be a persistent MRSA carrier escaping detection. To find this carrier we decided to screen all HCW's on 4 consecutive weeks and test for the presence of a MRSA by real-time PCR (RT-PCR) and culture techniques. When positive, HCW's were not allowed to work, and swabs were also obtained from their housemates.
Methods: Swabs were taken from nose and throat and were cultured overnight in an enrichment broth containing ceftizoxim. The enrichment broth was subcultered and DNA was isolated from it for RT-PCR based on the Huletski-protocol. MRSA-isolates were al SPA-typed.
Results: Based on RT-PCR results, four HCWs were MRSA positive on week 1, one on week 2, two on week 3 and two on week 4. In the end 11 (25%) HCWs were demonstrated to be colonized by a MRSA. From these 11 HCWs 5 (45%) had one or more MRSA positive housemates. Although many RT-PCR positive enrichment cultures were initially MRSA negative on subculture, isolates could be obtained by further subcultering in 90% of the HCWs. All were SPA-type T-026.
Conclusion: RT-PCR is a powerful tool for the detection of MRSA. Although initially many RT-PCR positive samples

were negative on subculture, by further subcultering an isolate could be obtained and typed in 90% of the cases. Also the repetitive screening demonstrates, that despite the availability of the RT-PCR, MRSA carriers can be missed. Therefore this combined approach offers promises for the investigation of MRSA outbreaks.

P050

Home cooking: a simple method to discriminate pig-farming associated methicillin-resistant *Staphylococcus aureus* from human strains

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A recent increase in incidence of community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in hospitalized patients in the Netherlands has been found to be mainly caused by strains associated with pig-farming. Hospitals mainly burdened with this problem are those with a large influx of patients from rural areas where pig farming is abundant. Typically, these hospitals do not have the advanced facilities needed to discriminate pig-farming associated MRSA strains from human strains.
In the setting of the Dutch MRSA search and destroy policy, correct and rapid identification of these strains is however of great importance. The need for an easily applicable rapid test to discriminate these organisms thus seems evident.
We have developed a test based on amplification and length analysis of the 16s-23s interspace (IS) region. Every *S. aureus* strain has 5 or 6 IS regions in it's chromosome. The length of these regions vary within the chromosome, so when these regions are amplified and sorted by length using polyacrylamide gel electrophoresis (PAGE), each strain produces a pattern of bands. Classification of 100 strains of methicillin-sensitive *S. aureus* and MRSA based on these patterns showed good agreement with AFLP based classification. What is more, we found that all 37 pig-farming associated strains we tested, showed an identical pattern, specific for these strains.
We have tried to construct the most simple and quick method to acquire these patterns. This method does not even require DNA isolation. Instead, a standardised amount of bacteria is lysed by making a suspension in milliQ water. After centrifugation of this suspension, the supernatant can be directly used for pcr. To sort the pcr products by length, they are applied to a 1,5% agarose gel and electrophorated during 1 hour. Classification of strains can be made using the patterns thus obtained. This whole procedure can be performed within 5 hours.

Conclusions: 1) Discrimination of pig-farming associated MRSA from other strains can be done based on length polymorphism of 16s-23s IS region. 2) This test can be performed within 5 hours by every bacteriological laboratory equipped with a pcr machine and gel-electrophoresis machine.

P051

Timesaving screening method for meticillin-resistant *Staphylococcus aureus* using HAIN GenoType MRSA direct after overnight incubation

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Methicillin resistant *Staphylococcus aureus* (MRSA) prevalence in the Netherlands is very low. Traditional culture methods to detect MRSA carriage in patients and health care workers are slow. We wanted to shorten the time between collection of screening samples and detection of MRSA presence. Swab samples of throat, nose and perineum (and wounds or urine if present) were collected in duplicate and incubated in broth for 18-24h at 36 °C. Then MRSA CHROMagar plates and blood agar plates were inoculated with the broth and incubated for 18-48 h.
Also the broth was lysed and the supernatant was tested using the HAIN GenoType MRSA Direct assay. In total 1071 swabs were tested from 160 patients.
Only culture-positive MRSA was considered to be real MRSA.
PCR is an expensive techniques. Nevertheless introduction of such a method might result in shortening the duration time of patient isolation and save expenses for the hospital organization.
Because there was no Real Time PCR we investigated an easy and fast method to fit in our laboratory possibilities. Hain Lifescience offers a test method (GenoType® MRSA Direct) consisting of a conventional PCR and detection of amplified product by LiPA.
High correlating results for all negative materials were achieved using the GenoType® MRSA Direct assay after incubation for 18-24 h. However application of the GenoType® MRSA Direct assay without incubation for 18-24 h diagnosed less positive samples (results not shown).
In addition incubation for 18-24 h was also better applicable in our laboratory setting.
Material resulting in a colored MRSA zone of the DNA strip will be confirmed by culture technique because of the low specificity. The result of the culture technique is obligatory.
By using the MRSA direct assay, the majority >95% of patients and health care workers were negative. Therefore results were faster available then by using only the culture method.

P052

A comparison of three commercial methicillin resistant *Staphylococcus aureus* chromogenic media

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Different methods for detection of methicilline reistant *Staphylococcus aureus* (MRSA) by culture media are known. We use blood agar based media and enrichment broth. A small amount of MRSA is very difficult to find between the competing bacterial flora. Becaus methicilline sensitive *S. aureus* (MSSA) and MRSA colonies look identical, lots of colonies have to be tested to determine whether it is a MRSA or not. By using media containing selective supplements this flora can be inhibited. Chromogenic media use certain sugars and chromogenic substrates which can be fermented by the pathogen of interest. So direct identification of MRSA can be made.
The aim of this study to test the practibility of different chromogenic media in our lab routine.
During winter 2005/2006 samples (nose, throat and perineum) were inoculated in MRSA-broth (Oxoid), incubated overnight and subcultured consecutively according to the in house method and to the new method. All evaluable chromogenic media were practicable for detection of MRSA. MRSA CHROMagar was the most specific in our hands.

P054

The change of methicillin-resistant *Staphylococcus aureus* clones in a Dutch University Hospital between 2002 and 2006

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Introduction: The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands is still low (circa 1%), but an increase in prevalence has been observed in recent years. This may be due to increased cross-border healthcare, which is especially important for our university hospital (azM) situated near the borders of three countries (Belgium, Germany and the Netherlands with a MRSA prevalence of 23.6%, 13.8%, and 0.6% respectively), or may be due to an increase of the prevalence of community-acquired (CA) MRSA. The aim of this study was to test this hypothesis by characterizing MRSA strains isolated in the azM during recent years.
Methods: MRSA strains (n=179), isolated between 2002 and 2006, were characterized by spa typing and SCC*mec* typing. The number of isolates per year were 32, 58, 35, 39, and 15. The presence of collagen adhesion (CNA), Panton Valentine leukocidin (PVL) and toxic shock syndrome toxin 1 (TSST-1) was investigated by real-time PCR. The antibiotic susceptibility was determined.
Results: In the azM, MRSA isolates associated with the Brazilian/Hungarian clone emerged in 2003 and disappeared thereafter, while MRSA strains associated with the Pediatric clone were found between 2002 and 2005. Minor MRSA clones, such as the Berlin, the Iberian, the New York/Japan, the Southern Germany, the UK EMRSA-3, the UK EMRSA-2/6 and the UK EMRSA-15 clone, were mainly found during the last years of the study. CNA was present among 33% of the strains and all strains associated with CC8 and CC45 harbored CNA. The prevalence of TSST-1 was 16%. Furthermore, 2% of the MRSA strains were PVL-positive, and belonged to the ST8 and ST80 clones. A correlation of approximately 83% was found between the SCC*mec* type and the antibiotic susceptibility pattern.
Conclusion: 1) MRSA strains associated with the New York/Japan, the Pediatric, the Southern Germany, the UK EMRSA-3, and the UK EMRSA-15 clone were newly introduced in the Netherlands, probably due to cross-border healthcare. 2) The low prevalence of PVL-positive MRSA strains suggest that the prevalence of CA-MRSA is low in the azM.

P055

An outbreak of methicillin-resistant *Staphylococcus aureus* in a Dutch nursing home during 2004

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Introduction: Nursing homes are considered an important source for both community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and hospital-acquired MRSA (HA-MRSA). The aim of the study was to investigate the genetic background of MRSA isolates that caused an outbreak of MRSA in a Dutch nursing home during 2004.
Methods: All MRSA isolates were characterized by *spa* typing and SCC*mec* typing. The presence of, the virulence factors Panton Valentine leukocidin (PVL) and toxic shock syndrome toxin 1 (TSST-1) was investigated by a real-time PCR assay.
Results: A MRSA outbreak took place in a nursing home in the south of the Netherlands. In addition to the index case, six other inhabitants and three staff members were found to be MRSA positive. A second surveillance revealed the presence of a further eight inhabitants and three staff members positive for MRSA. All 15 inhabitants and nine staff members colonized with MRSA were given mupirocin

treatment. Furthermore, infection control measurements were implemented according to the guidelines of the Dutch Working party on Infection Prevention (WIP) for MRSA in nursing homes. After six weeks, the MRSA outbreak was under control due to the implemented infection control measurements. All MSRA isolates belonged to either *spa* type t442 or t447, and both are related in their spa repeat pattern. SCC*mec* typing revealed the presence of *mecA*, *ccrAB2* and IS*1272*, and the absence of *mecI*, suggesting the presence of SCC*mec* type IV in the MRSA isolates. The *spa* typing and SCC*mec* typing showed that the MRSA strains were associated with the Pediatric clone (ST5-MRSA-IV). All isolates were found to be negative for PVL and TSST-1 genes.

Conclusion: A MRSA outbreak in a Dutch nursing home has been caused by a strain belonging to the HA-MRSA Pediatric clone.

P056

Methicillin-resistant *Staphylococcus aureus spa*Clonal complexes in the Euregion Meuse-Rhine

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Introduction: The Euregion Meuse-Rhine (EMR) consists of the border regions of Belgium, Germany and the Netherlands. Cross-border patient mobility and free access to hospitals is an important issue in the EMR, but concern is rising about the possible dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA), since the prevalence of MRSA is different in the three countries (23.6%, 13.8%, and 0.6% in Belgium, Germany, and the Netherlands, respectively). The aim of the study was to investigate the dissemination of MRSA in the EMR and the possible spread of community-acquired (CA) MRSA strains in hospitals in the EMR.

Methods: A total of 257 MRSA strains (44 from Belgian, 93 from German and 121 from Dutch hospitals in the EMR), isolated between July 2005 and April 2006, were characterized by *spa* typing and SCC*mec* typing. The presence of collagen adhesion (CNA), Panton Valentine leukocidin (PVL) and toxic shock syndrome toxin 1 (TSST-1) was investigated by real-time PCR, The antibiotic susceptibility patterns were determined.

Results: MRSA strains associated with clonal complex (CC) 5, 8, 30 and 45 were disseminated in the EMR. The Dutch isolates were associated with ST5-MRSA-II, ST5-MRSA-IV, ST8-MRSA-IV, ST30-MRSA-IV, ST36-MRSA-II and ST45-MSRA-IV, while the Belgian and German isolates were mainly associated with ST45-MTSA-IV and ST5-MRSA-II, respectively. CNA was present among 36% of the isolates and all isolates associated with CC30 and CC45 harbored CNA. TSST-1 was present among 3% of the isolates. Furthermore, 5% of the MRSA isolates were PVL-positive. These isolates were classified as CA-MRSA and had a diverse genetic background, associated with ST1, 8 and 80. A correlation of approximately 78% was found between the SCC*mec* type and the antibiotic susceptibility pattern.

Conclusion: 1) The Dutch isolates had a more diverse genetic background compared to the Belgian and German isolates. 2) MRSA clones associated with ST5-MRSA-II, ST5-MRSA-IV, ST30-MRSA-IV and ST36-MRSA-II have not been described previously in the Netherlands. 3. CA-MRSA has entered the hospital environment in the EMR.

P057

A positive culture with *Staphylococcus aureus* from the tip of an intravascular catheter: to treat or not to treat?

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Introduction: It has not been determined whether patients with positive results of catheter cultures (but with negative blood culture results) and no other obvious site of infection need to be treated with antibiotics (IDSA guidelines for the Management of Intravascular Catheter-Related Infections). If not, then there may be no need to obtain samples from catheters for culture at all. We aimed to quantify the occurrence of bacteremic complications, and the effects of antibiotic treatment hereon, in patients with line cultures positive for *Staphylococcus aureus* but without accompanying documented *S. aureus* bacteremia (SAB) at the time of line removal.

Methods: The database of the clinical microbiology laboratory was searched for patients who had positive cultures of an intravascular catheter with *S. aureus* between 1 June 2001 to 1 October 2006. Patients with SAB in the week preceding until one day after removal of the catheter were excluded (since the guidelines are clear on the fact that these patients should always be treated with antibiotics). Patient charts were retrospectively reviewed to determine whether patients had received antibiotic treatment, whether localized and systemic signs of infection were present, whether blood cultures were drawn up to the day after removal, and whether SAB developed as a complication. Bacteremic complications were defined as a combination of 1) SAB two days or more after intravascular catheter removal and 2) clinical deterioration of the patient.

Results: There were 190 patients with positive intravascular catheter cultures with *S. aureus*; 85 patients (45.7%) had

SAB before or within one day after catheter removal. One patient was lost to follow-up. Of 104 patients who fulfilled the inclusion criteria, 56 were treated with antibiotics; in this group 1 patient had a bacteremic complication (1.8%). This patient had received a new intravascular catheter before the start of antibiotic treatment and developed intravascular catheter associated SAB after cessation of treatment.

Among the 48 patients who were not treated, 10 developed complications with *S. aureus* (20.8%); this included: SAB of unknown origin (5), catheter-related SAB, arthritis, an abcess of the elbow, osteomyelitis, and an infected bone graft. All complications occurred within a range of 2 to 14 days after catheter removal. Two patients succumbed to their complications. Most, but not all, of these 47 patients had documented local signs of infection (tenderness, rubor or purulence) at the time of catheter removal.

Conclusion: Untreated positive intravascular catheter cultures with *S. aureus* were associated with a high percentage of complications (20.8%), even in patients who had no apparent signs or symptoms of systemic infection. Although limited by its retrospective nature and biased by the clinician's decision whether to culture a removed intravascular catheter, the results of this study strongly indicate that, when encountered, a positive line culture with *S. aureus* is an indication for antibiotic treatment.

P058

The distribution of immune evasion cluster and chemotaxis inhibitory protein among *Staphylococcus aureus* nose isolates is comparable to clinical isolates

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Background: Several *Staphylococcus aureus* virulence factors are bacteriophage encoded. β-Haemolysin-converting phages harbour genes that are involved in modulating the innate immune response. The best known example is Chemotaxis Inhibitory Protein of *S. aureus* (CHIPS), an excreted innate immune modulating protein located on an immune evasion cluster (IEC). Seven IEC types have been described, each containing at least two virulence genes involved in immune evasion. Recently it was described that 90% of clinical strains carry one of these IEC types.

Here we studied the distribution of IEC-types and CHIPS production of clinical and nose isolates of *S. aureus*.

Methods: From 246 clinical and 77 nose isolates the IEC and Multi Locus Sequence Type (MLST) were determined. Ninety-eight randomly chosen clinical and nose isolates derived from adult patients with diverse S. aureus diseases (UMCU 2002-2006), were used for determining CHIPS production.

Isolates were grown overnight in Luria Broth at 37 °C, 250 rpm. Fresh LB was inoculated at OD600 0.0025 and bacteria were grown for 6 hours and used to inoculate a second culture which was grown for 6 hours. Supernatants were collected every hour and stored at 4 °C. A CHIPS capture enzyme-linked immunosorbent assay was performed to determine CHIPS concentrations in the supernatants as described by de Haas et al. Three independent replicates were performed.

Results: Ninety percent of nose and clinical isolates were positive for IEC, but no significant differences in distribution of IEC types between nose and clinical isolates was observed.

Forty-six percent of all 322 isolates were positive for IEC type A, B, C or F. Type B was the dominant IEC type in the chp-positive collection (IEC type A, B, C or F) and total collection (present in 63% and 36% of the isolates, respectively). The distribution of the other IEC types types were as follows: type A: 19% and 11%, C: 7%; 4%, D: 0; 16%, E: 0; 13% F: 11%; 7% and G: 0; 3% whereas untypable isolates accounted for 0; 11% respectively. No correlation was found between IEC type and Clonal Complex (CC).

CHIPS production started during the mid-exponential phase and highest production was measured in the late exponential phase. CHIPS production varied heavily in and between CC's and IEC-types (31-124 ng/ml CHIPS). The average CHIPS production was 74±29 ng/ml CHIPS. Nose isolates had a slight tendency for lower CHIPS production (71±29 ng/ml) compared to clinical isolates (86±44 ng/ml), but other differences were not seen between CC or IEC.

Conclusions: 1) The distribution of IEC among *S. aureus* nose isolates is comparable to clinical isolates. 2) IEC type B is the most abundant type at the UMCU, whereas type C and G are less abundant. 3) IEC types and CHIPS production in vitro was not linked to MLST based CC's suggesting horizontal transfer of IEC harbouring phages. 4) Similarly, no association was found between strains containing IEC types A, B, C or F and CHIPS production. 5) Nose isolates showed a somewhat lower CHIPS production compared to clinical isolates, but the difference was not significant.

P059

Staphylococcus epidermidis isolated from human peri-catheter tissue harbour biofilm-associated genes as well as *agrC*

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Background: *Staphylococcus epidermidis* is responsible for the majority of biomaterial associated infections. Biofilm formation on the surface of biomaterials is considered the major cause of persistent infection. However, in experi-

mental biomaterial-associated infection, *S. epidermidis* predominantly colonizes the tissue surrounding implants, and also in deceased human patients we recently found that the tissue surrounding catheters is an important niche for staphylococci. Assuming that *S. epidermidis* will initially adhere to the biomaterial, their detachment, which depends on a functional *agrC*, is essential for colonization of the tissue. In the present study we therefore aimed to assess whether the strains isolated from tissue harboured the known *S. epidermidis* biofilm genes, as well as *agrC*.

Methods: Six strain pairs retrieved from catheters and corresponding tissue sections from 4 deceased patients were identified by 16S sequencing and API Staph, and typed by amplified fragment length polymorfism (AFLP). Presence of the virulence genes *agrC, aap, sarA, icaA/B/C, atlE*, and of the insertion element IS256 was investigated by PCR. For 2 strain pairs *agrC* expression in planktonic culture as well as in biofilm mode of growth was assessed by RT-PCR.

Results: All strains were identified as *S . epidermidis*, and strains from the catheters had the same AFLP patterns as the corresponding tissue strains. PCRs for *agrC, aap, sarA, icaA/B/C, atlE*, and IS256 were positive for all strains. *agrC* was expressed in the strains isolated from the catheters and from the surrounding tissue, under planktonic condition as well as in the biofilm mode of growth.

Conclusion / discussion: *S. epidermidis* isolates obtained from peri-catheter tissue of humans all harbour genes associated with biofilm formation and virulence, and do not differ from the *S. epidermidis* isolates from the catheter surface. This implies that *S. epidermidis* are capable of initially colonizing the biomaterial surface, to subsequently detach and invade the surrounding tissue.

P060

Potential of medical grade honey for prevention of catheter-associated infections

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Introduction: Catheter-related bloodstream infections are a serious problem in critically ill patients. These infections may originate either from the skin microflora (extraluminal), or from contaminated hubs or fluids (intraluminal). Although the skin is intensively disinfected prior to catheter insertion and a sterile dressing is applied, microorganisms residing in e.g. hair follicles, re-colonize the skin under the catheter dressing. Skin colonization at catheter insertion sites is associated with bloodstream infections, suggesting that bloodstream infections may arise from recolonized insertion sites. Local application of antibiotics and antiseptics reduces skin colonization and associated catheter-related infections, but increases the risk for resistance development. An antimicrobial preparation not associated with resistance development is medical grade honey. Honey has potent antimicrobial activity owing to (i) its high sugar content, (ii) the presence of glucose-oxidase producing hydrogen peroxide, (iii) low pH and (iv) additional as yet unidentified bactericidal compounds. We therefore assessed the potential of medical grade honey to prevent microbial skin colonization.

Methods: We assess the microbicidal spectrum of medical grade honey using quantitative killing assays in liquid medium, and in 'direct contact' microbicidal assays. The efficacy of honey to reduce skin colonization was studied in healthy volunteers, according to a protocol approved by the AMC medical ethics committee. Cultured bacteria were identified by routine microbiological diagnosis and sequencing of their rDNA.

Results: Medical grade honey had broad-spectrum microbicidal activity in vitro. Multiresistant Staphylococcus epidermidis (MRSE) were killed after 24 h by 30% honey (v/v), while *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VREF) were killed by 40% honey. Application of honey for 2 days on 2x2 cm patches of forearm skin of healthy volunteers reduced the colonization frequency from 79% (37/47) for controls to 13% (6/47) for the honey-treated group. Median numbers of colony-forming units (CFU) decreased from 100 CFU for the control group, to 0 CFU for the honey-treated group.

Conclusions: Medical grade honey had potent microbicidal activity in vitro and in a clinically relevant setting. A trial to assess the efficacy of honey to reduce skin colonization at potential catheter insertion sites of ICU patients is in preparation.

P061

The interaction of a 15 kDa *Ixodes* salivary gland protein, Salp15, with *Borrelia burgdorferi* sensu lato

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Spirochetes belonging to the *Borrelia burgdorferi* sensu lato group are the causative agents of Lyme borreliosis, and are predominantly transmitted by *Ixodes* ticks. In Europe, the three major *Borrelia* genospecies, *B. burgdorferi sensu stricto* (Bbss), *Borrelia garinii* (Bg) and *Borrelia afzelii* (Ba) are spread by *Ixodes ricinus*. In contrast, only *B. burgdorferi* sensu stricto strains are evident in the United States and transmitted by *Ixodes scapularis*. Bbss enhances expression

of *I. scapularis* genes that are beneficial for spirochete transmission and arthropod survival. One of these genes codes for Salp15, a 15 kDa feeding induced salivary gland protein, and has been shown to inhibit T lymphocyte activation, and bind Bbss Outer Surface Protein (Osp)C protecting Bbss from antibody mediated killing.

In the current study, we assessed the binding capacity of Bbss strain N40, Bg strain PBi, and Ba strain pKo to recombinant – *Drosophila* expressed – *I. scapularis* Salp15 by solid phase overlays, *in vitro* protection assays against *Borrelia* specific antiserum and Enzyme Linked ImmunoSorbant Assays (ELISA) with – *E. coli* expressed – recombinant OspC. The ELISA and solid phase overlays show that *I. scapularis* Salp15 has more affinity for N40-OspC than for pKo-OspC. Salp15 shows intermediate binding to PBi-OspC. These data indicate that *I. scapularis* Salp15 has more affinity for Bbss than for the two European *Borrelia* strains. Moreover, Salp15 was able to protect N40 from killing by N40-specific antiserum, where as Salp15 was unable to protect pKo from killing by pKo-specific antiserum.

Since, in nature *I. scapularis* does not transmit Bg and Ba, we also searched for Salp15 homologues in *Ixodes ricinus*. We performed reverse transcriptase polymerase chain reaction (RT-PCR) on RNA from salivary glands from fed and unfed female adult *I. ricinus* ticks with primers based on *I. scapularis salp15*. Thus, we identified the presence of three feeding induced Salp15 homologues in *Ixodes ricinus*. One of the predicted proteins shows 80% similarity to *I. scapularis* Salp15, evenly distributed over the entire amino acid sequence, where as the two other predicted proteins show approximately 60% similarity, mainly confined to the signal sequence and C-terminus. Comparison of the DNA and protein sequences with those deposited in several databases reveal a Salp15 family that is conserved among different *Ixodes* species, all capable of transmitting *B. burgdorferi* sensu lato. Recently, it was shown that the C-terminus of *I. scapularis* Salp15 binds murine $CD4^+$ T cells by binding to the CD4 co-receptor, thereby inhibiting T cell activation. Interestingly, all three Salp15 homologues in *I. ricinus* share great homology at the C-terminus.

These data show that several Salp15 homologues are present in *Ixodes* ticks and could play a role in the transmission of diverse *Borrelia* species. Further studies are ongoing to assess the interaction of the Salp15 homologues in *I. ricinus* with the different *Borrelia* species.

P062

Insertional inactivation of the enterococcal surface protein gene, *esp*, reduces biofilm formation of *Enterococcus faecium*

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Background: Most hospital outbreak and invasive *Enterococcus faecium* isolates cluster in clonal complex-17 (CC17) and most CC17 isolates harbour a putative pathogenicity island containing the *esp* gene. We have shown that Esp expression levels correlate with the degree of biofilm formation, supporting a role for Esp in the pathogenesis of *E. faecium*. Definitive conclusions about the role of Esp in pathogenesis is hampered by the lack of an isogenic *esp* deletion mutant. Up till now, it appeared extremely difficult to inactivate genes by allelic exchange in *E. faecium* clinical isolates due to poor transformation capacity, plasmid incompatibility and the lack of selective markers because of multiple antibiotic resistances. To verify the role of *esp* in biofilm formation, we aimed to construct an *esp* knock-out in an *E. faecium* clinical isolate.

Methods: *Esp* gene fragments were cloned into an improved temperature-sensitive vector (Nallapareddy et al. Appl Environ Microbiol, 2006) and used to introduce a deletion mutation in the *esp* gene of a clinical *E. faecium* isolate. DNA sequencing and Southern blotting were performed to verify correct deletion of *esp*. Western blotting and flowcytometry were carried out to determine Esp expression. In addition, initial adherence to polystyrene and biofilm formation assays were performed.

Results: Allelic replacement resulted in insertional inactivation of *esp* that completely abolished Esp expression intracellularly as well as on the cell surface. Both initial adherence to polystyrene and biofilm formation were significantly reduced by 30 and 75%, respectively in the deletion mutant relative to wild-type and dropped to levels seen in *esp* negative *E. faecium* isolates.

Conclusion: An *esp* deletion mutation was successfully created in a clinical *E. faecium* isolate resulting in abolished Esp expression, initial adherence and biofilm formation on polystryrene. These data illustrate that allelic replacement technology is available for constructing isogenic deletion mutants in *E. faecium* clinical isolates. Furthermore, these results denote a role of Esp in the pathogenesis of *E. faecium* CC17 infections.

P063

Expression of cpsA in response to various carbon sources

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Capsule is a condition 'sine qua non' in *Streptococcus pneumoniae* virulence and its regulation is an important part of the ability of *S. pneumoniae* to cause invasive disease. Its main purpose is to inhibit complement-mediated opsono-phagocytosis and a-capsular mutants are avirulent. There is a direct correlation between the amount of capsule produced and virulence and an inverse relation with the ability to adhere and transcytose. Capsule

expression varies throughout infection and responds to the proximity of eukaryotic cells. Taken together, these data argue that the amount of capsule is under complex regulatory control and that this regulation is an important aspect of the infection process. There are about 90 different capsule types (serotypes), encoded by 90 different gene clusters. Strikingly, the sequence of the promoter and first gene of all these clusters, cpsA, are fairly conserved throughout all serotypes, suggesting that there are common regulatory influences in all strains.
An important signal for bacteria inside the host is probably the available carbon sources in each niche. Therefore, we investigated the influence of various sugars on the expression of the capsule locus. To study this we cloned the cpsA promoter in front of a promoterless gfp gene and studied its expression using flow cytometric analysis in response to a) various simple sugars, b) to various subunits of mucin and c) mucin. There was a clear influence on the expression of the cpsA promoter in response to various sugars, with glucose and glucose- derivatives inducing the highest expression.
Thus, the carbon source available to the bacterium has a significant impact on the expression of cpsA and thus probably on the capsule locus.

P064

Gastric *Helicobacter* species colonizing strict carnivores express two different, functional urease enzymes

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The nickel-dependent enzyme urease is an essential virulence factor of *Helicobacter* species colonizing the gastric environment. Concerted expression of virulence factors is often a requirement for colonization by bacterial pathogens. In *H. pylori*, urease expression is induced by the NikR regulatory protein in response to availability of the nickel cofactor. Nickel is scarce in the mammal host, and is mostly acquired via vegetarian dietary sources. This is a potential problem for gastric *Helicobacter* species colonising the nickel-limited gastric environment of strict carnivores. Recently, a putative second urease gene cluster (ureA2B2) was detected in three gastric *Helicobacter* species colonizing carnivores. This gene cluster was not detected in other *Helicobacter* species. The aim of this study was to characterize the transcriptional regulation and contribution to urease activity of both urease systems using H. mustelae as model organism.
To study expression of both urease homologs *Helicobacter mustelae* NCTC 12198 and its isogenic nikR mutant were cultured under nickel-restricted and nickel-supplemented conditions. Regulation of ureB and ureB2 transcription was assessed by Northern hybridization and immunoblotting. UreA2B2 and UreAB urease activity was measured in nikR/ureB and nikR/ureB2 double mutants. A nikR/ureB/ureB2 triple mutant was used as negative control.
Both urease homologs of H. mustelae were expressed in nickel-restricted conditions, but were conversely regulated upon nickel-supplementation. Addition of nickel to a final concentration of 100 nM resulted in complete transcriptional repression of UreA2B2, whereas UreAB expression was induced. Insertional mutagenesis of nikR resulted in constitutive expression of both urease homologs, independent of the nickel concentration in the medium. Maximum activity of UreA2B2 in H. mustelae was ~0.5 U, whereas UreAB activity could reach up to 20 U of activity in nickel-supplemented conditions. Three carnivore-colonizing *Helicobacter* species express a second urease system, which is only active at severe nickel-restriction. This second urease system seems absent from *Helicobacter* species colonizing omnivores and herbivores. The expression of UreA2B2 is possibly an adaptation of carnivore-colonising *Helicobacter* species to the limitations of the diet of their carnivorous host, by using a urea-degrading enzyme with biochemical properties well suited for low nickel availability.

P065

Acute phase response impairs host defense against *Enterococcus faecium* peritonitis in mice

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Introduction: Multiresistant *Enterococcus faecium* is an important cause of hospital-acquired infections. In particular patients with a pre-existing illness are susceptible to infections with *E. faecium*. Such predisposing diseases are almost invariably associated with an acute phase protein response (APR). We here tested the hypothesis that a sterile acute phase response renders the host more vulnerable to *E. faecium* infection. Therefore, we used two well-established models to induce a sterile APR, namely subcutaneous injection of either turpentine or casein.
Methods: One day prior to intraperitoneal infection with 10^8 CFU *E. faecium*
C57BL/6 mice were injected subcutaneously with either turpentine in both hind limbs, or casein in the back/neck region. Control mice were injected with saline, or bicarbonate respectively. Mice were sacrificed at different time points up to one week after infection to determine immune responses and bacterial loads in blood, peritoneal lavage fluid, liver and lung.

Results: At the sites of the subcutaneous turpentine injections abscesses were formed, which was not the case for the casein injection sites. Both turpentine and casein induced an acute phase protein response as reflected by a transient weight loss and strong increases in the plasma levels of serum amyloid P and C3. Additionally, these mice had less circulating granulocytes and less peritoneal granulocyte influx compared to the control mice. Both turpentine and casein injected mice showed a significant delay in clearing the enterococci from all tested organs. It could not be demonstrated whether this was accompanied by reduced cyto- and chemokine responses, since *E. faecium* does not cause impressive levels of these immune proteins. All differences relative to controls were more profound in the turpentine-injected mice.
Conclusion: These data suggest that a pre-existing sterile acute phase protein response, such as occurs after trauma or major surgery, impairs host defense against *E. faecium* peritonitis.

P066
Urease-induced calcium precipitation by *Helicobacter* species may initiate gallstone formation

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Background: Gallstones are crystalline bodies formed by accretion or concretion of bile components. Bacteria are able to initiate calcium precipitation mediated by the activity of the enzyme urease. *Helicobacter* species can colonize the gastrointestinal and hepatobiliary tract of many mammals, and often cause inflammation-associated gastrointestinal and hepatobiliary diseases. Recently, infection with the enterohepatic *Helicobacter* species *H. bilis* and *H. hepaticus* has been linked to the formation of cholesterol gallstones in mice fed with a lithogenic diet (Gastroenterology 2005;128:1023-33), but the mechanism underlying this phenomenon was not described. In this study we have investigated whether *Helicobacter* urease activity is involved in precipitation of calcium salts.
Methods: A precipitation agar was developed which allowed for simultaneous growth of *Helicobacter* species and testing of their ability to precipitate calcium.
Results: All three urease-positive *Helicobacter* species were capable of precipitating calcium in our assay after overnight growth, as was detected by light microscopy. In contrast, isogenic ureB urease-negative mutants of this same species and natural urease negative *Helicobacter* species were unable to do so. Addition of purified urease enzyme also resulted in precipitation of calcium.
Conclusion: Urease-positive *Helicobacter* species which are able to colonize the bile ducts may initiate the formation of gallstones via their urease activity. This provides a possible mechanism explaining the association between hepatobiliary colonization with urease-positive *Helicobacter* species and gallstone formation.

P068
Elucidating the metagenome of the anammox bacterium *Kuenenia stuttgartiensis*

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Anaerobic ammonium oxidation (anammox) has become a main focus in oceanography and wastewater treatment. It is also the nitrogen cycles major remaining biochemical enigma. Among its features, the occurrence of hydrazine, the biosynthesis of ladderane lipids and the role of cytoplasm differentiation are unique in biology. To elucidate these intriguing properties, an environmental genomics approach was used to assemble the genome of the anammox bacterium Kuenenia stuttgartiensis from a complex microbial community. The genome data were able to trace the evolutionary history of the PVC superphylum. The genetic blueprint revealed the identity of candidate genes responsible for ladderane biosynthesis and biological hydrazine metabolism. In addition, an unexpected metabolic versatility was discovered that could be verified experimentally. Anammox cells were able to use a variety of organic acids to reduce nitrate via nitrite into ammonia. Labeling experiments with 15N-nitrate confirmed that anammox cells can produce 30N2 gas. Further anammox cells can reduce iron(III) and manganese (IV) with formate as electron acceptor. Recently we were able to functionally express several anammox proteins in *Escherichia coli*. Based on the metabolic inventory dedicated enrichment cultures were initiated to select for specialized new anammox bacteria. In this way, we were able to identify *Anammoxoglobus propionicus* and *Scalindua marina*.

P070
Anaerobic methane oxidation coupled to denitrification by a freshwater microbial consortium

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Modern agriculture has accelerated biological methane and nitrogen cycling on a global scale. Freshwater sediments often receive increased downward fluxes of nitrate from agricultural runoff and upward fluxes of methane generated by anaerobic decomposition of organic matter. Based on free energy calculations, the anaerobic oxidation

of methane coupled to nitrate or nitrite reduction should be feasible, but organisms carrying out this process have neither been observed in nature nor isolated in the laboratory. Thus, microbial oxidation of methane was believed to proceed only with oxygen or sulfate as electron acceptors. We used sediment of a Dutch canal (Twentekanaal) as inoculum for an enrichment culture that was supplied with methane, nitrate, nitrite and a bicarbonate buffered mineral medium. After 16 months a stable consortium was established that oxidized methane to carbon dioxide and reduced nitrite and nitrate to dinitrogen gas in the complete absence of oxygen. The enrichment culture was dominated by two species, a bacterium of the NC10 phylum lacking any cultured representatives and an archaeon of the order *Methanosarcinales*, distantly related to marine methanotrophic *Archaea*. The detection of closely related sequences of these prokaryotes in different freshwater ecosystems worldwide indicates that anaerobic oxidation of methane coupled to denitrification may contribute substantially to biological methane and nitrogen cycles. Currently, the diversity of genes involved in anaerobic methane (mcr*A*) and nitrite (nirK and nirS) conversion in the enrichment culture are investigated.

P072

Unexpected cutaneous *Leishmania* isolates from patients visiting Surinam

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Introduction: Cutaneous leishmaniasis is endemic in many parts of the world. The distribution of different *Leishmania* species depends among others on geography and reservoir. In Surinam, *Leishmania braziliensis* and *Leishmania guyanensis* are the main causes of cutaneous leishmaniasis. Two patients who visited Surinam presented with lesions suspected of cutaneous leishmaniasis. Patient V. was a 22 year old Marine, stationed on the Netherlands Antilles, who visited Surinam for jungle training. Patient O. was a 44 year old male returning from his third business trip to Surinam in a period of 2 years. During this period he also visited Ibiza.

Methods: *Leishmania* infection was investigated by microscopy, culture on NNN-medium, and PCR on biopsies from suspected lesions. The mini exon repeat sequence was amplified by PCR, products were sequenced and analyzed with CodonCode Aligner, ClustalW and MEGA.

Results: Patient V. had 2 suspected lesions (1.5 and 2 cm diameter) on the leg. Both were *Leishmania* positive by microscopy, culture and PCR. Sequence analysis identified *Leishmania naiffi*. The patient was successfully treated with pentamidine.

Patient O. had had a 4 mm papular lesion on the arm for approximately one year. Microscopy and culture were negative, but PCR and sequence analysis showed *Leishmania infantum*-complex. The lesion was treated with local cryotherapy and intralesional pentostam.

Conclusion: Patient V. was infected with *L. naiffi*, representing the first confirmed case of *L. naiffi* infection from Surinam. This species is a rare cause of cutaneous leishmaniasis, as only 8 patients have been described worldwide. The natural host of *L. naiffi*, *Dasypus novemcinctus*, (the nine-banded armadillo), is considered abundant in rural areas of Surinam.

It is unclear whether patient O. was infected with *L. infantum* on Ibiza or in Surinam. Cutaneous leishmaniasis due to *L. infantum* is usually found in the Mediterranean, but a recent report showed infection of local dogs with *L. infantum* in French Guyana, east of Suinam. Spread of *L. infantum* in this part of South America therefore seems a possibility.

P074

Comparison of microscopy, culture and Real-Time PCR results for the detection of *Mycobacterium tuberculosis* complex in clinical samples

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Introduction: Rapid and reliable laboratory diagnosis of *Mycobacterium tuberculosis* infections is essential for the prevention and treatment of tuberculosis. Molecular tools allow quicker detection of *M. tuberculosis* infections than conventional techniques.

The routine methods used in medical microbiological laboratories are microscopy, culture and/or PCR. By collecting test results of six Dutch laboratories, that used comparable diagnostic tests, we evaluated the importance of each test for a large series with a variety of clinical samples.

Methods: The six laboratories performed microscopy with Auramine and/or Ziehl-Neelsen staining and cultures with liquid and solid media. Similar methods were used for sample decontamination, the NaLC/NaOH procedure,

and real-time PCR. The method for DNA isolation and the media used for culturing were varied in all laboratories. The real-time PCR, targeting the IS*6110* and used by all six laboratories, was described by Savelkoul *et al.*

Results: A total of 14,360 samples, 6371 respiratory and 7989 non-respiratory samples, was compared to assess the ability of microscopy, culture and real-time PCR to detect the *Mycobacterium tuberculosis* complex. Of all samples 1.6% were positive according to all three methods, 3% (427) culture and PCR-positive but smear-negative, and 1.9% (275) were positive according to either culture or PCR and smear-negative.

Of the 702 samples that tested positive (culture and/or PCR), 33.9% were positive in the microscopic tests, 56.3% negative and 9.7% not performed. Of the culture and/or PCR-positive samples, 275 (39.2%) showed discrepancies between the two tests, 145 (52.7%) to the advantage of the PCR method and 130 (47.3%) to the advantage of culture.

Conclusion: Microscopic detection of *M. tuberculosis* is used as a fast method to indicate the presence of a mycobacterial infection; the sensitivity for this test in this study was 37.6%. The PCR method is a good and rapid method which can answer the question about the presence of tuberculosis much better, compared to the microscopic method, in one working day.

Analysis of the culture/PCR discrepancies showed that no infection was missed at the patient level due to multi-sampling of specimens. At the level of specimens the results of culture were comparable to those of real-time PCR with the restriction that both methods yielded false-negative results, probably due to sampling error. It can be concluded that the real-time PCR is comparable to culture in its performance but for good clinical practice, for the detection of *M. tuberculosis,* more than one sample is required in order to perform both culture and real-time PCR.

P076

Soya bean extracts inhibit adhesion of enterotoxigenic *Escherichia coli* K88 on intestinal epithelial cells

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Introduction: Diarrhoea is a major health problem worldwide, particularly during the weaning period of infants as well as of farm animals such as piglets. Earlier research indicated that tempe (fungal fermented soya bean) reduces the severity of *Escherichia coli* induced diarrhoea in piglets (Kiers *et al.*, 2003). Pediatric research in Indonesia indicated that in infants, the recovery after acute bacterial diarrhoea was faster when tempe was consumed as an ingredient of the infant food formula (Karmini *et al.*, 1997). To study this health promoting effect, pig intestinal brush border and Caco-2 intestinal epithelial cells were exposed to different soya bean extracts and the effect on the adhesion of *E. coli* was investigated.

Methods: Tempe was prepared at controlled laboratory scale. Soya beans were soaked overnight, cooked, cooled by evaporation of adhering water, inoculated with *Rhizopus microsporus* var. *microsporus* (LU 573) and incubated for 48, 72, 96 and 120 hours at 30 °C. Water soluble extracts were prepared from raw, soaked, cooked and fermented soya beans.

Pig intestinal brush border cells were treated with 0.01, 0.25 and 1% of soya bean extracts and incubated with an *E coli* (O149:K91:K88ac) strain ID1000 (ETEC) suspension (10^9 CFU/ml) at room temperature for 1 hour. Adhesion was determined with phase contrast microscopy.

Caco-2 cells were treated with 0.25% soya bean extracts and ETEC suspension and incubated at 37 °C for 1 hour. Adhesion was determined by plating of the adhered ETEC.

Results: Incubation of brush border vesicles with ETEC resulted in an adhesion of approximately 10 bacteria to one brush border; this value was used as a fully occupied control, i.e. 100% adhesion. Treatment with 0.25% of soya bean extracts results in reduced bacterial adhesion. Raw, soaked and cooked soya beans decrease adhesion to about 60% compared to the control and the different tempe samples reduced adhesion to less than 20%. Treatment with 1% soya bean extracts completely inhibited the adhesion. The inhibition of the adhesion of ETEC to brush borders was related to the concentration of the soya bean extracts. Treatment of Caco-2 cells resulted in an inhibition of adhesion to about 50% for the tempe extracts compared to a control without added soya bean extract. Addition of raw, soaked and cooked soya bean extracts resulted in a slightly increased adhesion compared to the control. Longer fermentation times did not change the activity of the adhesion inhibiting effect in both experiments.

Conclusions: 1) Soya bean extracts protect against the adhesion of ETEC to pig intestinal brush borders. 2) Fermented soya bean extracts also protect Caco-2 intestinal epithelial cells against adhesion of ETEC. 3) Duration of tempe fermentation does not affect the adhesion-reducing activity of the extracts.

P077

Characterisation of *Bacillus* spores obtained from the canning industry

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Introduction: Spore-forming bacteria can be a problem in the food industry, especially in the canning industry. The spores, which they form, severely challenge the preservation process since their thermal resistance may be very high. We asked the questions which type of spore-forming bacteria are present in the manufacturing process of industrial soups. The spores can be introduced in the production process in two ways. The first way is through the ingredients which may be contaminated with bacterial spores. A second way may be through growth and sporulation in line of the vegetative bacteria.

Methods: In dry ingredients mesophilic and thermophilic spores were analysed by plate counting.

The heat resistance of spores obtained from *Bacilli* which were isolated from the ingredients or the end product was assessed. The *Bacilli* were sporulated in a commercial medium (Nutrient broth) containing extra metal ions, and in a Mops based medium to which various additions were made. The additions made to the Mops based media were calcium chloride and ingredients used in the manufacturing of soups. A selection of ingredients was measured for the presence of divalent cations, as it is known that these may be involved in the development of heat resistance of bacterial spores. The strains obtained from the ingredients or the product were characterised at the species level using the following techniques: fatty acid analysis, 16S rRNA gene sequencing and DNA/DNA hybridisation. Furthermore Ribotyping and AFLP® were used for sub-clustering of strains within species.

Results: In some dry ingredients very high numbers of both mesophilic and thermophilic spores were analysed. Some strains which were isolated from ingredients or products were sporulated under different conditions and the heat resistance of these spores was assessed. The results showed that in particular spores formed in the presence of divalent metal ions were characterised by a high heat resistance. The divalent cations were therefore measured in a selection of ingredients. The data showed that calcium was found in high concentration in cumin which correlated with the high heat resistance of spores obtained in Mops medium with cumin. For calcium it is known that it can increase the heat resistance of spores. Species identity was confirmed using DNA-DNA hybridisation. Also molecular typing experiments using Ribotyping and AFLP® were performed. The data showed that the various Bacillus species and strains within the species can be clustered using AFLP® generated banding patterns according to the thermal resistance properties of their spores.

Conclusions: This study shows that it is important to know which types of *Bacilli* are present in ingredients used to produce soups. It is shown in the results that spores cultivated under conditions which are high in divalent cations show a high heat resistance. *Bacilli* of which the spores have the same thermal resistance characteristics clustered together using AFLP®. The combined results will allow the manufacturer to come to a more knowledge-based design of process settings, avoiding unnecessary over processing in obtaining commercially sterile products.

P078

Microbial distributions in food products

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Introduction: Foods are subjected to microbiological investigations at various stages of production. Importing countries, industrial clients, food business operators, food inspection services and consumer organizations sample food commodities from their own perspective and according to different sampling plans. Statistical concepts underlying these sampling plans often assume that the levels of microorganisms in foods are distributed log-normally. Knowledge of both physical and statistical distributions of microorganisms is of eminent importance for food safety management. However the effect of heterogeneity on the resulting distributions is rarely studied and important data are missing. This study aims at finding the statistical distributions that fit the experimental numbers of microorganisms best.

Methods: Batches of powdered infant formula were inoculated with known numbers of *Enterobacter sakazakii* ATCC 29544. Small batches of 5 g powder spiked with *E. sakazakii* were added to 1 kilogram and mixed to various degrees: limited and thorough mixing. From each batch numerous samples were taken and the numbers of *E. sakazakii* were determined by plate counting on Trypton Soy Agar (TSA).

Results: In all samples taken from the thoroughly mixed powder *E. sakazakii* was detected, while 77% of the non-thoroughly mixed samples were found to be contaminated. The numbers of organisms counted as colony forming unit (cfu)varied from 2.6 to 4.3 (log cfu)/g in the thoroughly mixed powder. In the not-well mixed powder the range of 1.8 to 5.1 (log cfu)/g was observed. The numbers of organisms were analysed by forming an experimental distribution function (EDF) and various statistical distributions were fitted to the data. The goodness-of-fit was assessed by several statistical tests.

Conclusion: After thorough mixing the numbers of *E. sakazakii* in the dry matrix could by described by the lognormal distribution.

P079

Metabolic capacity of two *Bacillus cereus* strains interlinked with comparative genomics

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Introduction: *Bacillus cereus* is a ubiquitous, Gram-positive, spore forming, food-borne human pathogen that is associated with two types of diseases: emesis and gastroenteritis. The ability of the vegetative cells to grow on numerous substrates and the ability to survive mild stresses make this organism an expert in survival and commonly found in various environments. To identify the metabolic potential of *B. cereus*, two sequenced strains, ATCC 14579 and ATCC 10987, were investigated for their capability to utilize several nutrient sources such as amino acids, saccharides and di-peptides. Furthermore, the response of these to strains was assessed to stresses such as low pH, and high osmolarity. More genome sequences become available each day and this data is used to predict metabolic capacities. In contrast, our approach not only predicts the metabolic capacity, but also experimentally verifies the predicted phenotypic differences.

Methods: The algorithm INPARANOID was used to reveal the strain specific orfs. The metabolic capacity and the responses to several stresses were investigated with the Phenotypic Microarray (PM) by Biolog. With this technique the performance of both strains was assessed for 1920 different phenotypes. A number of food associated phenotypic differences between the strains was verified in minimal medium (M0). M0 was designed to include only one carbon and one nitrogen source, which could be substituted by a substrate of interest.

Results: The genome comparison of ATCC 14579 and ATCC 10987 revealed 983 and 1360 strain specific orfs, respectively. 105 phenotypic differences were revealed, the most remarkable were: saccharide and di-peptide usage and osmo-tolerance. Many observed phenotypic differences could be interrelated with strain specific orfs. The usage of carbohydrates (fructose, glucose, trehalose, amylose and sucrose) by ATCC 14579 corresponds with several strain specific orfs (fructokinase, glucose ABC transporter, glycosidase and sucrose PTS system).

Discussion: Numerous strain-specific orfs have been identified of which a large number strain specific orfs were not identified previously in other studies. Here INPARANOID was used to compute the genetic differences by taking gene context into account, whereas previous studies were based on orf sequences only. It has been suggested that *B. cereus* shows prevalence for the metabolism of proteins, peptides and amino acids over the metabolism of carbohydrates. Here we provide evidence that *B. cereus* ATCC 14579 can also metabolize a large number of carbohydrates besides utilizing a broad range of peptides and amino acids for growth. The used approach, by establishing phenotypic differences independent of genetic variety, gives indications were to expect differences, which could be missed in a conventional approach based on comparative genome sequence analysis solely.

Conclusion: Interlinking genetic information and corresponding phenotypes, is a promising approach to assess the growth performance and metabolic capacity of micro-organisms and can supply indications for the performance of these bacteria in different environments.

P081

A high-throughput method for screening compounds against *Streptococcus mutans* biofilms

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The fact that bacterial cells are more resistant to antimicrobial treatment than their plantonic counterpart indicates that the efficacy of an antimicrobial agent should be evaluated with biofilm cells. Current evaluation methods for biofilms are, in general, very time-consuming. Therefore our aim was to develop a high-throughput screening method, by using a green fluorescent protein (GFP) reporter strain, to evaluate the inhibitory effects of antimicrobial agents on *Streptococcus mutans* biofilms. *S. mutans* is a dental pathogen that produces lactic acid in dental plaque biofilms and hence is responsible for dental caries.

A synthetic constitutive promoter was fused with a GFP-gene and inserted in the shuttle vector pVA838, resulting in plasmid pDM15. This plasmid and pVA838 (as a negative control) were transformed to *S. mutans* UA159. The two strains were then grown in black 96-well microtiter plates using semi-defined biofilm medium containing 0.2% sucrose and 0.3% yeast extract. 24h biofilms were treated with serial two-fold dilutions of chlorhexidine (CHX) or sodium chloride (NaCl), in the presence of 0.3% glucose. Average fluorescence intensity (FI) changes were measured kinetically for 5 h in a fluorimeter. Biomass was determined by a crystal-violet staining method and metabolic activity was assayed as lactic acid production using an enzymatic method.

The FI of the negative control was approx. 30 arbitrary fluorescence units (AU) and remained constant throughout the experiments. The FI of the strain carrying pDM15 was around 50 AU at the beginning of the measure-

ments and almost tripled towards saturation (140 AU) in the 5 hours of the experiment. This increase in FI was inhibited by increasing concentrations of CHX or NaCl in a dose-responsive manner. The lowest concentration at which GFP-production was completely blocked in the biofilms was 0.005% for CHX and 1M for NaCl. Similar dose-dependences were recorded in inhibition of lactic acid production. No significant differences in biomass were observed throughout wells. Inhibition of GFP-production from a constitutive promoter in the *S. mutans* biofilm reflects inhibition of metabolic activity and hence this GFP reporter microplate assay has great potential as a high-throughput method for screening antimicrobial agents against *S. mutans* biofilms.

P082

Transcriptomic analysis of interactions between *Streptococcus* mutans and *Veillonella parvula* grown in dual species biofilm

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Introduction: We have previously shown that *Streptococcus mutans* grown together with *Veillonella parvula*, a bacterium that utilizes lactic acid produced by *S. mutans* in dental plaque biofilm, has an altered acid production and decreased chlorhexidine susceptibility compared to *S. mutans* grown in single species biofilm (Kara *et al.*, 2006). The aim of the present study was to determine which gene transcript levels are changed in *S. mutans* when grown together with *V. parvula* and whether the expression of these genes may be responsible for the change in acid production and decreased susceptibility to chlorhexidine.
Materials and methods: Single species biofilms and dual species biofilm of *S. mutans* and *V. parvula* were grown on polystyrene in half strength BHI supplemented with lactic acid and PIPES-buffer pH 7.2. Growth was assessed by plate counting.
Differences between *S. mutans* mRNA levels grown in the absence and presence of *V. parvula* were evaluated by using 70-mer *S. mutans* microarrays (NIAIDs Pathogen Functional Genomics Resource Center), appropriate controls and statistics.
Results: 48-h biofilms contained 3x10^8-10^9 CFU/cm^2. In *S. mutans* the transcript levels of 15 genes were significantly higher in the presence of *V. parvula* than in the absence of *V. parvula* and 19 were lower. The changes in transcript levels were similar to those of *Streptococcus pneumoniae* and *Bacillus subtilis* exposed to antibiotics (Ng *et al.* 2003, Dandliker *et al.* 2003). In addition, the alpha subunit of the F0F1 membrane-bound proton-translocating ATPase and two other genes necessary for growth at low pH were down-regulated in the presence of *V. parvula*.
Conclusions: 1) Gene expression in *S. mutans* grown in dual species biofilm with *V. parvula* differs from that in *S. mutans* grown in single species biofilm. 2) Our results suggest that the presence of *V. parvula* may cause a similar response in *S. mutans* as antibiotics do in other Gram-positive bacteria. This may explain the decrease in chlorhexidine susceptibility. 3) Furthermore, *S. mutans* may experience a higher local pH when *V. parvula* is present in the biofilm, which possibly effects its acid production profile.

P083

The road to PCR-based serotyping of *Porphyromonas gingivalis*

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Periodontitis is an infection of the tooth supporting tissues and is characterized by severe alveolar bone loss. It is one of the most common infectious diseases in humans. Periodontitis is a complex multifactorial disease in which bacteria play an essential role. Host susceptibility is determined by behavioral and genetic factors. Recently, periodontitis has been linked with cardiovascular diseases and preterm low birth-weight. The Gram-negative, oral, strict anaerobic bacterium *Porphyromonas gingivalis* is strongly associated with periodontitis. *P. gingivalis* displays a wide variety of virulence factors. These include Arg- and Lys- gingipains, fimbriae, haemagglutinins, and lipopolysaccharides (LPS), and capsular polysaccharides (CPS).
To date, seven capsular polysaccharides serotypes (K1-K7) have been described (van Winkelhoff *et al.* 1993, Laine *et al.*, 1997, d'Empaire *et al.*, 2006). Although differences in morphology between serotypes are obvious, little is known about the differences in capsule structure, chemical composition and genetic variance between serotypes. A recent study reported on K1 serotype strains that induce chemokine expression in phagocytic cells more than other serotypes do (d'Empaire *et al.*, 2006). This demonstrates the importance of insights into the mechanism and into the differences in mechanisms that underlie the capsule biosynthesis of the different strains. To gain these insights the genomic sequence of the K1 W83 strain can be used to find potential target genes involved in capsular polysaccharide biosynthesis.
In previous work, a potential genomic CPS (K-antigen) biosynthesis locus (PG0106-PG0120) was found. Deletion of parts of this locus resulted in non-encapsulated mutants (Aduse-Opoku *et al.*, 2006). Differences between strains in this locus might play an important role in the serotype specificity of the capsular polysaccharides.

We will use these differences between serotypes to set up a PCR-based method for rapid identification of the serotype of *P. gingivalis* strains. This would only be possible if we would find a homogenous group of K-antigen loci within a serotype, but heterogeneity between serotypes. For that reason, we characterized the above mentioned locus of 10 strains of the K1 serotype by Restriction Fragment Length Polymorphism analysis (RFLP) and by gene specific Polymerase Chain Reaction (PCR) analysis. 1) Seven out of ten K1 strains have exactly the same RFLP pattern. 2) The heterogeneity within the K1 serotype does not involve gene replacements or deletions, but we found a gene insertion in two strains. 3) These finding enables the development of a PCR-based capsular serotyping technique for *P. gingivalis*.

P085

Investigation of the potential for competence development in *Bacillus* species

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Natural competence is the ability to take up and incorporate DNA from the environment. Competence development within the *Bacillus* family has so far been described for *Bacillus subtilis, Bacillus licheniformis* and *Bacillus amyloliquefasciens*, but not for *Bacillus cereus* or other *Bacillus* species. A large number of proteins required for transformation have been identified in *Bacillus subtilis*, that can be separated into 4 categories: 1) the master regulator: ComK. 2) the DNA uptake machinery: ComC, ComEABC, ComFABC, ComGABCDEFG. 3) proteins involved in modification and recombination of incoming DNA: RecA, NucA, Nin, YwpH/Ssb. 4) proteins involved transcriptional or post-translational regulation of ComK levels: DegSU, SinRI, Rok, CodY, AbrB (transcriptional regulation); MecA, Clp complex, ComS (regulation of ComK levels); ComXQ, ComAP, RapC, PhrC (quorum sensing apparatus important for the modulation of ComS levels). Various other genes regulated by ComK and possibly related to transformation have also been identified.
We have performed BLAST analyses against the genome of several fully sequenced *Bacillus* species (*B. antracis, B. cereus, B. thuringiensis, B. clausii, B. halodurans* and *B. licheniformis*) with the sequences of proteins involved in competence development of *B. subtilis*. Results were visualized with Genesis software.
By screening the genome of the fully sequenced *Bacillus* species, we have identified many genes encoding putative homologues of the proteins involved in competence development, raising the possibility that the former ones may also be able to acquire natural competence. The comK gene was missing in *B. clausii* and *B. halodurans*, while comFB and comGEFG was missing in all 'thought to be non-competent' *Bacilli*. Interestingly, several *B. cereus*, *B. antracis* and *B. thuringiensis* strains seem to contain two homologues of ComK. The putative ComK proteins show 61-62% (ComK1) and 44-48% (ComK2) homology to *B. subtilis* ComK. While ComK1 proteins have similar length to *B. subtilis* ComK protein, ComK2 proteins of the *B. cereus* group are 24 amino acids shorter.
Our comparative study helps to extend our understanding natural competence in general and competence in these *Bacillus* species specifically. Natural competence would greatly facilitate molecular genetic studies with these organisms.

P086

Antimicrobial activity of porcine defensin 2 (pBD-2) against pathogenic bacteria in the gastrointestinal tract

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Introduction: Defensins are antimicrobial peptides with activity against a broad range of bacteria, viruses and fungi. pBD-2 is a recently discovered new defensin in pig. The aim of our study was to determine the localization of pBD-2 in the intestine and to determine its antimicrobial activity against pathogenic gastrointestinal-bacteria.
Results: Gene transcripts of pBD2 were found throughout the whole intestine with a relatively high expression in the duodenum compared to the ileum and jejunum. The pBD2 peptide was found to be highly bactericidal against the porcine pathogens Salmonella typhimurium, Listeria monocytogenes and Erysipelothrix rushiopathiae and to a lesser extent *Escherichia coli* and *Yersinia enterocolitica*. Electron microscopy showed that the initial damage caused by pBD2 is disruption of the bacterial membrane. Antimicrobial activity was strongly reduced at high ionic strength of the test medium, with no residual activity at 150 mM NaCl. Haemolytic activity of pBD-2 was undetectable at physiological conditions.
Prospects: The activity of pBD2 could be an important factor determining the dynamics of porcine intestinal microbiota. Dietary modulation of pBD-2 may be a way to improve intestinal health and food safety.

P087

Carbon disulfide conversion by an extremely acidophilic *Acidithiobacillus* strain

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Carbon disulfide (CS_2) is an odorous and toxic gas, which is emitted to the atmosphere from both natural and anthropogenic sources. Natural CS_2 sources include soils,

marine sediments, marshes, oceans and plants, while the anthropogenic emissions mainly originate from the viscose industry. Reduction of the industrial emissions of CS_2 is required because of its toxicity and environmental impact as a greenhouse gas. Incineration of CS_2 containing air streams is expensive because the generated air streams are large and have relatively low CS_2 concentrations. Therefore biological treatment using biotrickling filters are being developed as an alternative. The number of microorganisms known to be able to grow chemolithoautotrophically on CS_2 is very limited; this trait has only been reported for *Thiothrix ramosa, Thioalkalivibrio* spp., *Paracoccus denitrificans* and some *Thiobacillus* species. These bacteria convert CS_2 to hydrogen sulfide, which is further oxidized to sulfuric acid to yield energy. Most of the described CS_2 utilizing bacteria grow under neutrophilic conditions. However, application in trickling filters would favor acidophilic bacteria due to the inherent acidification of the trickling water. Consequently, the microorganisms in such systems should be able to tolerate extremely low pH values accompanied with a high CS_2 affinity because of the low concentration in the waste gas. So far only one bacterium (*Thiobacillus* strain TJ330) capable of growth under acidic conditions (as low as pH 0.5) has been described. The present research describes the isolation of a new extremely acidophilic *Acidithiobacillus* strain able to grow on CS_2 at a sulfuric acid concentration of 0.75 M (pH about 0.1). We also show that this microorganism converts CS_2 to hydrogen sulfide (H_2S) via hydrolysis with carbonyl sulphide (COS) as intermediate (equations 1 and 2).

$CS_2 + H_2O \text{ -->} COS + H_2S$ (1)

$COS + H_2O \text{ -->} H_2S + CO_2$ (2)

Further the isolated strain is characterized by a constant respiration rate (0.245-0.30 $\mu mol\ O_2.\mu g^{-1}.min,^{-1}$) over a wide pH range (0.7-5). The growth rate was relatively high, 0.16 h^{-1} (determined at pH 2.5) and the affinity constant (K_s) for CS_2 was below 0.2 μM. This indicates that this bacterium is very well adapted for CS_2 conversion at extremely low pH.

P088

Complete denitrification in eukaryotic benthic foraminifera

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The anaerobic respiration with nitrate as an electron acceptor was generally regarded as a bacterial process. However, using $_{15}N$ activity tests, we have recently discovered species of eukaryotic benthic foraminifera with the ability to fully denitrify in the oxygen-free zone of marine sediments. In addition these species accumulated and stored high concentrations of nitrate in their cells. The amount of stored nitrate would be sufficient to support anaerobic respiration for over a month. Further, the intracellular nitrate pool of the dominant foraminifera accounted for 20% of the large cell-bound nitrate pool present in the oxygen-free zone of the investigated sediments. Electron microscopy and fluorescence in situ hybridisation indicated that the ability of the foraminifera to denitrify was not the result of a symbiosis with bacteria. Given the high abundance of foraminifera in anoxic marine environments, these new findings suggested that foraminifera may play an important role in global nitrogen cycling and indicated that our understanding of the complexity and evolution of the marine nitrogen cycle is far from complete.

P089

Detection and quantification of genes conferring bacterial heavy metal resistances in metal-polluted marine sediments

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Many of Europe's coastal sediments are contaminated with heavy metals due to industrial discharges, waste disposal streams and atmospheric deposition of exhaust gasses. Particularly harbour sediments often contain high concentrations of heavy metals, especially copper and tin, which is attributed to the application of antifouling paints on ship hulls of sea vessels. The objectives of this study were to develop a Quantitative-PCR (Q-PCR) assay for the detection of genes encoding bacterial metal transporters and to investigate whether a correlation existed between copy numbers in marine sediments and concentration of the corresponding metal. From comparative genome analysis two genes were selected based on their wide spread occurrence: 1) copA, a P-type ATPase transporting Cu(I), and 2) czcA, a proton/cation antiporter for Co(II), Zn(II) and Cd(II). In both cases, primers were designed to amplify genes from selected members of the Alteromonadaceae and the Vibrionaceae. Primer specificity was tested on control strains and confirmed by sequencing of the products.
In the first experiment a silty sediment from an industrial harbour was studied in detail, while in a second test a range of 11 European sediments was analysed. High resolution metal analysis in the silty marine sediment showed that heavy metals as copper, cobalt, cadmium and zinc resided in tightly defined horizons, determined by redox gradients and mobilization/precipitation reactions. The number of 16S rRNA genes per ng DNA decreased with depth from $(1.27\pm0.08)*10^{10}$ at the surface to $(5.83\pm0.18)*10^{9}$ at 4cm depth. For czcA copy numbers throughout the column ranged from 6 to 220 per 10^{10}copies of 16S rRNA. Maximal copy numbers of copA were found in the 1st cm

of the sediment, i.e. 1190 per 10^{10} copies of 16S rRNA, coinciding with the peak in copper concentration at that depth (r^2=0.76). In the second experiment sediments differing in heavy metal load, grain size and salinity were compared. Statistical analysis elucidated a correlation between copynumbers of copA and extracted copper, but only for the sandy sediments (n=5, r^2=0.95, p=0.004). A moderately strong but significant correlation was revealed between czcA copynumber and extracted cadmium in all tested sediments (n=11, r^2=0.75, p=0.0006).
These results provide convincing evidence that quantification of metal transporter encoding genes in environmental samples is feasible, and moreover they suggest that the copynumber of copA or czcA can be used as an indicator for bio-available heavy metals.

P090

Combined 3D structural analysis of a bacterium with an organelle

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Anaerobic ammonium oxidation (anammox) is a recently discovered pathway of the biological nitrogen cycle and is currently estimated to be the major source of gaseous nitrogen on Earth. Anammox is performed by a clade of planctomycete bacteria, which divide only once per two weeks at maximum speed. These bacteria possess an organelle, the 'anammoxosome', an intracytoplasmic compartment surrounded by a bilayer membrane. This organelle is dedicated to anammox catabolism and is the locus of respiration by these bacteria, analogous to the mitochondria in Eukaryotes. The anammoxosome membrane consists mainly of so-called ladderane lipids that make this membrane almost impermeable to protons and the toxic intermediates of anammox catabolism.
With their small size (<1 µm) and spectacular cell biology, these prokaryotes are an ideal target for tomographical investigation. Here we used electron tomography to compile an image of the anammox cell and its organelle. Different stages of the one-month cell cycle were investigated and compared to resolve how the anammoxosome divides and is passed on to the daughter cells. Many structures were observed in the cytoplasm and in the anammoxosome, such as glycogen storage particles, iron containing particles, membrane foldings and fibrous protein structures. Electron tomography was combined with energy dispersive x-ray analysis, immunogold localization of anammox enzymes, and histochemical staining methods to provide the link to the functional significance of the structures visualized. The recently completed genome of the anammox bacterium *Kuenenia stuttgartiensis* provided us with many leads for the functional unraveling of anammox cell biology.

P091

Insertion/deletion polymorphism in the heterolysis-associated cibABC region of *Streptococcus pneumoniae* serotype 7 strains

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Background: Competence, required for gene exchange in *Streptococcus pneumoniae*, is associated with cell lysis for the release of DNA. Lysis of pneumococci also plays an essential role in virulence. Prior to autolytic degradation, cells are killed by the action of the Competence Stimulating Peptide-inducible 2-peptide bacteriocin CibAB, produced by a subpopulation of induced cells which kill non-induced cells (heterolysis). Coexpression of the cognate resistance protein CibC renders CSP-induced cells resistant for CibAB. The apparent requirement of CibABC for fratricide, and the need of lysis for virulence suggest that CibABC is required for virulence. We therefore aimed to analyse cibABC in different *S. pneumoniae* serotypes.
Methods: S. pneumoniae isolates from the blood of 74 patients with invasive pneumococcal disease were included in the study. Isolates were of serotypes 2(2 isolates), 4(3), 6(8), 7(15), 8(8), 9(4), 11(2), 12(1), 13(1), 14(6), 15 (3), 16(1), 17(1), 19(3), 21(1), 23 (12), 25(1), 35(1), 37(1), 42(1).The presence of cibABC was assessed by conventional and Expand Long Template PCR, and sequencing.
Results: PCRs for cibA, cibAB, and cibABC were positive for all isolates, except for the 15 serotype 7 isolates. Based on the available genome sequences of S. pneumoniae primers were designed to amplify sequences flanking the cibABC operon. PCRs for the regions directly upstream and downstream of cibABC were negative in the serotype 7 isolates, implying a deletion and/or insertion. Expand Long Template PCR with DNA of 8 of the *S. pneumoniae* serotype 7 isolates as template, and using primers annealing approximately 1 kb upstream and downstream of the cibABC operon yielded identical fragments, which were approximately 4 kb larger than amplicons obtained with serotype 2 strain D39 DNA as template. Partial sequencing of the amplicons of 2 of the serotype 7 isolates revealed a deletion comprising the open reading frames of cibA and most of cibB, leaving the cibABC promoter and putative translation start site of cibC intact. Downstream a cibC

sequence encoding a putative CibC protein with a modified C-terminus is present, followed by a large insertion, which is presently being sequenced.

Conclusion/discussion: In all 15 *S. pneumoniae* serotype 7 isolates analysed, cibA and cibB are deleted. However, serotype 7 isolates do show lysis in culture, indicating that either a functional copy of cibAB is present elsewhere in the genome, or that CibAB-independent lysis also occurs. Provided that the modified CibC of the serotype 7 isolates is functional, CSP-induced cells would be protected against CibAB from other isolates, and take advantage from the fratricide of these other isolates in a competitive cocolonization situation.

P092

Functionality of the pig gastrointestinal microbiota in response to alternatives for in-feed antimicrobials

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Introduction: Weaning of piglets at an early age causes gastrointestinal disturbances and an increased susceptibility to infection. Control measures rely on the addition of microbial growth promoters (AMGPs) in feeds. The EU-ban on AMGPs has increased the interest on alternative ways to stimulate and stabilize the autochthonous gastrointestinal microbiota. In the framework of the EU-funded project 'FeedForPigHealth', the current study aims to detect, localize and quantify key microbial species, their metabolic activity and interaction with the host, as affected by PENS (plant extracts and other natural substances) supplemented to diets, using an in vitro batch culture system in combination with conventional cultivation and direct molecular techniques (fingerprinting and detection). The overall microbial activity will be assessed by chemical analyses of the digesta for microbial metabolites. In addition, attempts will be made to identify molecular functional markers for the detection of specific changes in gene expression profiles in key populations, in response to PENS.

Methods: 1) PENS trials: Molecular fingerprinting techniques (DGGE of total bacterial and *Lactobacillus* communities) were used to investigate how complex microbial populations respond to the inclusion of PENS. Faecal inoculum was obtained per rectum from piglets (two weeks post-weaning). Some PENS (from a total of 42) were included as a whole substrate in the in vitro system, to be tested for their fermentative ability. Others (e.g., organic oils, sanguinaria, carotenoids) were tested in combination with substrates at different concentrations. 2) It has been described that fructooligosaccharides (FOS) affect the gut community. The fact that the porcine gut commensal *Lactobacillus sobrius* is able to use FOS as carbon and energy source could indicate the presence of an operon closely related with the one found in *Lactobacillus acidophilus*. Primers were designed for highly conserved regions in two genes (bfrA, fructosidase gene and gtfA, sucrose phosphorylase gene) from the gene cluster involved in FOS utilization by *L. acidophilus*.

Results: 1) Microbiological analyses of the fermented samples demonstrated that in general, incubation with different substrates (i.e. SBP, chyme and starch) lead to substrate-specific shifts in community profiles, while most of non-fermentable additives had no significant effect on microbiota composition. Only oregano oil (10% solution) additive had a significant effect on the composition, increasing the microbiota diversity. No additive-specific changes were observed in *Lactobacillus* spp. profiles. Further studies are currently underway to assess the effect of selected PENS on microbiota composition and activity *in vivo*. 2) The primers designed for the conserved regions of the gene cluster confirmed the presence of gtfA in the genome of *L. sobrius*.

Conclusion: 1) Community profiles are dependent on the substrate present during fermentation. 2) Addition of non-fermentable PENS does not have a qualitative effect on the microbiota. 3) Several PENS cause changes in the fermentation parameters but only concentrated Oregano oil provoked a significant change of microbiota composition. 4) Genes encoding for proteins potentially involved in FOS metabolism detected in *L. sobrius*.

P093

Expression analysis of formate dehydrogenases and hydrogenases in *Syntrophobacter fumaroxidans* with quantitative PCR

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Syntrophic consortia of propionate oxidizing bacteria and methanogenic archaea are crucial for high rate wastewater treatment performance by the Upflow Anaerobic Sludge Bed (UASB) reactor. To keep anaerobic propionate oxidation energetically favourable, interspecies electron transfer, either as hydrogen or formate, is essential. We study the hydrogen and formate transfer between *Syntrophobacter fumaroxidans* (propionate oxidizer) and Methanospirillum hungatei (methanogen).

Genome analysis of *S. fumaroxidans* revealed the presence of four distinct gene clusters coding for formate dehydrogenases and six distinct gene clusters coding for hydrogenases, including five [NiFe]-hydrogenases and one [FeFe]-hydrogenase. Primers were designed, specific for each formate dehydrogenase and hydrogenase gene cluster and tested with quantitative PCR. During syntrophic

growth with *Methanospirillum hungatei*, the expression level of [FeFe]-hydrogenase was higher than the expression levels of the five [NiFe]-hydrogenases, whereas expression levels were similar during axenic growth. This strongly suggests that the [FeFe]-hydrogenase of *S. fumaroxidans* plays an important role during syntrophic growth.

P094

Comparison of five Epstein Barr virus (EBV) enzyme immunoassays, an automated chemiluminescence assay and immunoblot assay with the EBV immunofluorescence assay as a reference

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Background: Enzyme immunoassays (EIAs) from Serion (Se), Panbio (Pa), DiaSorin (Di), Novitech Immunowell (No), Genzyme Virotech (Vi), the Virotech immunoblot assay (IB) and the DiaSorin automated chemiluminescence assay (Liaison) were evaluated with an in-house EBV VCA IgM, VCA IgG and EBNA IgG immunofluorescence assay (IFA) as the reference test.

Methods: Serum samples from immunocompetent and organ transplant (Tx) patients were assigned to different groups depending on their EBV IFA patterns. Group 1: IgM positive, IgG positive, EBNA negative (n=80), group 2: IgM, IgG and EBNA negative (n=103), group 3: IgM negative, IgG and EBNA positive (n=27). Group 4: IgM negative, IgG positive, EBNA negative (n= 10). Group 5: IgM negative, IgG and EBNA positive (n=25; Tx patients). Group 6: IgM negative, IgG positive, EBNA negative (n=22; Tx patients). Group 7: IgM, IgG and EBNA positive (n=25). Samples tested positive for Toxoplasma gondii IgM, (n=25) and hepatitis A IgM (n=17) were included to assess interference with EBV IgM (group 8, all IFA IgM negative). A subset of samples (n=319) was evaluated in the Liaison. Grey zone results were considered negative.

Results: IgM sensitivities (group 1) were 78-95% (Vi and Se), 93% for Liaison and 100% for IB. IgM specificities (groups 2-6 and 8) were 93-97% (Vi and No/PA), 95% for Liaison and 92% for IB. IgG sensitivities (groups 1 and 3-7) were 84-97% (Vi and Se), 91% for Liaison and 98% for IB. IgG specificities (group 2) were 87-92% (Vi and Se), 90% for Liaison, 88% for IB. EBNA sensitivities (groups 3,5 and 7) were 75-96% (Se and No), 87% for Liaison and 92% for IB. EBNA specificities (groups 1 and 2) were 78-97% (No and Se), 85% for Liaison and 89% for IB. The serological status of recent infection (A), past infection (B) or seronegative (C), were concordant with IFA for A in 30-72% (No and Pa), in 67% for Liaison and 88% for IB. Concordance with IFA for B was found in 78-96% (Se and Pa), in 85% for Liaison and 96% IB and for C in 77-88% (Pa and Se), 82% for Liaison and 84% IB. Intrarun coefficients of variation (CVs) for IgM were 8% to 41% (Se and No), for IgG 5% to 57% (Vi/Pa and No) and for EBNA 10% to 28% (Di and Se). Interrun CVs for IgM were 7% to 24% (Di and No), for IgG 7% to 52% (Pa and No) and for EBNA 10% to 42% (Pa and Vi).

Conclusions: The performance of the evaluated assays varied for each individual EBV parameter. Overall, Se and IB performed best compared to IFA, with respect to sensitivity and specificity. However, when regarding the EBV serological status only IB showed an acceptable concordance with IFA for recent infection, whereas Pa and IB showed excellent concordance with IFA for past infection. All assays showed acceptable concordance with IFA for the seronegative status. Lowest interrun and intrarun CVs were observed for Pa and Di.

P095

Successful control of *Clostridium difficile* type 027 outbreak by cohort isolation of infected patients

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Objectives: Since 2005, outbreaks due the hypervirulent *Clostridium difficile* type 027 were recognized in the western part of the Netherlands. The outbreaks are difficult to control and advises are contradictory with respect to isolation protocols. We performed a prospective comparative study of cohort isolation with isolation of a patient in a single room to the incidence of CDAD.

Methods: From January until December 2005, an outbreak of CDAD occurred in a 400 beds general hospital encompassing 129 patients in total. The mortality attributable to CDAD was 3.1%. Of 17 available strains, 12 (71%) belonged to PCR ribotype 027. The departments of Internal Medicine (81 beds) and Surgery (76 beds) were the two most affected departments with 42 and 60 patients, respectively. At week 18, cohort isolation was introduced at the Surgery department, whereas isolation on a single room was continued at the Internal Medicine. Patients were isolated on a clinical suspicion of CDAD and/or a positive toxin test of a faeces sample by enzyme immunoassay. Data on the antibiotic use were obtained from the pharmacy database and calculated as daily defined doses (DDD). Patients characteristics were obtained using a home made standardized questionnaire.

Results: Before week 18 of the outbreak, the incidence of CDAD was 38.5 per 1000 admissions at the Department of Internal medicine and 35.4 per 1000 admission

at the Surgery department. The construction of both departments was identical. Patients did not differ in mean age, gender, classification of American Society of Anaesthesiology characteristics, previous antibiotic use and days of admission before CDAD developed. After introduction of cohort isolation, the incidence decreased in the following 20 weeks to 4.4 per 1000 admissions whereas the incidence remained unchanged to 39.8 per 1000 admission at the Internal Medicine. Patients with CDAD after week 18 did not differ from patients with CDAD before week 18. Environmental disinfection with hypochlorite was similar at the two departments, as were handhygiene with water and soap and the use of protective clothing. DDD of both cephalosporines and fluoroquinolones was 11.0 at the Internal Medicine per 100 bed-days and 10.5 and 11.0 at the Surgery department.

Conclusion: In contrast with isolation on a single room, cohort isolation resulted in a rapid decrease of the incidence of CDAD during an outbreak of the hypervirulent type 027.

P096

Elimination of *Staphylococcus aureus* nasal carriage in health care workers of a burn unit: effect on *S. aureus* burn wound colonization

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Introduction: *Staphylococcus aureus* colonization and infection of burn wounds increases morbidity and delays wound healing. *S. aureus* burn wound colonization may result from nasal and pharyngeal colonization of patients as well as health care workers (HCW).

The aim of this study was to evaluate the effect of eradication of nasal *S. aureus* in HCW with mupirocin on the incidence of S. aureus burn wound colonization.

Methods: HCW nasal *S. aureus* was eradicated with one course of mupirocin in July 2004. From July 2003 to June 2006 patients were extensively screened on admission for *S. aureus* carriage and burn wounds were cultured weekly. *S. aureus* burn wound colonization during the year following mupirocin treatment (July 2004 to July 2005, n=72) was compared with two control periods, C1 (July 2003 to June 2004, n=54) and C2 (August 2005 to June 2006, n=57).

Results: Forty-three (93%) HCW have received the mupirocin course. Nasal eradication was proven successful in 13/15 (87%) of the nasal carriers. HCW nasal carriage rate dropped from 35% to 2%, and gradually increased from 12% after 6 months to 25% after one year.

The incidence of burn wound colonization during admission in the year after the mupirocin course was 27/56 (48%) and for control groups C1 and C2 12/42 (29%) and 14/44 (32%) respectively.

Conclusion: Eradication of *S. aureus* nasal carriage among health care workers did not reduce *S. aureus* burn wound colonization.

P097

Staphylococcal modulation of the Lectin Pathway of complement

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Introduction: The Lectin Pathway (LP) triggers activation of the complement system and is therefore considered to be important for innate immune defences. In the LP, recognition of foreign antigens is provided by different carbohydrate recognition molecules: Mannose binding lectin (MBL) and ficolins (H-, L-, and M-ficolin). Binding of MBL and ficolin to microbes results in activation of MBL-associated serine proteases (MASPs). In humans, four different MASPs were described: MASP-1, MASP-2, MASP-3 and a truncated MASP, Map19. Only MASP-2 is known to activate the complement system by cleavage of C4 and C2 resulting in the formation of C3 convertases (C4b2a). The C3 convertases cover microbes with C3b molecules which is crucial for phagocytosis and activation of C5. The role of the other MASPs remains unknown. Staphylococcus aureus is well-known for the excretion of numerous small molecules that specifically block critical steps of the innate immune defence. Since the LP is crucial in our defence against *Staphylococcus aureus*, we investigated the presence of specific LP inhibitors in *S. aureus*.

Methods: Thirty clinical *S. aureus* isolates were grown in Iscove's Modified Dulbecco's Medium (IMDM) for 16 hours at 37 °C. Bacterial supernatants were collected after centrifugation and subsequently dialyzed against Veronal Buffered Saline, 5 mM CaCl2, 2,5 mM MgCl2, pH 7.4 (VBS++). Mannan-coated microtiter wells were incubated with IgG-depleted human serum at 37 °C in the presence of bacterial supernatants. LP-mediated deposition of C4b or C3b deposition was detected using specific antibodies. Activation of the classical pathway was performed on IgM-coated wells.

Results: Analyses of thirty different *S. aureus* supernatants in a LP ELISA led to the identification of three supernatants that could block LP-mediated C4b deposition by 50%. These supernatants also prevented C3b deposition by the LP. Since these supernatants did not affect C4b deposition in the Classical Pathway, complement inhibition is specific for the LP.

Conclusion: *S. aureus* excretes specific inhibitors of the LP. Purification of the active component from staphylococcal supernatants will be needed to get insight into the exact

inhibitory mechanisms. Identification of bacterial LP inhibitors will increase our understanding of the role of the LP in our defence against bacteria.

P098

Functional analysis of PcsB in pneumococcus

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PcsB is a protein with putative murein biosynthesis function that influences the cell morphology of several pathogenic species of *Streptococcus* and other related bacteria. Homologues of PcsB are essential in *Streptococcus pneumoniae* (pneumococcus), *Enterococcus faecium*, conditionally essential in *Streptococcus agalactiae*, but non-essential in *Streptococcus mutans* and *Staphylococcus aureus*. In pneumocuccus, PcsB is regulated by the two-component regulatory system designated YycF/G (TCS) and has been suggested to act as a cell wall hydrolase with a dual function in i) controlling the extent of cell wall synthesis during cell division and ii) participation in cell separation. Pneumococcal PcsB contains conserved protein domains found in other bacterial murein hydrolases. Evidence for its peptidoglycan hydrolase activity in pneumococcus has also come from PcsB depletion studies, although definitive biochemical data are still lacking. Furthermore, it remains unknown why pneumococcal PcsB expression is strongly and directly regulated by TCS, and why the closest homologues of PcsB from *S. mutans* and *S. agalactiae* failed to complement mutations in pneumococcal PcsB. To further elucidate the biochemical functions and interactions of pneumococcal PcsB, its gene was targeted for cloning and overexpression in *Escherichia coli* using the pET-51b+ streptag system. Results will be presented on structural and functional studies of purified PcsB. The PcsB expression during the pneumococcal cell cycle will be further analyzed using a quantitative real-time PCR. The physiological significance of these results will be depicted in our current network model of gene regulation by YycFG in pneumococcus.

P103

Similar early viral kinetics of hepatitis C virus genotypes 1 and 4

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Background: Patients infected with hepatitis C virus genotype 2 (HCV-2) or HCV-3 respond better to interferon alfa (IFN-α) treatment than HCV-1 or HCV-4 patients. The mean initial decline in HCV RNA during IFN-α therapy is faster for HCV-2 and HCV-3 compared to HCV-1 patients. Little is known about early viral kinetics in patients with HCV-4.
Aims: The aim of our study was to determine genotype specific differences in early viral kinetics in HCV-1 and HCV-4 patients during a modified treatment regimen with a high initial dose of interferon (induction).
Methods: We treated naïve patients with HCV-1 (n=42) or HCV-4 (n=12) with triple antiviral therapy consisting of amantadine hydrochloride and ribavirin, combined with 6 weeks of IFN-2b induction (week 1-2: 18 MU/day, week 3-4: 9 MU/day, week 5-6: 6 MU/day), thereafter combined with weekly Peg-IFN-α 2b, for 24 or 48 weeks. HCV RNA was assessed at baseline, day 1, 2, week 1, 2, 4, 6, 8, and then every 4 weeks until end of treatment by: quantitative bDNA (LLD 615 IU/ml), qualitative PCR (LLD 50 IU/ml), and TMA (Transcription-Mediated Amplification, LLD 5 IU/ml). Viral dynamics were estimated using the bi-phasic model for HCV during treatment with IFN-α.
Results: Baseline HCV RNA levels, and the 1st and 2nd phase decline in HCV RNA, were similar in HCV-1 and HCV-4 patients (Figure). Mean time to reach a TMA negative status in patients with subsequent SVR was shorter in HCV-4 (4.3±2.3 weeks) compared to HCV-1 (6.4±4.5 weeks), this difference was not significant. SVR was achieved by 43% of HCV-1 and 50% of HCV-4 patients.
Conclusions: Early viral kinetics are similar in HCV-1 and HCV-4 patients, these results confirm that HCV-4 patients should be treated as HCV-1.

P105

Early exposure to rota- and norovirus infections and the development of atopic manifestations and recurrent wheeze in infants during he first two years of life

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The incidence of atopic manifestations (eczema, wheeze and sensitization) amongst children in the developed world has increased over the past decades. This increase has been associated with decreasing incidence of childhood infections, however most evidence in favor of this association has been obtained from cross-sectional studies, where the causal relationship is difficult to establish. The aim of this prospective study was to investigate the relation between early intestinal viral infections and the development of atopic symptoms in 2 year old infants.

612 infants were followed from birth till 2 years by repeated ISAAC questionnaires establishing the presence or absence of eczema and wheeze. Rota- and Norovirus seropositivity and the presence of total and specific IgE were measured by ELISA assays at 1 and 2 years of age.
During the first 2 years of infant life, 32% developed atopic dermatitis, 9% experienced recurrent wheezy attacks and 24% showed atomic sensitization, Rotavirus seropositivity at age 1 year (39%) was significantly associated with recurrent wheeze in infants 1 and 2 years of age (ORadj 2.0 (95% CI; 1.1-3.9) and 2.1 (95% CI; 1.1-3.8) respectively). Norovirus genotype I.1 seropositivity (19%) showed a trend for a negative association with increasing concentrations of total and specific IgE at 1 year of age. No further associations where found between intestinal viral seropositivity and atopic manifestations during the first two years of life.
Conclusions: Infants who experienced a Rotavirus infection during the first year of life showed significantly more recurrent wheeze at 1 and 2 years of age when compared to infants without Rotavirus infections. Norovirus GI.I showed a weak negative association with total and specific IgE, indicating that infants exposed to a Norovirus GI.1 rich environment are protected against early atopic sensitization. However, this cohort needs to be followed up to older ages in order to establish the true importance of intestinal viral infections in atopic disease etiology.