NEDERLANDS TIJDSCHRIFT VOOR

MEDISCHE MICROBIOLOGIE

SUPPLEMENT BIJ TWAALFDE JAARGANG, APRIL 2004

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) in samenwerking met:
Secties Algemene en Moleculaire Microbiologie, Microbiële Ecologie, Technische Microbiologie en Mycologie; Sectie Algemene Virologie; Sectie Levensmiddelenmicrobiologie; Nederlandse Vereniging voor Medische Mycologie; Werkgemeenschap Microbiële Pathogenese; Werkgroep Epidemiologische Typeringen; Werkgroepen Oost en West Medische Microbiologie; Nederlandse Werkgroep Klinische Virologie; Stichting Kwaliteitsbewaking Medische Microbiologie

Papendal, 6 en 7 april 2004

adv Clindia

Inleiding

Inmiddels is het traditie aan het worden: de Voorjaarsvergadering van de Nederlandse Vereniging voor Microbiologie (NVvM) en de Nederlandse Vereniging voor Medische Microbiologie (NVMM), en wordt op 6 en 7 april 2004 te Papendal gehouden. Ook de formule wordt een traditie: een plenair symposium op dinsdagochtend, dit jaar met als thema 'Communication', gevolgd door overwegend thematisch ingedeelde parallelsessies.
De multidisciplinaire, interactieve sessie van vorig jaar was dermate succesvol, dat deze sessievorm dit voorjaar wordt verdubbeld: één over mycobacteriële infecties bij kinderen en de ander over *Chlamydia trachomatis* en infertiliteit.

AIO's blijven zeer welkom op de Voorjaarsvergadering: zij worden vrijgesteld van inschrijfkosten, middels de 'Young Investigators Grant', op voorwaarde dat zij een presentatie houden. Uiteraard is alleen de presenterende auteur van een voordracht of poster vrijgesteld.

'Communication' is het onderwerp van het plenaire symposium op dinsdagochtend: communicatie tussen micro-organismen onderling en tussen micro-organismen en gastheer. We hopen dat dit thema de hele Voorjaarsvergadering zal beheersen en dat communicatie tussen de verschillende geledingen die in de Voorjaarsvergadering participeren optimaal zal zijn.

Voorbereidingscommissie

Prof. dr. C.M.J.E. Vandenbroucke-Grauls, voorzitter
Dr. T. Boekhout
Dr. C.H.E. Boel
Prof. dr. S. Brul
Dr. R.J.A. Diepersloot
Prof. dr. L. Dijkhuizen
Mw. dr. L.Dijkshoorn
Prof. dr. J.M.D. Galama
Dr. P.W.M. Hermans
Mw. drs. L.M. Kortbeek
Prof. dr. H.J. Laanbroek
Dr. J.A.G. Strijp
Dr. P.E. Verweij
Prof. dr. W. de Vos
Prof. dr. E.J.H.J. Wiertz
Dr. H.A.B. Wösten

De NVMM organiseert deze bijeenkomst in samenwerking met

Nederlandse Vereniging voor Microbiologie
Secties Algemene en Moleculaire Microbiologie, Microbiële Ecologie, Technische Microbiologie en Mycologie
Sectie Algemene Virologie
Sectie Levensmiddelenmicrobiologie
Nederlandse Vereniging voor Medische Mycologie
Werkgemeenschap Microbiële Pathogenese
Werkgroep Epidemiologische Typeringen
Werkgroepen Oost en West Medische Microbiologie
Nederlandse Werkgroep Klinische Virologie
Stichting Kwaliteitsbewaking Medische Microbiologie

ZonMw ALW NWO

Congressecretariaat

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Congress Care

Dates

6 and 7 April 2004

Venue

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

Website

Please check *www.nvmm.nl* for up-to-date programme information.

Language

The language during the scientific sessions will be English (unless in the programme mentioned otherwise).

Accreditation

The 'Wetenschappelijke Voorjaarsvergadering 2004' will be accredited by the NVMM with 5 points per day and maximal 10 points for the whole meeting.

Name badges

All participants are requested to wear their name badges throughout the congress.

Registration desk

The registration desk will be open on Tuesday and Wednesday during congress hours.

Audiovisual equipment

PowerPoint

All meeting rooms are equipped with computers and data projectors. Please bring your presentation on CD-rom or memory-stick to the meeting room of your session in the break before your presentation.

Poster session

Posters will be on display throughout the congress in the foyer of meeting rooms 6/7 and 8/9. Please mount the poster before the end of the lunch break on Tuesday 6 April and according to the poster number, as confirmed to the presenting author. The numbers on the poster boards correspond with the abstract numbers in the programme/abstract book. Posters can be removed Wednesday 7 April after the lunch break.
The poster boards are 90 cm wide and 150 cm high.
Poster authors are requested to man their posters on Tuesday evening 6 April. The *odd numbers* from 20:30-21:15 hours and the *even numbers* from 21:15-22:00 hours.

Poster prices

Yakult Nederland sponsors the poster prices. The following 3 prices are available:
1st price of € 350
2nd price of € 225
3rd price of € 115
The poster price ceremony will be held on Tuesday 6 April at 22:00 hours, in the foyer of meeting rooms 6/7 and 8/9. The winners have to be personally registered and be present.

Exhibition, lunch break, coffee/tea break

Coffee/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.

3W Informed
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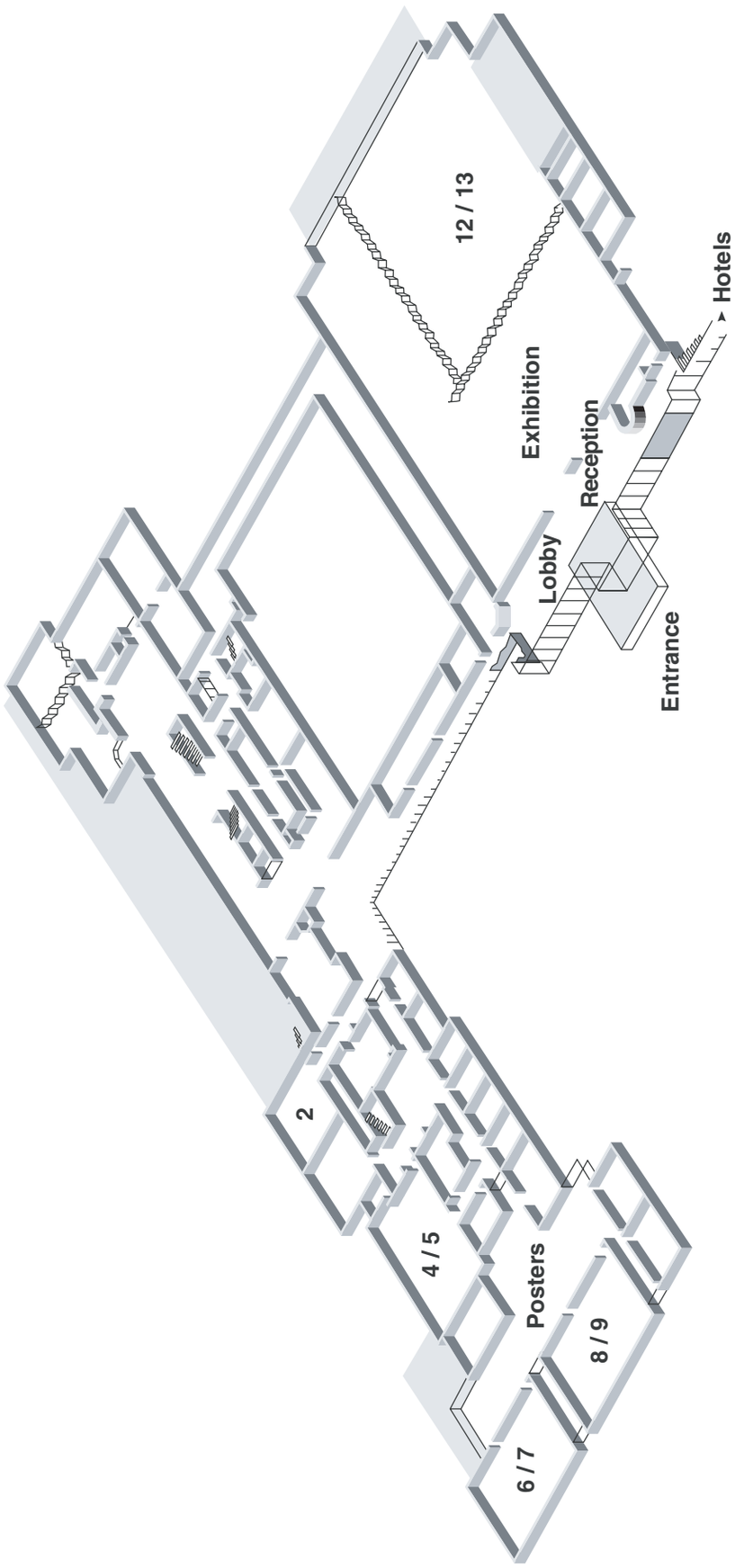
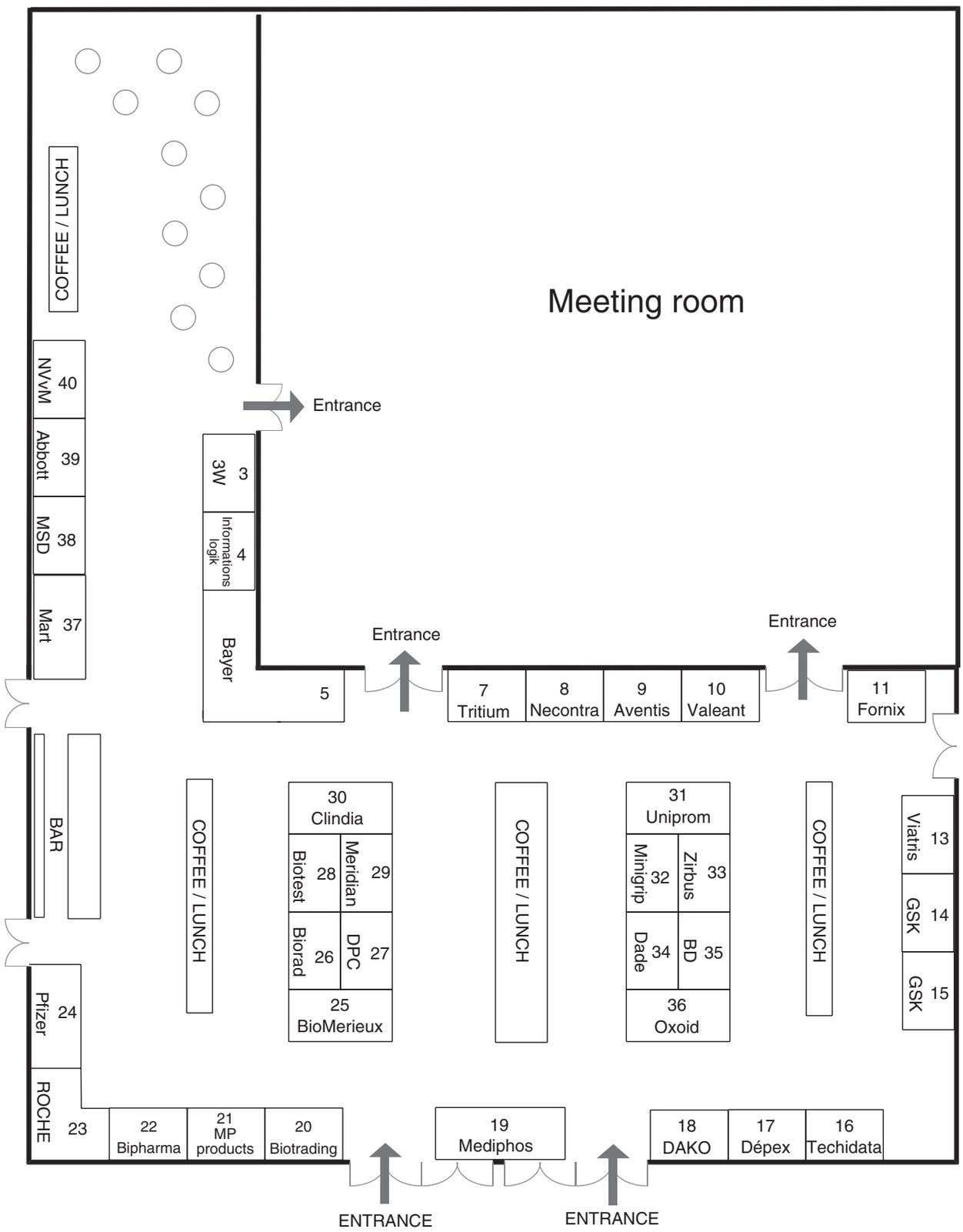
Sponsor poster prices

FLOORPLAN CONGRESS CENTRE



Floorplan congresscentre



TUESDAY 6 APRIL 2004

09:00 - 09:30	Registration and coffee/tea			
	Room 12/13			
09:30 - 10:15	Plenairy Session A01		P. Williams	
10:15 - 11:00	Plenairy Session A02		B.B. Finlay	
11:00 - 11:15	Coffee/tea			
11:15 - 12:00	Plenairy Session A03		H.P. Spaink	
12:00 - 12:45	Plenairy Session A04		L.A. Casselton	
12:45 - 14:00	Lunch			
	Lunchsymposium Bayer Diagnostica *(Room 12/13)*			
	Beroepsbelangencommissie niet-registerleden *(Room 2)*			
	Room 4/5	*Room 6/7*	*Room 8/9*	*Room 12/13*
	Working Group Epidemiological Typing	Diagnostics 1	Pathogenesis 1	Progress in Microbiology 1
14:00 - 14:15	B01/02	C01	D01	E01
14:15 - 14:30		C02	D02	E02
14:30 - 14:45	B03	C03	D03	E03
14:45 - 15:00	B04	C04	D04	E04
15:00 - 15:15	B05	C05	D05	E05
15:15 - 15:45	Coffee/tea			
	Vergadering werkgroepleiders Microbiële Pathogenese *(Room 8/9)*			
	Epidemiology	Workshop Microbiology Laboratory Services	Pathogenesis 2	Progress in Microbiology 2
15:45 - 16:00	G01	H01/02/03	D06	E06
16:00 - 16:15	G02		D07	E07
16:15 - 16:30	G03		D08	E08
16:30 - 16:45	G04	H04/05/06	D09	E09
16:45 - 17:00	G05		D10	E10
17:00 - 17:15	G06		D11	E11
	Room 12/13			
17:15 - 17:45	Plenairy Session A05		Kluyver Prize	
17:45 - 18:15	Plenairy Session A06		Hot News	
18:30 - 19:00	Drinks			
19:00 - 20:30	Dinner			
	Room 6/7 and 8/9			
20:30 - 22:00	Poster Presentations			
22.00	Poster Prices			

WEDNESDAY 7 APRIL 2004

	Room 4/5	*Room 6/7*	*Room 8/9*	*Room 12/13*
	Medical Mycology	Diagnostics 2	Functional Genomics 1	Multidisciplinaire Sessie: Lymphadenitis
09:00 - 09:15	J01	K01	L01/02/03	M01/02
09:15 - 09:30	J02	K02		
09:30 - 09:45	J03	K03		M03/04
09:45 - 10:00	J04	K04	L04/05/06	
10:00 - 10:15	J05	K05		M05/06
10:15 - 10:30	J06	K06		
10:30 - 11:00	Coffee/tea			
11:00 - 11:15	J07			
11:15 - 11:30	J08			
11:30 - 11:45	J09			
	Case Presentations	ICT in de Medische Microbiologie	Functional Genomics 2	Multidisciplinaire Sessie: Chlamydia
11:00 - 11:15	N01	O01/02	L07/08/09	Q01
11:15 - 11:30	N02			Q02
11:30 - 11:45	N03	O03/04		Q03
11:45 - 12:00	N04		L10/11/12	Q04
12:00 - 12:15	N05	O05/06		Q05
12:15 - 12:30				
12:45 - 13:15	Ledenvergadering NVvM *(Room 2)*			
13:00 - 14:00	Lunch			
	Therapy	De opleiding tot arts-microbioloog	Pathogenesis 3	Progress in Microbiology 3
14:00 - 14:15	R01/02	S01	T01	V01
14:15 - 14:30		S02	T02	V02
14:30 - 14:45	R03	S03/04/05	T03	V03
14:45 - 15:00	R04		T04	V04
15:00 - 15:15	R05		T05	V05
15:15 - 15:30	R06	S06	T06	V06
15:30 - 16:00	Coffee/tea			
16:00 - 18.00	Vergadering bestuur NVvM *(Room 10a)*			
	Ledenvergadering NVMM *(Room 4/5)*			
				Progress in Microbiology 4
16:00 - 16:15				V07/08/09
16:15 - 16:30				
16:30 - 16:45				

TUESDAY 6 APRIL 2004

A Room 12/13 **Plenary Session 'Communication'**

Chairman: W. de Vos

09:30 - 10:15	P. Williams (Nottingham, United Kingdom) Cell-to-cell communication in the bacterial world: small talk, straight talk and cross talk	A01
10:15 - 11:00	B.B. Finlay (Vancouver, Canada) Pathogenic *E. coli*: from molecules to vaccine	A02
11:00 - 11:15	Coffee/tea	

Chairman: A. Voss

11:15 - 12:00	H.P. Spaink (Leiden) Similarities of microbial recognition by plants and animals	A03
12:00 - 12:45	L.A. Casselton (Oxford, United Kingdom) The role of pheromones in fungal mating	A04

Room 12/13 **Lunchsymposium Bayer Diagnostica**
Monitoring voor de juiste koers
'de noodzaak van resistentie vaststelling'
(Dutch spoken session)

Chairman: C.A.B. Boucher

13:00 - 13:20	J.W. Mouton DUEM (DUtch In-Vitro Evaluatie van Moxifloxacin)
13:20 - 13:40	C.A.B. Boucher HIV-resistentie: back to the future?
13:40 - 14:00	H.L. Zaaijer Variabiliteit van HBsAg

B Room 4/5 **Working Group Epidemiological Typing**

Chairman: L. Dijkshoorn

14:00 - 14:30	B.R. Bochner (California, USA) Phenotype MicroArrays™ for phenotypic analysis of *E. coli*, *S. cerevisiae* and other microbial species	B01/02
14:30 - 14:45	J. Top, L.M. Schouls, M.J.M. Bonten, R.J.L. Willems Multi-locus variable-number tandem repeat analysis: a new fast, robust and discriminatory typing scheme for studying the genetic relatedness of *Enterococcus faecium* isolates	B03
14:45 - 15:00	A.T.A. Box, E. van Duijkeren, A.C. Fluit Great diversity within the staphylococcal cassette chromosome *mec* in methicillin-resistant staphylococci from human and veterinary origin	B04

15:00 - 15:15	E.W. Tiemersma, C.E.M. Moolhuijzen, M.E.O.C. Heck, G.N. Pluister, W.J.B. Wannet, A.J. de Neeling Origin of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands: results from a random sample of MRSA isolates	B05

C Room 6/7 Diagnostics 1

Chairman: A.C.M. Kroes

14:00 - 14:15	A.V.M. Möller, H. Geerligs, B.G. Dijk, B.P. Overbeek Few chromogenic agars perform well for rapid detection of *E. coli* in urine and are cost-effective when a high percentage of cultures is positive for *E. coli*	C01
14:15 - 14:30	Y.J. Debets-Ossenkopp, J. Mulder, H.M. Gittelbauer, P.H.M. Savelkoul, C.M.J.E. Vandenbroucke-Grauls Simple, rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* with cefoxitin	C02
14:30 - 14:45	M.F.C. Beersma, K. Dirven, H.J. Gerritsen, A.P. van Dam, E.C. Claas, H. Goossens Evaluation of *Mycoplasma pneumoniae* commercial tests for detection of serum antibodies using PCR as gold standard	C03
14:45 - 15:00	A.P. van Dam, G.A. Oei, J.F.P. Schellekens Evaluation of the C6-peptide ELISA in patients with Lyme borreliosis and controls	C04
15:00 - 15:15	R.P. Schade, C.J. Schinkel, F. Roelandse, R.B. Geskus, L.G. Visser, J.M.C. van Dijk, H. van Pelt, E.J. Kuijper Results of cerebrospinal fluid-analysis have no predictive value for external drain-related bacterial meningitis	C05

D Room 8/9 Pathogenesis 1

Chairman: J.G. Kusters

14:00 - 14:15	P.A. Berk, R. de Jonge The relation between acid adaptation and virulence of *Salmonella enterica* serovar Typhimurium DT104	D01
14:15 - 14:30	C.A.N. Broekhuizen, L. de Boer, K. Schipper, J.J. Weening, J. Dankert, S.A.J. Zaat Tissue colonization is more important than biofilm formation in *Staphylococcus epidermidis* experimental biomaterial-associated infection	D02
14:30 - 14:45	F.D. Ernst, E.L. Benanti, E.J. Kuipers, A. Heijens, J. Stoof, J.G. Kusters, P.T. Chivers, A.H.M. van Vliet The NikR regulatory protein governs transcriptional regulation of NixA-mediated nickel-uptake in *Helicobacter pylori*	D03
14:45 - 15:00	P.H.S. Kwakman, A.A. te Velde, J. Dankert, S.J.H. van Deventer, S.A.J. Zaat Antimicrobial and anti-inflammatory activity of Bactericidal Peptide 2 *in vitro*	D04

15:00 - 15:15 — S. van Selm, P.V. Adrian, S.C. Estevao, M. van Roosmalen, J. Neef, H. Metselaar, S. Audouy, E.E.S. Nieuwenhuis, P.W.M. Hermans, K. Leenhouts — D05
Lactococcus lactis ghosts displaying multiple *Streptococcus pneumoniae* protein antigens elicit protective immunity in a murine pneumonia model

D Room 8/9 Pathogenesis 2

Chairman: S.A.J. Zaat

15:45 - 16:00 — W.T. Hendriksen, A. de Jong, P.V. Adrian, R. de Groot, O.P. Kuipers, P.W.M. Hermans — D06
CodY-transcriptional regulation in *Streptococcus pneumoniae*

16:00 - 16:15 — V. Hira, P.V. Adrian, M. Sluijter, R.F. Kornelisse, R. de Groot, P.W.M. Hermans — D07
Selection of surface-exposed proteins for development of protective antibodies against coagulase-negative staphylococcal infection

16:15 - 16:30 — A.M. Abdallah, A.M. van der Sar, M. Sparrius, E. Reinders, C.M.J.E. Vandenbroucke-Grauls, W. Bitter — D08
Mycobacterium marinum strain-variation is an important factor in the pathology of fish tuberculosis

16:30 - 16:45 — M. Wosten, A. Wagenaar, P. Putten — D09
The FlgS/FlgR two-component signal transduction system regulates the FLA regulon in *Campylobacter jejuni*

16:45 - 17:00 — Y. Pannekoek, J. Spaargaren, A.A. Langerak, J. Merks, S.A. Morre, A. van der Ende — D10
Mutations in the *incA* gene of *Chlamydia trachomatis* and association with non-fusogenicity and silent infection

17:00 - 17:15 — S. Ouburg, A. van de Ende, J.B.A. Crusius, Y. Pannekoek, R.W.M. van der Hulst, A.S.P. Peña, S.A. Morré — D11
Host factors, *IL1B-511 & IL-1RN* gene polymorphisms, and bacterial factors of *Helicobacter pylori, cagA* and *vacA* subtypes, in peptic ulcer disease and non-ulcer dyspepsia: a synergistic effect

E Room 12/13 Progress in Microbiology 1

Chairman: H.A.B. Wösten

14:00 - 14:15 — I. Cirpus, H. Harhangi, H. op den Camp, M. Schmid, J.G. Kuenen, D. LesPaslier, M. Wagner, M. Strous, M. Jetten — E01
Biochemistry and electron transport of the anammox bacterium *Kuenenia stuttgartiensis*

14:15 - 14:30 — M. Papadimitriou, S. Brul — E02
Expression and role of the Pdr12 membrane protein in yeast cells under sorbic acid stress

14:30 - 14:45 — A.H.A.M. van Hoek, I.M.J. Scholtens, H.J.M. Aarts — E03
Micro-array analysis for the screening of *Salmonella* strains for antibiotic resistance genes

14:45 - 15:00 — J.L.W. Rademaker, F. Driehuis, J. Hoolwerf, W.H. Noordman, A. Wagendorp, M.C. te Giffel — E04
Application of DNA fingerprinting in the food supply chain

15:00 - 15:15	W. van Schaik, M.H. Zwietering, W.M. de Vos, T. Abee The stress sigma factor SigmaB of *Bacillus cereus*: regulation and regulon	E05

E Room 12/13 **Progress in Microbiology 2**

15:45 - 16:00	J. Siebring, A.M.C.R. Alves, L. Dijkhuizen, G. van Keulen Functional analysis of phosphofructokinase orthologues in *Streptomyces coelicolor*: the multiplicity phenomenon	E06
16:00 - 16:15	S.A. Dar, J.G. Kuenen, G. Muyzer Diversity analysis of sulphate-reducing bacteria using a nested PCR-DGGE approach	E07
16:15 - 16:30	E. Kuramae, V. Robert, B. Snel, T. Boekhout Fungal phylogenomics: linking evolution and function	E08
16:30 - 16:45	G. Roeselers, M.C.M. van Loosdrecht, G. Muyzer Microbial diversity of phototrophic biofilms from wastewater treatment plants	E09
16:45 - 17:00	G. Zwart, M. Kamst-van Agterveld, I. van der Werff-Staverman, F. Hagen, H. Hoogveld, H. Gons Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake	E10
17:00 - 17:15	P. de Vreugd, H. Kloosterman, L. Dijkhuizen Novel teicoplanin and vancomycin derivatives using mutant glycosyltransferases	E11

G Room 4/5 — Epidemiology

Chairman: P. Savelkoul

15:45 - 16:00	D. Bogaert, A. van Belkum, M. Sluijter, A. Luijendijk, R. de Groot, H.C. Rumke, H.A. Verbrugh, P.W.M. Hermans Natural competition between *Streptococcus pneumoniae* and *Staphylococcus aureus* during colonisation in healthy children	G01
16:00 - 16:15	S. Nijssen, A. Fluit, A. Florijn, M. Bonten Epidemiology of integron-associated resistance in 2 ICUs during a non-outbreak period	G02
16:15 - 16:30	J.J.C. de Vries, W.H. Baas, J.E. Degener, J.P. Arends Outbreak of *Serratia marcescens* traced to a health care worker with at least 3 months carriage on the hands	G03
16:30 - 16:45	B.L. Herpers, B.M. de Jongh, H. van Velzen-Blad, J.C. Grutters, B.A.W. de Jong, E.J. van Hannen Mannose-binding lectin polymorphisms in Löfgren's syndrome	G04
16:45 - 17:00	G.A. Kampinga, C.A.M. Ossendrijver, G.T. Noordhoek, S. Mulder, P.C. Caesar An epidemic with a non-MRSA but multiresistant *S. aureus*	G05
17:00 - 17:15	B.J.M. Vlaminckx, W. van Pelt, L.M. Schouls, A. van Silfhout, E. Mascini, J.F.P. Schellekens Epidemiological features of invasive and non-invasive group A streptococcal disease in the Netherlands, 1992-1996	G06

H Room 6/7 — Workshop: Reïnforcement of Microbiology Laboratory Services for Public Health

Chairman: P.M. Schneeberger

15:45 - 16:30	B.I. Duerden (Cardiff, United Kingdom) Infectious diseases - a public threat The organisation of health protection in England	H01/02/03
16:30 - 17:15	J. Schellekens Surveillance of pathogens. Creating a nation-wide laboratory response network	H04/05/06

A Room 12/13 — Plenary Session

17:15 - 17:45	Kluyver Price	A05
17:15 - 18:15	Hot News	A06

P Foyer Room 6/7 and 8/9 — Poster Session and Presentation Yakult Poster Prices

20:30 - 21:15	Poster presentations odd poster numbers
21:15 - 22:00	Poster presentations even poster numbers
22:00	Presentation Yakult poster prices

WEDNESDAY 7 APRIL 2004

J Room 4/5 **Medical Mycology**

Chairman: J.F.G.M. Meis

09:00 - 09:15	M. Bovers, F. Hagen, F. Coenjaerts, R. May, T. Boekhout Using the model organism *Caenorhabditis elegans* to study the pathogenesis of *Cryptococcus neoformans*	J01
09:15 - 09:30	R.R. Klont, N.M.A. Blijlevens, J.P. Donnelly, P.E. Verweij Failure of pre-emptive management strategy to detect cerebral aspergillosis early	J02
09:30 - 09:45	W. Becker, R. Horre Recognition of *Pseudallescheria boydii* in the clinical laboratory	J03
09:45 - 10:00	D.T.A. te Dorsthorst, J.W. Mouton, J.F.G.M. Meis, P.E. Verweij Correlation between *in vitro* MIC and *in vivo* activity of flucytosine	J04
10:00 - 10:15	F. Hagen, E.J. Kuijper, J. Dankert, T. Boekhout Genotyping by amplified fragment length polymorphism (AFLP), mating type- and serotype-diversity, and susceptibility to fluconazole among Dutch isolates of *Cryptococcus neoformans*	J05

10:15 - 10:30	A. Warris, M.G. Netea, J.W.M. van der Meer, P.E. Verweij, B.J. Kullberg *A. fumigatus* evades immune recognition through loss of TLR4-mediated signal transduction	J06
10:30 - 11:00	coffee/tea	
11.00 - 11.15	A.H.G. Gerrits van den Ende, G.S. de Hoog Species diagnostics in Fonsecaea	J07
11.15 - 11.30	G. Fernandez-Zeppenfeldt, A.H.G. Gerrits van den Ende, R. Yegres, G.S. de Hoog Cladophialophora agents of human chromoblastomycosis have a natural reservoir in cactus plants	J08
11.30 - 11.45	R.R. Klont, C.A. Eggink, A.J.M.M. Rijs, P.E. Verweij Successful treatment of *Fusarium keratitis* with cornea tranplantation and topical and systemic voriconazole	J09

K Room 6/7 Diagnostics 2

Chairman: H.A. Verbrugh

09:00 - 09:15	A.J.C. van den Brule, G. Onland, L. Bartels, C.H.E. Boel Comparison of the NucliSens Magnetic Extraction Reagents to reference extraction methods the isolation of RNA and DNA from various sample types	K01
09:15 - 09:30	T. Schuurman, R.F. de Boer, P.P.H. Peters, P.H.M. Savelkoul, A.M.D. Kooistra, A.A. van Zwet DNA recovery of the automated Roche MagNA pure total nucleic acid isolation kit as compared to the manual BOOM extraction method	K02
09:30 - 09:45	K. Boutaga, A.J. van Winkelhoff, C.M.J.E. Vandenbroucke-Grauls, P.H.M. Savelkoul Quantitative analysis of periodontal pathogens by real-time PCR	K03
09:45 - 10:00	M.A.P. van Bergen, F.J. van der Wal, G. Simons, L. van der Graaf-van Bloois, J. Wagenaar Identification of specific markers for *Campylobacter fetus* subspecies by amplified fragment length polymorphism	K04
10:00 - 10:15	C. Mayer, P. Roosken, G. Muyzer Micro-arrays for the detection of the abundance and distribution of pathogenic protozoa	K05
10:15 - 10:30	E.S. Bruijnesteijn, J.A. Lindeboom, J. Prins, E.C.J. Claas, E.J. Kuijper Detection of mycobacteria with real-time PCR in children with cervical lymphadenitis	K06

L Room 8/9 Functional Genomics 1

Chairman: L. Dijkhuizen

09:00 - 09:45	P. Daran-Lapujade Transcriptomics in chemostat cultures of *Saccharomyces cerevisiae*	L01/02/03
09:45 - 10:30	F.O. Glöckner Genome analysis of environmentally relevant marine bacteria - lessons from the *Pirellula* sp. strain 1 genome	L04/05/06

L Room 8/9	**Functional Genomics 2**	
11:00 - 11:45	Ph. Glaser *Listeria* host pathogen interaction: insights from analysis of evolution and adaptation	L07/08/09
11:45 - 12:30	M.J. van der Werf, H. Meerman Analyzing the metabolome of *Trichoderma reesei*	L10/11/12
M Room 12/13	**Multidisciplinaire Sessie: Een Kind met Lymphadenitis** (Dutch spoken session) *Chairmen: J. van Dissel, E.J. Kuijper*	
09:00 - 09:30	J. van Dissel Lymphadenitis: verwekkers en gastheerfactoren	M01/02
09:30 - 10:00	D. van Soolingen Identificatie en resistentiebepalingen van verwekkers van mycobacteriële lymphadenitis	M03/04
10:00 - 10:30	J. Lindeboom Diagnostiek en therapie van mycobacteriële lymphadenitis in de praktijk	M05/06
N Room 4/5	**Case Presentations** *Chairman: J. Verhoef*	
11:00 - 11:15	J.M. Ossewaarde, R.F. Nieuwenhuis, J. Dees, H.B. Thio, M. Thomeer, H.A.M. Neumann, W.I. van der Meijden An outbreak of Lymphogranuloma Venereum proctitis among homosexual males in the Netherlands	N01
11:15 - 11:30	B.U. Ridwan, J.M. Vogten, B.C. van Hees, J. Wille, B.P.J. Keller, W.J.B. Wannet, B.M. de Jongh A case of severe skin infection caused by a panton valentine leucocidin (PVL) producing *Staphylococcus aureus*	N02
11:30 - 11:45	C.M.A. Swanink, E.T.T.L. Tjwa, J.W.R. Meijer, C. Richter Fever and lymphadenopathy associated with HHV-8 infection in a HIV-positive patient	N03
11:45 - 12:00	A.P. van Dam, M. Pruijm, L.B.S. Gelinck, B. Harinck, E.J. Kuijper Successful treatment of pulmonary mucormycosis and nocardiosis after near-drowning	N04
12:00 - 12:15	E. van Duijkeren, A.T.A. Box, M.E.O.C. Heck, M.J.H.M. Wolfhagen, W.J.B. Wannet, A.C. Fluit Methicillin-resistant *Staphylococcus aureus*: a zoonosis?	N05
O Room 6/7	**Nieuwe ICT ontwikkelingen in de Medische Microbiologie** (Dutch spoken session) *Chairman: E. Boel*	
11:00 - 11:30	S. Thijssen Gebruiksmogelijkheden van een personal digital assistant (PDA) in de medische microbiologie	O01/02

11:30 - 12:00	M. Visser Papierloos werken in het microbiologisch-diagnostisch laboratorium: praktische aspecten bij de implementatie van een laboratoriuminformatiesysteem	O03/04
12:00 - 12:30	P. de Clerq Ontwikkeling en toepassing van een consultmodule in de medische microbiologie	O05/06

Q Room 12/13 **Multidisciplinaire Sessie: Chlamydia**
(Dutch spoken session)

Chairman: F. van Tiel

11:00 - 11:15	F. van Tiel Inleiding	Q01
11:15 - 11:30	J. Land Chlamydia serologie en subfertiliteit: gynaecologische praktijk	Q02
11:30 - 11:45	J. den Hartog Chlamydia serologie en subfertiliteit: nieuwe ontwikkelingen	Q03
11:45 - 12:00	A. Smismans Chlamydia serologie en subfertiliteit: microbiologische praktijk	Q04
12:00 - 12:15	A.J.C. van den Brule Natuurlijk beloop van *Chlamydia trachomatis* infecties	Q05

R Room 4/5 **Therapy**

Chairman: J.M.D. Galama

14:00 - 14:30	A. Meijer, J.A. van der Goot, G. Koch, M. van Boven, T.G. Kimman Oseltamivir reduces transmission, morbidity and mortality of highly pathogenic avian influenza in chickens	R01/02
14:30 - 14:45	J. Kalpoe, M. van Tol, R. Bredius, D. van Baarle, N. Annels, E. Claas, A. Kroes, A. Lankester Pre-emptive treatment with rituximab© efficiently prevents EBV lymphoproliferative disease (EBV-LPD) in pediatric allogenic stem cell transplantation	R03
14:45 - 15:00	A. Riezebos-Brilman, J. Regts, J. Wilschut, T. Daemen Immunisation strategy against cervical cancer involving a Semliki Forest virus vector expressing Human papillomavirus 16 E6 and E7	R04
15:00 - 15:15	M.M. Gerrits, E.J. van der Wouden, D. Bax, A.A. van Zwet, A.H.M. van Vliet, J.C. Thijs, J.G. Kusters, A. de Jong, E.J. Kuipers Anaerobic incubation of metronidazole-resistant *Helicobacter pylori*: understanding the mechanism of metronidazole resistance	R05
15:15 - 15:30	A.M.J. Wensing, D.A.M.C. van de Vijver, E.L.M. op de Coul, R. Schuurman, C.A.B. Boucher, for the SPREAD-network Analysis from 2208 patients newly diagnosed with HIV from 19 countries show that 10% carry primary drug resistance: the CATCH-study	R06

S Room 6/7 **De opleiding tot arts-microbioloog: van A naar Beter**
(Dutch spoken session)

Chairmen: P.E. Verweij, J.W. Mouton

14:00 - 14:05	Inleiding	
14:00 - 14:20	Prof. dr. J.E. Degener (Hoogleraar Medische Microbiologie, AZG) De opleiding in Europees perspectief: de visie van de subcommissie medische microbiologie van het UEMS	S01
14:20 - 14:35	Dr. F. van Tiel (Arts-microbioloog, AZM) Meten is weten: toetsing van de opleiding	S02
14:35 - 15:15	Dr. C.W.G.M. Frenken (Secretaris van de Medisch Specialisten Registratie Commissie) Opleiden: zorg en vreugde voor twee, opleider en MSRC	S03/04/05
15:15 - 15:30	Discussie	S06

T Room 8/9 **Pathogenesis 3**

Chairman: P.W.M. Hermans

14:00 - 14:15	M.L. Laine, S.A. Morré, A.J. van Winkelhoff, A.S. Peña Polymorphisms in the innate immunity genes are associated with the susceptibility to periodontitis	T01
14:15 - 14:30	C. Belzer, J. Stoof, E.J. Kuipers, J.G. Kusters, A.H.M. van Vliet Urease activity in *Helicobacter hepaticus* is nickel-responsive, but acid-independent	T02
14:30 - 14:45	B.L. Herpers, M.M. Immink, B.M. de Jongh, H. van Velzen-Blad, B.A.W. de Jong, E.J. van Hannen Identification of polymorphisms in the FCN2 gene encoding the human lectin pathway activator ficolin-2 of innate immunity	T03
14:45 - 15:00	R.G.J. Pot, J.J. Briede, E.J. Kuipers, A.H.M. van Vliet, J.C.S. Kleinjans, J.G. Kusters The presence of the *cag* pathogenicity island is associated with increased ROS scavenging by *Helicobacter pylori*	T04
15:00 - 15:15	Y. Pannekoek, V. Heurgue-Hamard, A.A.J. Langerak, R. Buckingham, A. van der Ende Methylation in *Chlamydia trachomatis*: identification of a functional *S*-adenosyl-L-methionine-dependent methyl-transferase encoded by *prmC* (CT024) in *C. trachomatis*	T05
15:15 - 15:30	J. van Strijp, S. Rooijakkers, P.J. Haas, B. Postma, W. van Wamel, C. de Haas, K. van Kessel Three novel complement modulators from *Staphylococcus aureus*	T06

V Room 12/13 **Progress in Microbiology 3**

Chairman: R. Laanbroek

14:00 - 14:15	E. Vijgenboom, B.J.F. Keijser, M. Machczynski, A.V. Cherepanov, S.M. Bialek, G.W. Canters Respiration and copper metabolism: checkpoints in morphological development of *Streptomyces*	V01

14:15 - 14:30	H.J. Deelstra, J. Dijksterhuis, J. Kruijtzer, R.M.J. Liskamp, H.A.B. Wösten Repellents and hydrophobins have a different mode of action	V02
14:30 - 14:45	A. Bart, M.W.J. van Passel, K. van Amsterdam, A. van der Ende Direct detection of methylation in genomic DNA	V03
14:45 - 15:00	J.F. de Jong, O.M.H. de Vries, R. Jalving, J. Dijksterhuis, H.A.B. Wösten, J.G.H. Wessels, L.G. Lugones SC15 of *Schizophyllum commune* mediates formation of aerial hyphae and attachment to hydrophobic surfaces in the absence of SC3	V04
15:00 - 15:15	J.T. Keltjens, A.G.M. Janssen, P.J.M. Steenbakkers, W.J. Geerts, M.S.M. Jetten Differential expression of methanogenes in *Methanothermobacter thermautotrophicus*	V05
15:15 - 15:30	M.A.S.H. Mennink-Kersten, R.R. Klont, D. Ruegebrink, H.J.M. op den Camp, P.E. Verweij False-positive reactivity in *Aspergillus* antigen detection	V06

V Room 12/13 **Progress in Microbiology 4**

16:00 - 16:45	J.D. van Elsas Microbial adaptation to soil - what do we know and challenges	V07/08/09

A01
Cell-to-cell communication in the bacterial world: small talk, straight talk and cross talk

P. Williams
Institute of Infection, Immunity and Inflammation, University of Nottingham, Nottingham, UK

In diverse Gram-negative and Gram-positive bacteria, many different physiological processes including secondary metabolite production, motility, conjugal plasmid transfer, biofilm maturation and virulence are regulated through cell-to-cell communication or 'quorum sensing'. At the molecular level, quorum sensing depends on the activation (or repression) of a sensor kinase or response regulator by a small diffusible signal molecule. Several chemically distinct families of quorum sensing signal molecules have been described including the *N*-acylhomoserine lactones (AHLs), the γ-butyrolactones, the peptide thiolactones and the hydroxyalkylquinolones. In the pseudomonads for example, AHL-dependent quorum sensing contributes to environmental adaptation by facilitating the elaboration of virulence determinants in pathogenic species and biocontrol characteristics in beneficial species as well as directing biofilm maturation and colony escape. Quorum sensing also crosses the prokaryotic-eukaryotic boundary in that quorum sensing signal molecules influence the behaviour of eukaryotic organisms in both plants and animals. Certain quorum sensing signal molecules also possess potent immune modulatory, pharmacological and antibacterial activities such that they may directly promote bacterial survival by promoting an advantageous lifestyle within a given environmental niche.

A02
Pathogenic *E. coli*: from molecules to vaccine

B.B. Finlay
Biotechnology Laboratory, University of British Columbia, Vancouver, Canada

Pathogenic *E. coli* cause much morbidity and mortality worldwide. Two types of *E. coli* (enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC, or O157)) cause severe diarrhoea, with EHEC also causing haemolytic uremic syndrome in a subset of cases. These pathogens subvert host epithelial cells, exploiting host processes to build a cellular protrusion (pedestal) on the cell surface upon which they sit. Pedestal formation requires host signal transduction activation and major actin cytoskeletal rearrangements, which are mediated by bacterial proteins that are inserted into the host cell via a bacterial type III secretion system. One of these injected proteins, Tir, is inserted into the host cell membrane, which for EPEC, but not EHEC Tir, becomes tyrosine phosphorylated. This transmembrane form of Tir is a multifunctional bridging protein, acting both as the bacterial receptor on mammalian cell surfaces for intimin, a bacterial outer membrane protein, and nucleating the actin cytoskeleton beneath adherent bacteria. The various features of pedestal formation will be discussed. In addition, we have been using this fundamental information to pursue development of a various potential therapeutics against O157. These include a bovine vaccine using bacterial components that are involved in pedestal formation. Results of vaccine studies with cattle and O157 shedding will also be discussed, illustrating the progression from fundamental scientific knowledge to commercialization.

A03
Similarities of microbial recognition by plants and animals

H.P. Spaink
Leiden University, Institute of Biology, Leiden, the Netherlands,

It is well known that symbiotic interactions between plants and microbes are based on a molecular dialogue between both partners. For the rhizobium-plant interaction, various signal molecules, produced by the microbial partner, have been identified. One group - the Nod factors - have attracted much attention because they specifically trigger the host plants (belonging exclusively to the Leguminosae) to produce a specialized microbe-accommodating organ (the root nodule). In the last three years the molecular basis of recognition of Nod factors secreted by the symbiotic bacteria has been revealed. It was shown that specific recognition involves the function of several family members of the serine/threonine receptor kinase family. One of the major classes of these receptors is characterized by the presence of an extracellular domain that is called a leucine-rich repeat (LRR) domain. This domain is related to the extracellular domains of other plant kinases involved in defence against pathogens and the animal Toll like receptors that function in the innate immune system. This shows that the microbial recognition systems based on LRR receptors already evolved over 1.8 billion years ago in a common eukaryotic ancestor. Plants and animals also share common components in downstream signalling through these receptors. I will illustrate that even the resulting responses of specific recognition of microbes by plants and animals share surprising similarities.
Reference: Spaink HP. Plant-microbe interactions: a receptor in symbiotic dialogue. Nature 2002;417:910-1.

A04
The role of pheromones in fungal mating

L.A. Casselton
Department of Plant Sciences, University of Oxford, Oxford, UK

Detecting a compatible mating partner may require signalling across a distance or taking a chance and fusing with any cell that comes into close contact. In either case, pheromones play an essential role in fungal mate recognition and elicit developmental changes that are a prerequisite for sexual reproduction. Our understanding of pheromone signalling and the intracellular signal transduction pathway that it activates came initially from studies with the ascomycete yeast *Saccharomyces cerevisiae*. In this unicellular fungus, there are just two mating types and each secretes a mating type-specific pheromone that can bind a surface receptor

produced on cells of the opposite mating type. Pheromone-binding activates a MAP kinase cascade and resulting changes in gene expression cause cells to alter shape, grow towards each other and to then fuse. In filamentous ascomycete and basidiomycete fungi, there is a considerable delay between mating cell fusion and nuclear fusion. Here we see an additional role for pheromone signalling in maintaining the integrity of a specialised mycelial state, the dikaryon, in which the nuclei from each mate remain paired and divide in synchrony in each cell. Dikaryotic cell division may be complex, there is a need for different outputs from the pheromone response pathway and these are affected by different transcription factors. The mushroom fungi are of particular interest because they may have several thousands of different mating types that, in part, are determined by large families of pheromones and receptors. These fungi do not use pheromones as attractants. Random cell fusion is recognised as compatible if it brings together pheromones and receptors that can activate each other. By looking at pheromone sequence variation we can gain insight into determinants of pheromone-receptor specificity.

B01/02
Phenotype MicroArrays™ for phenotypic analysis of *E. coli*, *S. cerevisiae* and other microbial species

B.R. Bochner
Chairman and Vice President of R&D, Biolog Inc., Hayward, California, USA

Phenotype MicroArray (PM) technology allows a biologist to test 2,000 properties (phenotypes) of a cell. Testing involves about 30 minutes of actual labour and 24 to 48 hours of incubation. The phenotypic assays are designed from a physiological perspective to survey *in vivo*, the function of diverse biological pathways, including both metabolic and regulatory pathways. Included in the phenotypes are basic cellular nutritional pathways for C, N, P, and S metabolism (800 tests), pH growth range and regulation of pH control (100 tests), sensitivity to NaCl and various other ions (100 tests), and sensitivity to chemical agents that disrupt various biological pathways (1,000 tests). PM technology can be used to complement genetics and genomics. A change in genotype of a cell should lead to one or more changes in phenotype, if the gene has a real function. PMs allow testing of knockout or overexpression mutants to discern the biological changes that occur consequent to genetic changes. Examples will be discussed primarily using *Escherichia coli* and *Saccharomyces cerevisiae* as models, where knockout mutants have been phenotyped. The technology has been applied to diverse microbial species including *Salmonella typhimurium, Pseudomonas aeruginosa, Burkholderia cepacia, Vibrio cholerae, Helicobacter pylori, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Listeria monocytogenes, Bacillus subtilis, Bacillus cereus, Corynebacterium striatum,Candida albicans, Ustilago maydis,* and *Aspergillus nidulans*. Prototype PMs have also been developed for human cells.

B03
Multi-locus variable-number tandem repeat analysis: a new fast, robust and discriminatory typing scheme for studying the genetic relatedness of *Enterococcus faecium* isolates

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Enterococcus faecium has emerged as an important cause of nosocomial infections. Recently developed typing schemes like AFLP and MLST have revealed the existence of genogroups among *E. faecium* and a distinct genetic lineage associated with hospital outbreaks. AFLP, however, does not provide an unambiguous nomenclature of genotypes, which is a prerequisite for studying global and long-term epidemiology while MLST is quite labour-intensive and expensive. Here we describe Multiple-Locus Variable-Number Tandem Repeat (VNTR) Analysis (MLVA) as a new typing scheme for *E. faecium*. A search for tandem repeats in the genome sequence of *E. faecium* strain DO revealed six different repeat loci with repeat sizes ranging from 121 to 279 bp. The six VNTR-loci were amplified with primers flanking the repeat regions. The resulting fragments were separated on agarose gels and fragment sizes converted into repeat numbers. The number of repeats for the six VNTR-loci resulted in a MLVA profile, which was used for cluster analysis. MLVA profiles of 392 isolates including 70 isolates from different animals, environment and food, 17 community survey isolates, 122 clinical isolates, 66 hospital survey isolates and 117 isolates from 29 different hospital outbreaks were analysed. The number of alleles for each VNTR-locus varied strongly from 3 for VNTR-9 up to 13 for VNTR-2. In total 127 different MLVA profiles were discerned. Cluster analysis of MLVA profiles confirmed the genogroups A-D found with MLST, including the C1 group of clinical/epidemic isolates distinct from the community survey and animal isolates. Isolates from a single outbreak exhibited an identical VT. Comparison of MLVA with MLST showed that MLVA was able to discriminate between isolates that were identical by MLST. This demonstrated that although MLVA is a highly discriminatory typing method it is still able to identify isolates belonging to a single outbreak. To conclude, we developed a new MLVA-based typing scheme for *E. faecium*, which is a robust and portable method that combines the high throughput and discriminatory power of AFLP with the ability to provide the unambiguous nomenclature of MLST.

B04
Great diversity within the staphylococcal cassette chromosome *mec* in methicillin-resistant staphylococci from human and veterinary origin

A.T.A. Box[1], E. van Duijkeren[2], A.C. Fluit[2]
[1]Eijkman-Winkler Institute, University Medical Centre Utrecht, Utrecht, the Netherlands, [2]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) is a mobile genetic element that carries the *mecA* gene mediating methicillin resistance in staphylococci. For *Staphylococcus aureus* four SCC*mec* types have been described, however, little is known about their distribution in staphylococci from veterinary origin. We investigated the diversity of SCC*mec* in 55 different methicillin-resistant and *mecA*-positive staphylococcal strains (38 *S. aureus* (MRSA), 17 coagulase-negative staphylococci) of both human and veterinary origin. Strains were epidemiologically unrelated and, in case of MRSA, represented the major widespread clonal lineages. The molecular typing of SCC*mec* was based on the cassette chromosome recombinase gene (*ccr*), the *mecA* gene complex from which different types have been described based on the presence or absence of regulatory sequences, and the detection of specific genetic determinants (loci) as defined by Oliveira and de Lencastre (AAC 46 (2002) p. 2155). The three known *ccr* gene types were detected in combination with a variety of *mec* gene complexes and loci combinations. In 5 veterinary strains *ccr* genes were not detected. At least 39 distinct SCC*mec* variants were found. Different staphylococcal species contained an identical SCC*mec* (type II, *mecA* gene complex A, loci ABCDEG; type IV, *mecA* gene complex B, loci AD). Different SCC*mec* types contained identical *mecA* gene complexes and loci combinations. Human and veterinary strains shared identical SCC*mec* types, although their genetic markers differed. In conclusion a great diversity of SCC*mec* types was found in methicillin-resistant staphylococci of both human and veterinary origin. The occurrence of different SCC*mec* types containing identical genetic markers and the presence of identical SCC*mec* types in different staphylococcal species of both human and animal origin, support the hypothesis of intra- and interspecies transfer of SCC*mec* and that SCC*mec* is a hot-spot for the loss or integration of DNA sequences. The presence of methicillin resistance in veterinary isolates may become a major point of concern.

B05
Origin of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands: results from a random sample of MRSA isolates

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[1]National Institute for Public Health and the Environment, Centre for Infectious Diseases Epidemiology, Bilthoven, the Netherlands, [2]National Institute for Public Health and the Environment, Laboratory for Infectious Diseases and Perinatal Screening, Bilthoven, the Netherlands

Introduction: All first MRSA isolates from the Netherlands are sent to the National Institute for Public Health (RIVM) for confirmation, typing and epidemiological analysis. According to standard questionnaires accompanying this collection, the proportion of isolates related to stay in foreign hospitals decreased from 45% in 1995 to 10% in 2002. However, the response to the standard questionnaires decreased from about 60% to 44% in the same period. To investigate the possible reasons for the decreasing proportion of isolates associated with foreign countries, we investigated the epidemiological background of a limited sample of strains more intensively.

Methods: A random sample of 108 isolates was drawn from a total of 1081 isolates sent to the RIVM between September 2001 and September 2002. PCR tests showed that 99 isolates were *mecA* positive and had a minimum inhibitory concentration of equal to 4 mg/L or greater. Improved questionnaires were mailed to the laboratories submitting the MRSA isolates and to the corresponding hospital hygienists. Senders were recalled by telephone if questionnaires had not been returned within two weeks.
Results: The additional survey increased the response from 44% to 67%. The majority of the questionnaires (67%) was returned by hospital hygienists. Seventy-three questionnaires (74%) contained sufficient information about the origin of the isolates. Out of these 73, 11 isolates (15%) had a direct relation to a recent stay in a foreign hospital, 2 isolates were from patients who had evidently been in contact with a patient repatriated from a foreign country, 2 isolates were from adopted children and 11 other isolates were from patients that had visited foreign countries, and had probably stayed with family. Thus, up to 26 isolates (36%) could have originated from foreign countries, although most MRSA carriers did not originate directly from foreign hospitals.
Conclusions: 1) Adaptation of the procedure of mailing and recalls significantly increased the response to the questionnaire. 2) Nevertheless, only a minority of isolates (15%) could be related to stay in foreign hospitals.

C01
Few chromogenic agars perform well for rapid detection of *E. coli* in urine and are cost-effective when a high percentage of cultures is positive for *E. coli*

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Introduction: *E. coli* is the most frequent cause of UTI. Various chromagarplates are used for direct identification of *E. coli*. We tested five chromogenic media on their usefulness and costs.
Methods: 26 *E. coli*, 25 *Proteus* spp, 12 *Citrobacter and 11 Klebsiella* spp were inoculated on chromogenic agar {UTI (Oxoid), UTI clear (Oxoid), CPS-ID2 (BM; BioMérieux), BBL CHROMAgar Orientation (BD) and Uriselect 4 (U4; Biorad)}. Plates were incubated for 18 h by 35°C and inspected for growth, visibility, colony colour and swarming. We analyzed our database for the total amount of urine cultures submitted in 2002 to two of our MMLs (one with GP urine cultures and one without), and the frequency of *E. coli* isolation. Also we calculated the costs of the standard use of a chromogenic medium, compared to determination by Vitek.
Results: For detection of *E. coli* all media performed relatively well. With *Citrobacter* both Oxoid media and the Bioradmedium showed colonies suspected for *E. coli*. Also, *Proteus* spp. swarmed on these media. CPS-ID2 and the BD-medium performed well on colour. Colonies were more easily observed on clear media (UTI clear, CPS-ID2 and BD). The costs using a chromogenic plate were lower than determination with Vitek in urine frequently positive for *E. coli*. That was the matter with urine cultures administered by general practioners. In the clinical lab without GP urine

cultures *E. coli* was found in 13.7% of 7436 urine cultures. In the MML with only GP urine cultures *E. coli* was detected in 28.2% of 9270 cultures. When the costs were calculated for the clinical lab the use of blood agar, MacConkey agar and determination by Vitek is cheaper than the standard use of chromogenic agar. In the 'GP' lab with a high percentage of *E. coli* in urine the use of CPS-ID2 (BioMérieux) or CHROMAgar (BD) resulted in a reduction of costs by 27.3%, compared to the conventional method.
Conclusions: 1) Only CPS-ID2 and BBL CHROMAgar Orientation performed well. 2) Standard use of chromogenic agar is only cost effective when a high percentage of urine is positive for *E. coli*.

C02
Simple, rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* with cefoxitin

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Introduction: Rapid detection of patients colonized or infected with MRSA is crucial in order to prevent and control outbreaks. Antibiotics of the cephamycin-group were chosen for rapid susceptibility testing because of there potential to induce production of PBP-2a.
Material/methods: 119 characterized clinical isolates of *S. aureus* were tested (53 MSSA and 66 MRSA). 8/66 MRSA showed low-level resistance with MICs ranging from 1 to 16 mg/L. Susceptibility to oxacillin (1 µg), cefoxitin (30 and 60 µg), and moxalactam (30 µg) was determined on Mueller Hinton agar. Plates were incubated for 18 hours at 30°C and 37°C. For oxacilline 1 µg, cefoxitin 30 µg and moxalactam 30 µg, NCCLS breakpoints were applied. For cefoxitin 60 µg screening breakpoint diameter recommended by the manufacturers of neo-sensitabs was applied. After this initial study, performance of cefoxitin 60 µg (30°C) in the routine setting was validated with a total of 650 *S. aureus* clinical isolates. MRSA screen latex agglutination test and *mecA* PCR were applied for confirmation of expression and presence of *mecA* consecutively.
Results: In the initial study the results of the susceptibility testing to cefoxitin after overnight incubation at both temperatures showed an overall accordance with the MRSA-screen latex agglutination test and *mecA* PCR. Oxacillin needed the full 24 hours of incubation to equal performance of cefoxitin. Moxalactam performed less well. The results in the routine setting with the 650 *S. aureus* clinical isolates (8 MRSA, 642 MSSA) showed an overall accordance between, cefoxitin-, oxacillin- and MRSA-screen agglutination findings. All eight MRSA strains were *mecA* positive with PCR. 87 randomly chosen, MSSA isolates (cefoxitin (S), oxacillin (S), and MRSA-screen negative) out of this collection were checked for presence of *mecA* with PCR, one out of these carried *mecA* .
Conclusion: Agar disk diffusion with cefoxitin is a simple, accurate and rapid method for detection of low-level methicillin-resistance in *S. aureus* and is accessible for any laboratory.

C03
Evaluation of *Mycoplasma pneumoniae* commercial tests for detection of serum antibodies using PCR as gold standard

M.F.C. Beersma, K. Dirven, H.J. Gerritsen, A.P. van Dam, E.C. Claas, H. Goossens
Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands

Introduction: Serological methods are most widely used for diagnosis of *Mycoplasma pneumoniae* (MP) infection, but very few studies have evaluated available commercial tests. We studied commercial MP IgG and IgM EIAs using PCR for detection of MP as gold standard.
Methods: Twenty-nine MP PCR-positive patients (49 serum samples) were included from two prospective studies on acute lower respiratory tract infections (LRTI). Control sera were tested from i: 20 patients with acute LRTI negative for M by PCR and from ii: 61 patients with microbiological documented LRTI other than MP, but without PCR exclusion. The different EIAs tested were Platelia (Bio-Rad); SeroMP (Savyon); Serion classic (Virion/Serion); Biotest EIA (Biotest); Ridascreen EIA (r-Biopharm); AniLabsystems EIA (Labsystems); Novum EIA (Novum Diagnostica); Diagnosys EIA (MP products); Genzyme/Virotech EIA; ImmunoWell EIA (Genbio); Immunocard EIA (Meridian). In addition, the complement fixation test (CFT) and Serodia-Myco II agglutination test (Fujirebio) were included.
Results: The sensitivities of IgM EIAs ranged from 7%-23% in the first 6 days after onset of disease to 29%-86% after more than 16 days of illness. IgG EIAs detected seroconversion or a significant rise of IgG titers in 47%-63% of the PCR-positive patients. IgM tests with the best results for both sensitivity and specificity were AniLabsystems EIA (86%/92%), Diagnosys EIA (71%/94%) and Serodia-MycoII (80%/87%), whereas other IgM tests failed to achieve satisfactory results for either the sensitivity (below 70% after 16 days) or specificity (below 80%). False-positive EIA IgM results occurred more frequently in the control patients with acute EBV infection. The sensitivity and specificity of the CFT (99%/95%) exceeded those of the commercial IgM tests at all clinical time-points.
Conclusion: Evaluation of the currently available serological EIAs, CFT, and agglutination test for *Mycoplasma pneumoniae* using PCR as gold standard, showed substantial differences between the performances of the assays.

C04
Evaluation of the C6-peptide ELISA in patients with Lyme borreliosis and controls

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Introduction: The diagnosis of Lyme borreliosis is usually based on a combination of clinical and serologic findings. Recently, a new ELISA in which the so-called C6-peptide from the VlsE antigen was used as an antigen became commercially available. The assay measures only IgG antibodies and can be completed within one hour.

Methods: Sera from patients with erythema migrans (EM, n=40), early disseminated Lyme borreliosis (EDLB, n=35) and acrodermatitis chronica atrophicans (ACA, n=20) as well as control sera (n=41) containing rheumatoid factors or from patients with syphilis, leptospirosis, CMV or EBV infections were tested in the C6-peptide ELISA (ITK), as well as in a flagellin-ELISA (Dako), a recombinant immunoblot (Microgen) and an in-house immunoblot.
Results: The C6-peptide ELISA was positive in 22.5%, 80% and 90% of sera from patients with EM, EDLB and ACA, respectively. In the flagellin-ELISA, 60%, 88% and 95% of these sera were positive either in the IgM or in the IgG test. In the recombinant immunoblot, these figures were 53%, 70% and 100%, and in the native immunoblot 50%, 56% and 95%, respectively. None of the control sera were positive in the C6-peptide ELISA, whereas 18%, 7% and 13% of the sera were positive in the flagellin-ELISA, recombinant and native immunoblot.
Conclusion. The C6 peptide has an excellent specificity. The sensitivity of the assay is significantly lower in patients with erythema migrans, but similar in patients with EDLB and ACA. However, if patients with EM are not routinely tested for antibodies to *B. burgdorferi*, as recommended in the recent CBO guidelines, and serologic testing for Lyme borreliosis is limited to cases of suspected disseminated or late Lyme borreliosis, the C6-peptide assay is a suitable alternative test.

C05
Results of cerebrospinal fluid-analysis have no predictive value for external drain-related bacterial meningitis

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Introduction: Drain-related bacterial meningitis (BM) is the most important complication of external drainage (ED) of cerebrospinal fluid (CSF). Regular microbiological and chemical analysis of CSF is often performed to diagnose ED-related BM (ED-BM) at an early stage. A cohort study was undertaken to investigate the diagnostic value of several commonly used CSF-parameters, and the inflammatory cytokine interleukin 6.
Methods: A consecutive cohort of 230 patients had ED using lumbar (54%) or ventricular (42%) catheters, in the period July 1999-January 2003. No antimicrobial prophylaxis was used. To screen for the development of ED-BM, cultures and Gram-stains of CSF samples were performed daily, and chemical analysis every weekday. Chemical CSF-results were evaluated longitudinally and transversally for predictive value for ED-BM.
Results: Twenty-three (10%) patients developed ED-BM, defined on the results of CSF-cultures. Results from chemical-analyses of the CSF-samples (n=1788) showed no significant differences between patients (n=23) and controls (n=195) at the first 2 days of positive CSF-cultures, with regard to total cell count, protein-level, glucose-level, glucose CSF/blood-ratio and interleukin 6. Furthermore, no significant differences were observed for the 3 days before the first positive CSF-culture. Conditional regression analysis showed that none of the 5 markers had significant predictive value for ED-BM, both using absolute values, ratios or differences with previous days. Comparison of Gram-stains and CSF-cultures showed a very high (100%) specificity of the Gram-stain. However, sensitivity was very low: only 35 (40%) of 87 positive CSF-samples had a positive Gram-stain. Sensitivity of the Gram-stain on the first day of infection was only 17% (4/23).
Conclusions: Due to the underlying disease, there are severe disturbances in CSF of patients with ED, which make routine chemical analysis of CSF of limited value to screen for ED-BM. Routine Gram-stain of CSF has, despite its high specificity, limited value in the screening for ED-BM due to very low sensitivity. We recommend frequent monitoring of growth in CSF-cultures to screen for ED-BM in a population of patients with ED.

D01
The relation between acid adaptation and virulence of *Salmonella enterica* serovar Typhimurium DT104

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Introduction: *Salmonella* enterica serovar Typhimurium DT104 (*S. typhimurium*) resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline (R-type ACSSuT) was first isolated in the UK in 1984. Since then the incidence of *S. typhimurium* DT104 is increased in many countries including the Netherlands. The first line of defence against food borne pathogens is the stomach with its acidic pH. *Salmonellas* possess several systems that can protect the against low pH environments. We investigated if acid adaptation increases virulence during other steps in the infection, besides enhanced survival of the stomach.
Methods: Two intestinal epithelial cell lines, (Caco-2 and IEC-18) were cultured in 12 wells plates for 12-19 days to differentiate. They were infected with 5 *S. typhimurium* DT104 strains with a multiplicity of infection of ± 10 bacteria per cell. Bacteria were cultured at pH 7 (non-adapted) and at pH 5 (adapted). Two hours after infection the amount of invasion and cell associated (adhesion and invasion) bacteria was determined.
Results: Of all strains about 10^7 bacteria adhered to the intestinal cell lines and about 10^4 bacteria invaded the cells. No differences were found between the different *S. typhimurium* DT104 strains nor between acid adapted and non-adapted bacteria. Also no significant differences could be found between the human and the rat intestinal epithelial cell line.
Conclusion: The ability to adapt to acidic conditions is important to survive the acidic pH of the stomach, but not for the adhesion or invasion into intestinal epithelial cells according to our results. The next step in pathogenesis is the survival within macrophages. Studies are undertaken to investigate if acid adaptation is important for survival in macrophages.

D02

Tissue colonization is more important than biofilm formation in *Staphylococcus epidermidis* experimental biomaterial-associated infection

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The majority of biomaterial-associated infections (BAIs) are cause by coagulase-negative staphylococci, particularly *Staphylococcus epidermidis*. Although biofilm formation on the biomaterial surface is considered to be the cause of these infections, we observed high numbers of bacteria in peri-implant tissue in previous studies. To investigate whether *S. epidermidis* predominantly resides in the tissue or in a biofilm on the implant, we investigated BAI of pvp-coated silicon elastomer (SEpvp) in a mouse model.
Experimental biomaterial-associated infection: Two SEpvp segments were implanted subcutaneously in the back of female C57Bl/6 mice. 25ul of inoculum of *S. epidermidis* strain RP62a or AMC5 was injected alongside each biomaterial segment. After 5-14 days standardized 12mm diameter tissue biopsies including the implanted SEpvp segments were collected and blood samples were obtained. Selected samples were taken for histology and stained using a haematoxylin-eosin and gram staining.
A dose-dependent infection frequency was observed, with an ID90 (90% infective dose) of 107 cfu (colony forming units). At all inocula from 106 up to 5x107 cfu, the number of culture positive biopsies was higher than of the corresponding explanted SEpvp segments, and more cfu were cultured. At an inoculum of 107 cfu, the frequencies of infection did not decrease between 5 and 14 days, but at both time points tissue biopsies had a higher frequency of culture positivity than the corresponding biomaterial segments. *S. epidermidis* appeared to be localized within host cells with macrophage morphology at a distance of several cell layers from the biomaterial-tissue interface.
Conclusion: Since the frequencies of infection and the numbers of cfu cultured from the peri-implant tissue were (much) higher than those from the implanted biomaterial, we conclude that tissue colonization may be equally or more important than biofilm formation in the pathogenesis of biomaterial-associated infection.

D03

The NikR regulatory protein governs transcriptional regulation of NixA-mediated nickel-uptake in *Helicobacter pylori*

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Introduction: *Helicobacter pylori* produces large amounts of the nickel-containing metalloenzyme urease, which plays an essential role in virulence of this human pathogen. To obtain nickel *H. pylori* requires efficient acquisition systems, but also mechanisms to maintain cytoplasmic nickel homeostasis to avoid cytotoxicity. The aim of this study was to determine the roles of the NikR protein as regulator of nickel homeostasis and NixA as nickel-uptake system.
Methods: Growth of *H. pylori* reference strain 26695 wild-type, *nikR* mutant, *nixA* mutant and a *nikR-nixA* double mutant was determined in Brucella media supplemented with $NiCl_2$ up to 1 mM. Transcription of the *nixA* gene was analyzed by Northern hybridization. Protein expression was determined by urease activity measurement and SDS-PAGE, while gel mobility shift assays and DNAse foot printing with the *nixA* promoter were used to characterize the interaction of NikR with the *nixA* promoter.
Results: The parental *H. pylori* strain 26695 was resistant to $NiCl_2$ concentrations of up to 750 μM, whereas an isogenic *nikR* mutant displayed a significant increase in nickel-sensitivity. Inactivation of the nickel-transporter gene *nixA* abolished the nickel-sensitivity of the *nikR* mutant. Transcription of the *nixA* gene was nickel-responsive in the wild-type strain, while in the *nikR* mutant the *nixA* gene was constitutively transcribed. Direct NikR-mediated regulation of the *nixA* promoter was confirmed using DNAse foot printing. Finally, nickel induction of urease activity was dependent on NikR and nickel, but not on *nixA*.
Conclusion: The NikR protein plays a central role in nickel-uptake, allowing nickel-uptake via *nixA* only when nickel is scarce. This regulatory pattern is similar to that described for iron via the Fur protein. The presence of this nickel-responsive system mediating regulation of the essential virulence factor urease may allow for the development of new therapeutic compounds for treatment of *H. pylori* infection.

D04

Antimicrobial and anti-inflammatory activity of Bactericidal Peptide 2 *in vitro*

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Antimicrobial peptides (AMPs) are an important first line of defence against micro-organisms and are produced in nearly all species tested, including humans. AMPs are generally small (<50 amino acids), net positively charged peptides, and contain both hydrophobic and hydrophilic domains. Specificity for micro-organisms is mediated by electrostatic interactions with negatively charged microbial membranes. It is generally thought that the microbial cell membrane is the primary target for most AMPs and killing is mediated by pore-formation or lysis. The synthetic alpha-helical antimicrobial peptide Bactericidal Peptide 2 (BP2) was designed based on conserved elements from various LPS-binding proteins (Abraham PR, Appelmelk BJ, Deventer SJH van. Patent application WO 99/06440).
To assess the antimicrobial spectrum of BP2 *in vitro*, a broad range of micro-organisms was tested in a Minimal Bactericidal/Fungicidal Concentration (MBC/MFC) assay. Kinetics of killing of bacteria was analyzed by quantitative determination of colony forming units after exposure to BP2. The anti-inflammatory potential of BP2 was analyzed by its ability to reduce cytokine production by THP-1 mononuclear cells stimulated with 10 ng/ml *E. coli* 0111:B4 LPS.

BP2 had broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, and yeasts with MBC/MFC values ranging from 0.5-7.5 micromolar. Susceptibility of antibiotic sensitive and resistant strains of *Staphylococcus aureus* and *Enterococcus faecium* to BP2 were similar. *Proteus mirabilis*, *Morganella morganii* and *Burkholderia cepacia* were not susceptible (MBC >30 micromolar). *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were killed within 1 minute after exposure to BP2 at or above its MBC value. IL-8 production by LPS stimulated THP-1 cells was reduced by 91% and 87% when 1 micromolar BP2 was added 30 minutes prior to or after stimulation, respectively.
In conclusion, BP2 has broad-spectrum antimicrobial activity and it can neutralize LPS at micromolar concentrations *in vitro*.

D05

Lactococcus lactis ghosts displaying multiple *Streptococcus pneumoniae* protein antigens elicit protective immunity in a murine pneumonia model

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Introduction: *Streptococcus pneumoniae* is the leading etiological agent of severe infections such as meningitis, pneumonia and septicemia. The development of an affordable effective vaccine against invasive pneumococcal disease will have major benefits in terms of reducing disease burden and healthcare costs in both developed and developing countries. The current 7-valent capsular carbohydrate conjugate vaccine is very effective against invasive pneumococcal disease. However, protection is limited to vaccine serotypes, and the current unit cost of the vaccine is likely to limit its widespread use in developing countries.
Methods: A new approach is the use of protein-based pneumococcal antigens. Lactococcus lactis, a non-pathogenic, non-colonizing food-grade Gram-positive bacterium sharing close homology with pathogenic streptococci, is an ideal organism for the production of pneumococcal vaccine antigens. Of particular importance is the potential to produce generally affordable mucosal vaccines based on pneumococcal protein antigens bound to non-genetically modified non-living lactococci (Ghosts) acting as carrier and mucosal adjuvant. We have constructed lactococcal Ghost-based multivalent mucosal vaccines containing different combinations of the surface-exposed pneumococcal proteins PspA, PsaA, CbpA, PpmA, SlrA and IgA1.
Results: The vaccines were evaluated for immunogenicity and protective efficacy in an intranasal challenge murine model for pneumococcal pneumonia. The results indicate that the immune-stimulating potential of the Ghost-based multivalent vaccines is very high, and that mucosal immunization results in an immune-protective response against invasive pneumococcal disease.
Conclusions: We conclude that the Ghost-binding- and display technology has great potential to develop a broadly applicable mucosal *S. pneumoniae* vaccine, which elicits systemic protection.

D06

CodY-transcriptional regulation in *Streptococcus pneumoniae*

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Introduction: *CodY* is a pleiotropic DNA-binding repressor, which is known to play a regulatory role in amino acid metabolism in *Bacillus subtilis* and *Lactococcus lactis*. In this study, we investigated the regulation of gene transcription by the CodY homologue in *Streptococcus pneumoniae* in order to predict its role in pathogenesis.
Methods: The gene encoding *codY* was inactivated in strain D39 (serotype 2). The *in vitro* growth characteristics of the D39 *codY* knock out mutant in THY-medium were compared with the parental strain by monitoring the optical density in time. Total RNA was isolated at various time points during growth of both wild-type and mutant strain. The RNA of both strains was reversely transcribed, fluorescently labelled using Cy3/Cy5, and applied to micro-array slides to identify the transcriptome profiles.
Results: D39 *codY* knock out mutant displayed a significant reduction in growth when cultured in THY-broth at 37 degrees Celsius in a static flask culture, which suggests that the *codY* mutation has severe effects on the global transcription. Several genes were upregulated by the disruption of *codY*. These genes comprised members involved in nitrogen/amino acid metabolism, metal uptake, competence energy transduction and fatty acid metabolism.
Conclusion: In *S. pneumoniae codY* regulates not only genes involved in peptide uptake, e.g. the *ami*-operon, *carA*, *thd1* and *brnQ*, which are up-regulated in the *codY* knock out mutant, but also other operons involved in thiamine synthesis (*thi*-operon), and acetoacetate synthesis (*ilv*-operon). Since pleiotropic regulators are usually involved in many different cellular and physiological processes, the relevance of *codY* in pathogenesis will be discussed.

D07

Selection of surface-exposed proteins for development of protective antibodies against coagulase-negative staphylococcal infection

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Introduction: For the prevention of coagulase-negative staphylococci (CoNS) mediated sepsis in neonates, we aim to develop a monoclonal antibody against CoNS for passive immune protection.
Materials: From the publicly available genome of *S. epidermidis* ATCC 12228, we selected genes that are predicted to be surface-exposed. The *in silico* selection was based on the predicted presence of a signal peptide, transmembrane domains, peptidoglycan-binding domains (PBD), choline-binding domains (CBD), lipobox- and LPxTG motives and protein length. Primers with His^6-tag sequences and NheI and BamHI restriction sites were designed for

amplification of the genes. PCR products were cloned into *E. coli* BL21 (DE3) using the pET11c vector. Protein expression was induced by IPTG, cells were centrifuged and pellet was solved in 10mM imidazole buffer. Cells were disrupted by sonification, centrifuged and supernatant was collected. Proteins were purified by nickel column chromatography. Optimal imidazole wash and elution concentrations were determined. For confirmation of the proteins, they were digested with trypsin and peptides were analyzed by MALDI-TOF mass spectrometry.
Results: 388 Proteins were predicted to have a signal peptide with a probability of 40% or higher. Of these proteins, 183 proteins were predicted to have 0 or 1 transmembrane domain, 32 had 2 domains. In these groups, there were 51 proteins with a lipobox motive, none had a CBD, 6 had a PBD and 9 had an LPxTG motive. All LPxTG proteins were selected, as well as the 6 largest proteins. These 6 proteins were all lipobox-containing proteins. So far ten genes were successfully cloned in *E. coli*.
Conclusion: *S. epidermidis* surface-exposed proteins can be produced and purified using *E. coli*. These recombinant proteins will be used as antigens to produce protective antibodies against CoNS infection.

D08
Mycobacterium marinum strain-variation is an important factor in the pathology of fish tuberculosis

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Mycobacterium marinum causes a systemic tuberculosis-like disease, characterised by granuloma formation in all organs, in a large number of poikilothermic animals. These granulomas share many morphological features with those found in persistent human tuberculosis infections and therefore *M. marinum* is used as a model for mycobacterial pathogenesis. In this study, we used the zebra fish (*Danio rerio*) to determine the effect of *M. marinum* strain variation on pathogenesis. Six *M. marinum* strains were each intraperitoneally (IP) injected in zebra fish. Depending on the *M. marinum* isolate used for the infection, an acute or chronic disease was evoked. Acute disease was characterised by an uncontrolled growth of the pathogen at the site of infection and all animals succumbed to the infection within 16 days. Chronic disease was characterised by granuloma formation in different organs and from 4-7 weeks on some animals were in a moribund state. Amplified fragment length polymorphism (AFLP) analysis of these and 12 other *M. marinum* isolates showed that these strains grouped in two main clusters. Cluster I contains predominantly human isolates whereas the majority of cluster II isolates originate from poikilothermic species. Interestingly, acute disease progression was correlated with cluster I. This difference in virulence could also be observed with the infection of cell cultures. Cluster I isolate Mma20 was able to infect and survive efficiently in human macrophages, whereas the total number of bacteria belonging to Mma11 of cluster II, steadily declined. In conclusion these data show that genetically different *M. marinum* strains evoke markedly different immunopathological events in zebra fish. Furthermore, the fact that cluster I gives rise to acute disease responses in zebra fish and also contains predominantly human isolates, suggests that cluster I is more pathogenic for humans.

D09
The FlgS/FlgR two-component signal transduction system regulates the FLA regulon in *Campylobacter jejuni*

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Flagella are the locomotory organelles of bacteria that propels them in directions dictated by diverse sensory signals. In many pathogenic bacterial species, flagellar motility is required in order to reach the ultimate infection niche and to establish infection. The human pathogen *Campylobacter jejuni* is a highly motile organism that carriers a flagellum on each pole. The flagellar motility is regarded as an important trait in *C. jejuni* colonization of the intestinal tract, however the knowledge of the regulation of this important colonization factor is rudimentary. The biogenesis of flagella is dependent on a highly regulated pathway of timed gene expression and protein synthesis. In the present study we unravelled the regulation of *C. jejuni fla* regulon and revealed that the FlgS/FlgR two component signal transduction system is essential for flagella biosynthesis. Our data indicate that, in response to thus far unidentified signal, the sensor kinase FlgS autophosphorylates and subsequently transfer its phosphate to its cognate response regulator FlgR. Phosphorylated FlgR is needed to activate RpoN-dependent genes of which the products form the hook-basal body filament complex. By real-time RT-PCR we identified that FlgS, FlgR, RpoN and FliA belong to the early flagellar genes and are regulated by sigma 70. FliD and the putative anti-sigma-factor FlgM are regulated by a sigma 54 and sigma 28-dependent promoters. Activation of the *fla* regulon is growth phase dependent and very energy consuming. Challenge experiments in chickens indicated that the FlgS/FlgR two-component system is important for the efficient passage of the gastro-intestinal effect but not for the persistence in the ceaca.

D10
Mutations in the *incA* gene of *Chlamydia trachomatis* and association with non-fusogenicity and silent infection

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C. trachomatis is an obligate intracellular pathogen causing sexually transmitted disease (STD) in Western countries. Fifty to 70% of the infections run an asymptomatic course. The bacteria multiply and divide in vacuoles termed inclusions.

Typical *C. trachomatis* isolates reside in inclusions that fuse with other *C. trachomatis* containing inclusions directly after infection of the cells with multiple bacteria (fusogenic phenotype). Recently, clinical isolates in were identified in the USA forming multiple-lobed, non-fusogenic inclusions. These non-fusogenic strains, were mainly isolated from patients with asymptomatic infection. Most of these isolates lack IncA in the inclusion membrane (IM) due to truncation of the gene.

To evaluate the relationship between mutations in *incA*, non-fusogenicity and the tendency of the *C. trachomatis* isolate to cause cryptic infection, the sequence of *incA* of *C. trachomatis* isolates from ~100 persons with a *C. trachomatis* infections, either symptomatic or asymptomatic was determined.

Sequence analysis identified two isolates (2%) with truncated *incA* genes. These strains were isolated from patients with symptomatic disease. In addition, a large number (~70%) of *incA* sequences contained non-synonymous mutations compared to the prototype sequence of a fusogenic strain. These strains were apparently randomly distributed among persons with or without symptoms. The relationship between IncA expression, its localization to the IM and fusogenicity of all studied isolates is currently being investigated by immunofluorescence microscopy and immunoblotting. So far, at least one isolate was identified exhibiting the non-fusogenic phenotype. This isolate contains a truncated *incA* gene.

D11

Host factors, *IL1B-511* & *IL-1RN* gene polymorphisms, and bacterial factors of *Helicobacter pylori, cagA* and *vacA* subtypes, in peptic ulcer disease and non-ulcer dyspepsia: a synergistic effect

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Introduction: Environmental, bacterial and host factors are linked to the susceptibility, severity and clinical outcome of infectious diseases. Disease associated bacterial loci have been identified in *Helicobacter pylori*, among them the polymorphic gene encoding cytotoxin (*vacA*) and the cytotoxin associated gene A (*cagA*). Both bacterial factors are associated with peptic ulcer disease (PUD). In addition, specific polymorphism of interleukin-1B promoter region (*IL1B-511*) and interleukin-1 receptor antagonist gene (*IL-1RN*) of the host also appear to be associated with PUD in *H. pylori* infected persons.

Aim: To determine the interrelationship between the genotype of *IL1B-511* and *IL-1RN* gene of the host, the *H. pylori* factors *cag*A status and *vacA* subtype and the clinical outcome in *H. pylori* related diseases, both by single trait and carrier trait analyses.

Methods: Ninety Dutch Caucasian consecutive patients, 43 diagnosed with PUD and 47 had non-ulcer dyspepsia (NUD), were included into the study. The *H. pylori cagA* and *vacA* status was determined using PCR and/or serology. DNA was isolated from sera to analyse *IL1B-511* and *IL-1RN* gene polymorphisms.

Results: All genotypes for the IL1 cluster genes were in Hardy-Weinberg equilibrium showing Mendelian inheritance. NUD vs PUD (p-value; OR): 1) *IL1B-511* allele 2 (*2): 56% vs 62% (0.5; 1.4); 2) *IL1RN*2*: 28% vs 43% (0.19; 1.9); 3) *cag*A+: 67% vs 91% (0.0073; 5.2); 4) *vac*A s1+ partially determined (in progress) for 25 NUD and 20 PUD patients: 12% vs 30% (0.45; 2.4). Carrier trait analyses NUD (25 patients) vs PUD (20 patients): 1) *IL1B*2/IL1RN*2*: 14% vs 30% (0.13; 2.5); 2) *IL1B*2/IL1RN*2/cag*A+: 5% vs 26% (0.008; 7.0); 3) *IL1B*2/IL1RN*2/cag*A+/*vac*As1+: 4% vs 30% (0.034; 10.2).

Conclusions: *cagA* is associated with the more severe clinical outcome PUD in *H. pylori*-infected patients. Carrier trait analyses showed that combining these bacterial and host factors provides the strongest risk profile, increasing ORs from 1.4 to 10.2.

E01

Biochemistry and electron transport of the anammox bacterium *Kuenenia stuttgartiensis*

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The planctomycete *Kuenenia stuttgartiensis* catalyses the anaerobic transformation of ammonium (NH4+) and nitrite (NO2-) into dinitrogen (N2) to yield energy. Important intermediates in this so-called anammox process are hydroxylamine, nitric oxide and hydrazine. The enzymes and corresponding genes involved in this catabolism are hydroxylamine oxidoreductase (HAO), nitrite reductase and hydrazine hydrolase. *Kuenenia stuttgartiensis* exhibits an unusually high content of cytochromes c. Already the HAO-enzyme (24 heme per trimer) constitutes about 10% of the total protein content which is located exclusively in anammoxosome. The special bacterial organelle is surrounded by a lipid bilayer composed of unique 5-cyclobutane (ladderane) lipids.

Analysis of the *Kuenenia stuttgartiensis* partial genome assembly revealed many ORFs with the classical heme C-binding motif, CXXCH. Two of the c-type cytochromes deduced from the genome analysis correspond to cytochromes previously isolated: the high molecular-mass cytochrome HAO, and a small cytochrome c with unknown function. Some of the other membrane and soluble c-type cytochromes are most likely involved in electron transfer of the anammox reactions. *Kuenenia stuttgartiensis* uses CO2 as major carbon source. For every 15 catabolic reactions, one CO2 can be fixed. The reducing equivalents (NADPH) needed for the CO2 fixation must be produced via reversed electron transport from the anaerobic oxidation of nitrite. The compounds, enzymes and corresponding genes potentially involved in this reversed electron transport could be nitrite oxidoreductase, cytochrome c, the BC1 complex, quinols and the NADH:ubiquinone oxidoreductase. Several component of this process are currently heterologously expressed in *E. coli* to obtain enough protein for localization and functional studies.

E02

Expression and role of the Pdr12 membrane protein in yeast cells under sorbic acid stress

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Introduction: Weak acid preservatives, such as sorbic acid, are used widely in the food industry. However, consumer demands for products with low content in chemical preservatives, has led to the need for more sophisticated preservation strategies. This requires an understanding of how these molecules affect the spoilage-causing micro-organisms. This study focuses on the behaviour of yeast cells in the presence of sorbic acid. Of particular interest is Pdr12, a protein previously reported as an anion-pump that is induced by sorbic acid in a low pH environment. The goal is to test whether Pdr12 is expressed in a variety of yeast species and examine its potential role.
Methods: 12 yeast strains were tested for Pdr12 expression under various conditions of sorbic acid stress. Protein extracts from whole yeast cells were immunoblotted using a polyclonal anti-Pdr12 antiserum. Growth patterns were also examined.
Results: - Pdr12 is induced by sorbic acid (0.9 mM at pH 4.2) in Saccharomyces strains (both S. cerevisiae and S. Bayanus). This induction is observed not only in the case of growth in rich media but also in tea.
- A band cross-reacting with the a-Pdr12 polyclonal antibody was detected in Zygosaccharomyces Bailii, Z. Lentus and Pichia Membranaefaciens strains. This band, however, is constitutively present even in the absence of sorbate stress and is not affected by it.
- Both laboratory strains and spoilage isolates from products were tested for Pdr12 expression and they exhibited very similar patterns.
- Pdr12 is induced rapidly and expression is maintained for the duration of the stress.
After removal of the stress, Pdr12 levels quickly drop.
- Comparing the behaviour of yeast cells pre-stressed with sorbic acid for two hours (so that Pdr12 is maximally induced) to non-prestressed cells, we did not observe significant differences with respect to their reaction to the application of a subsequent sorbic acid stress.
Conclusions: Pdr12 is a membrane protein that is rapidly induced by sorbic acid only in Saccharomyces strains. Its role appears to be important in the innate resistance of yeast cells to sorbic acid stress, but not in conferring acquired resistance (adaptation) to higher concentrations of sorbic acid.

E03

Micro-array analysis for the screening of *Salmonella* strains for antibiotic resistance genes

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Introduction: Due to the use of antibiotics the prevalence of antibiotic resistant micro-organisms has increased. Especially in the case of pathogenic bacteria antibiotic resistance is a major public health issue. For example, multi-drug resistant pathogens have emerged, such as *Salmonella enterica* serovar Typhimurium DT104. *S. typhimurium* DT104 possesses a gene cluster located within the Salmonella Genomic Island 1 (SGI1) that is responsible for resistance to five commonly used antibiotics (so-called R-type ACSSuT). For the detection of these genes and to investigate the applicability of the micro-array technology as a screening tool for antibiotic resistance genes a pilot micro-array was designed with 60-mer oligonucleotides directed against the 5 resistance genes present on the SGI1. Furthermore a second micro-array containing a larger set of oligonucleotides was designed to screen more than 20 different Salmonella strains.
Methods: Two sets of Salmonella strains were tested: set 1 consists of strains with known antibiotic resistance profiles and genotypes (determined by PCR), set 2 contains strains of which only genetic information was available. Micro-arrays were spotted on silylated slides using a Microgrid (Biorobotics). Fluorescently labelled DNA fragments were either generated by PCR or by direct labelling of total DNA using the BioPrime DNA labelling system (Invitrogen). After hybridisation the micro-arrays were scanned using a confocal laser scanner (ScanArray 3000 (General Scanning)).
Results: The micro-array results of the first set of strains (MDR mutants) perfectly matched with the phenotypic and genotypic information. Hybridisation signals were only found with those oligonucleotides representing genes present in the strains. For the second set of strains most of the micro-array data of were in concordance with the phenotypic data. Hybridisation signals were also found with oligonucleotides corresponding to resistance phenotypes that had not been determined in the investigated strains.
Conclusion: The oligonucleotide micro-array technology can serve as a rapid screening technique for the presence of antibiotic resistance genes in pathogenic bacteria.

E04

Application of DNA fingerprinting in the food supply chain

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Safe products with constant quality are important pre-conditions in the food chain. Micro-organisms play a crucial role with respect to quality and safety. Detailed characterization of the biodiversity of these microbes is essential to maintain a high standard of quality and safety in feed and food and to facilitate innovation. PCR-based fingerprinting is increasingly applied to characterize beneficial, spoilage and pathogenic microbes in the food supply-chain. Moreover, DNA population fingerprinting enables studying microbial population structure and dynamics.
GTG5 fingerprinting is a high resolution fingerprinting method that yields characteristic profiles from bacteria as well as fungi. High standardization and cluster analysis allow for comparative as well as surveillance or database typing. Selection of representatives of clusters of highly similar

isolates for DNA sequence analysis enables highly efficient diversity and identification screens.
DNA fingerprints of individual isolates are used to track sources of spoilage or pathogenic microbes in feed and food production processes. Cluster analysis of fingerprints of individual isolates of spore-forming bacteria in the farm environment including feed, manure and milk enables assessment of reservoirs. This can be used for implementation of specific control measures to improve quality and safety in the dairy supply chain. Alternatively, DNA fingerprinting has been applied to reconstruct defined starters from undefined mixtures. DNA population fingerprinting has been applied to assess the population structure and dynamics in processing of fermented products foods. The ratio of starters during production and ripening of a variety of products can be determined and related to individual strains and characteristic properties, like flavour or structure.
Conclusion: PCR-based fingerprinting, computer-assisted analysis and database construction are highly valuable tools for tracking and tracing unwanted microbes and to select beneficial microbes such as individual starter strains. Alternative methods allow determination of composition and dynamics of complex microbial populations.

E05
The stress sigma factor SigmaB of *Bacillus cereus*: regulation and regulon

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Introduction: *Bacillus cereus* is a toxin-producing, spore-forming pathogen that is associated with food spoilage and food-borne illness. The capability of vegetative cells of *B. cereus* to trigger a stress response, can lead to an increased survival during food processing and storage. In a number of Gram-positive bacteria the 'stress' sigma factor, SigmaB, plays a central role in regulating gene expression during stress response. Here, we describe the role of SigmaB in the stress response of *B. cereus*.
Methods: Western blotting with polyclonal anti-SigmaB antibodies was used to determine SigmaB-levels in cells upon various stress exposures. The transcription of the *sigB* operon was studied by Northern blotting and primer extension analysis. SigmaB-regulated genes were identified by a proteomics approach and bioinformatics-based searches. A *sigB* null mutant was constructed and the stress resistance of the *sigB* null mutant and its parent strain were compared.
Results: Heat shock had the largest SigmaB-activating effect, but other stresses (oxidative stress, ethanol shock, osmotic upshock and acid stress) also resulted in the activation of SigmaB. The *sigB* operon is transcribed from a SigmaB-dependent promoter upstream of the first open reading frame (*rsbV*) of the operon, indicating that transcription of *sigB* is autoregulated. Nineteen SigmaB-dependent genes have so far been identified. Some of the encoded proteins may have a role in the proteolytic turnover of misfolded proteins or in the regulation of metabolic fluxes of the cell.
SigmaB was found to play a role in the protective heat shock response of *B. cereus*. The *sigB* null mutant was less protected against the lethal temperature of 50°C by a pre-adaptation to 42°C than the parent strain.
Conclusion: SigmaB plays a considerable role in the stress response of *B. cereus* and its activation may lead to an increased survival of the organism during food processing and storage. Currently, the post-translational regulation of SigmaB-activity is studied. Furthermore, genome-wide transcription analysis is being performed to reveal the complete set of SigmaB regulated genes and analyze their contribution to the *B. cereus* stress response.

E06
Functional analysis of phosphofructokinase orthologues in *Streptomyces coelicolor*: the multiplicity phenomenon

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Streptomyces coelicolor A3(2) is the best studied *Streptomyces* species at the genetic level. Like other members of the order *Actinomycetales* it has a complex life cycle involving mycelial growth and spore formation. Streptomycetes are medically and industrially important because they produce pharmaceutically useful compounds including over two-thirds of naturally derived antibiotics currently in use for which several precursors are retrieved from the primary metabolic pathways. Therefore studies on central metabolism are a pre-requisite for a rational approach for the optimization of antibiotic production. With the availability of the complete genome sequence it became interesting to elucidate the numerous duplication events found in central metabolism in particular in glucose degradation. This project focuses on the four (putative) phosphofructokinase (PFK) genes annotated in the *S. coelicolor* genome, whereas prokaryotic organisms usually contain up to 2 *pfk* genes. PFKs catalyze an important regulatory step in glycolysis - the reaction fructose-6-phosphate => fructose-1,6-phosphate - making the enzyme an interesting subject concerning antibiotic production. Furthermore, the four *pfk* genes may be differentially expressed throughout the complex life cycle of *S. coelicolor* and/or when grown on different carbon sources. Two *pfk* knockout strains have been constructed and are currently being phenotypically characterized. The remainder of the knockout strains are still under construction. All four putative *pfk* genes will be expressed in *E. coli*, purified, and biochemically characterized, e.g. ATP/PPi substrate specificity. Thus far two gene copies have been successfully cloned into expression vectors. Finally, *pfk-EGFP* fusions are constructed permitting spatial and temporal expression studies.

E07
Diversity analysis of sulphate-reducing bacteria using a nested PCR-DGGE approach

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Sulphate-reducing bacteria (SRB) form a phylogenetically

diverse group of anaerobic bacteria using sulphate as a terminal electron acceptor in the degradation of organic matter. Different culture-independent methods have been used to study SRB populations in various ecosystems. Denaturing gradient gel electrophoresis (DGGE) is one of these methods.
However, DGGE mainly detects the major constituents of the analysed community, thus overlooking the less abundant species. Here we describe a strategy to overcome the difficulty in detecting low numbers of SRB in environmental samples. A three step nested PCR-DGGE approach was devised to detect SRB in complex microbial communities from industrial bioreactors which otherwise would not have been detected. In the first step nearly the complete 16S rRNA gene was amplified using bacterial primers. Subsequently, this product was used as a template in a second PCR with group specific SRB primers. A third round of amplification was conducted using DGGE primers and the product of the nested amplification as template to generate SRB specific 16S rDNA fragments suitable for DGGE analysis. Individual DGGE bands were excised and sequenced to determine the phylogenetic affiliation of the SRB. Comparative analysis showed that SRB were not the dominant community members. Only in some of the reactor samples a few low intense bands could be identified as those belonging to SRB. The three-step nested PCR strategy however enabled a greater resolution of SRB-specific bands. The largest number of bands was observed in DGGE patterns of products obtained with primers for the *Desulfovibrio-Desulfomicrobium* group, indicating a large diversity within this group. In addition, the nested PCR-DGGE approach revealed the presence of members of other phylogenetic SRB groups, i.e., members of the genera *Desulfotomaculum, Desulfobulbus* and *Desulfococcus-Desulfonema-Desulfosarcina,* which were not detected by the direct approach. Bands corresponding to *Desulfobacterium* and *Desulfobacter* were not detected in these bioreactor samples. The developed three step PCR-DGGE strategy is a welcome tool for studying the diversity of sulphate-reducing bacteria.

E08
Fungal phylogenomics: linking evolution and function

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Analyses of fungal phylogeny have been based on the ribosomal gene complex. The availability of fully sequenced genomes of several fungi belonging to different lineages now allows comparisons that are more meaningful than those performed before as they embrace the total genetic potential.
Complete fungal mitochondrial genomes and clusters of orthologous groups of proteins (KOGs) from complete fungal genomes were used for phylogeny analysis. For mitochondrial phylogeny, seven proteins sequences from nineteen fungi were concatenated. For KOG phylogeny, the analyses were done based on the presence or absence of KOGs and concatenation of KOGs present only in fungi. The distribution of KOGs among *C. neoformans, N. crassa, S. cerevisiae* and *S. pombe* in 24 different functional categories was analysed.
The mitochondrial tree places the Chytridiomycetes correctly at the base of the fungi. The basidiomycetes are basal to the yeasts and filamentous ascomycetes. The KOG phylogenetic tree supported the classification of the endomycetous yeasts (viz. *Saccharomyces* spp.) and the Euascomycete *Neurospora crassa* as a sister groups in a single clade, whereas *Schizosacharomyces pombe* and *Cryptococcus neoformans* seems to represent basal lineages. The distribution of the KOGs over the functional categories demonstrates that the genome of *C. neoformans* is more similar to that of the filamentous fungus *N. crassa*, whereas the yeasts *S. cerevisiae* and *S. pombe*, seem different. The *C. neoformans* and *N. crassa* genomes have more copies of genes of lipid metabolism, defence mechanisms, secondary metabolites biosynthesis, transport and catabolism, energy production and conversion, carbohydrate transport and metabolism, inorganic transport and metabolism than *S. cerevisiae* and *S. pombe*.
The fungal KOG-based tree largely corresponds with the previous mitochondrial and ribosomal trees. According to the functional analysis of KOGs, it seems that *C. neoformans* has the gene machinery of filamentous fungi, which may not be surprising if the dimorphic character of this pathogen is taken into account.

E09
Microbial diversity of phototrophic biofilms from wastewater treatment plants

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Phototrophic biofilms are microbial communities driven by light. Surface attached photosynthetic microbes, like cyanobacteria and diatoms derive energy from captured photons, reduce carbon dioxide, and provide organic compounds and oxygen that fuel the growth of heterotrophic micro-organisms in the biofilms. The phototrophic and heterotrophic micro-organisms produce an extracellular matrix of polymeric substances (EPS). This matrix enables the attachment of the micro-organisms to surfaces, which is crucial in biofilm development.
In this study the species composition of an aquatic phototrophic biofilm is determined. Biofilms recovered from a sedimentation tank of the wastewater treatment plant at Fiumicino Airport (Rome, Italy), and biofilms cultivated in a special designed incubator were analysed. DNA was extracted from these samples and PCR-amplified with primer sets specific to bacterial 16S rDNA, 16S rDNA of oxygenic phototrophs, and eukaryotic 18S rDNA. Denaturing gradient gel electrophoresis (DGGE) of the gene fragments obtained provided an overview of the microbial diversity in the phototrophic biofilms. Extraction and sequencing of the specific DGGE bands revealed the identity of the dominant species at different stages of biofilm development. It was obtained that the species composition changes drastically as the biofilm grows. Preliminary sequence comparisons revealed DNA sequences affiliated to chloroplasts, cyanobacteria and bacteroidetes to be dominant in the biofilm. These results suggest that a strong succession takes place within the developing biofilm.

E10

Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake

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We have studied the diversity of pelagic cyanobacteria in Lake Loosdrecht, the Netherlands, through recovery and analysis of small subunit ribosomal RNA gene sequences from lake samples and cyanobacterial isolates. We used an adapted protocol for specific amplification of cyanobacterial rDNA for denaturing gradient gel electrophoresis (DGGE) analysis. This protocol enabled direct comparison of cyanobacterial community profiles to overall bacterial profiles. The theoretical amplification specificity of the primers was supported by sequence analysis of DNA from excised DGGE bands. Sequences recovered from these bands in addition to sequences obtained by shotgun cloning from lake DNA as well as from cyanobacterial isolates from the lake, revealed a diverse consortium of cyanobacteria. The lake is dominated by filamentous species that originally have been termed Oscillatoria limnetica-like. We show that this is a group of several related species, co-occurring in the lake, which belong to the Limnothrix/Pseudanabaena group. Another important cyanobacterium is Prochlorothrix hollandica that apparently co-occurs with an unknown but related species. Furthermore, in agreement with microscopic observations, we detected species from the genera Aphanizomenon, Planktothrix and Microcystis. In addition, we detected two different taxa of the Synechococcus 6b group. The coexistence of many related filamentous species in the lake may be explained through different strategies towards limiting resources or through diversification driven by viral pressure.

E11

Novel teicoplanin and vancomycin derivatives using mutant glycosyltransferases

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The class of glycopeptide antibiotics is important due to their activity against Gram$^+$ bacteria, like the methicillin resistant *Staphylococcus aureus* (MRSA), interfering with the crosslinking of the cell wall components. Glycopeptide antibiotics are synthesized by actinomycetes via multienzyme pathways. These pathways also involve 1-3 glycosyltransferases (GTFs), that each couples a specific sugar to a specific site on the peptide aglycon (e.g. vancomycin and teicoplanin). These sugars are important for increasing solubility and dimerization constants, determining potency and restricting conformational flexibility of the aglycon scaffold.[1] Little is known about the biochemical and kinetic characteristics of these GTF enzymes. Two 3D structures have been published, both cGtfA and cGtfB of the chloroeremomycin cluster (*cep*).[2,3] We have cloned and expressed several of these genes from *Amycolatopsis mediterranei* (encoding bGTFA and bGTFB, both involved in balhimycin synthesis) and *Actinoplanes teichomyceticus* (tGtfA and tGtfB, involved in teicoplanin synthesis) into *E. coli* and determined enzyme activity. Both tGtf proteins responsible for attaching aminosugars at amino acid residues 4 and 6 of the teicoplanin aglycon, recognize UDP-N-acetylglucosamine as their substrate. Both tGtfs can glycosylate the aglycon, but tGtfA is also capable of glycosylating the pseudoaglycon, after glycosylation by tGtfB at residue 4, where tGtfB is unable to use the pseudoaglycon, glycosylated at residue 6, as a substrate.
To identify structural features in these enzymes, that determine their substrate specificity, mutant and hybrid GTF enzymes have been constructed rationally and randomly, followed by detailed biochemical characterization of mutant GTF enzymes with new substrate specificity.
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G01

Natural competition between *Streptococcus pneumoniae* and *Staphylococcus aureus* during colonisation in healthy children

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Introduction: Recently, a large randomised double-blind trial with a 7-valent pneumococcal-conjugate vaccine was conducted in children suffering from recurrent acute otitis media. A complete shift in pneumococcal colonisation towards non-vaccine serotypes was observed. In addition, an increase in *Staphylococcus aureus*-related acute otitis media was found after vaccination (Veenhoven. Lancet 2003;361:2189-95).
Methods: We investigated the prevalence and determinants of nasopharyngeal carriage of *S. pneumoniae* and *S. aureus* in 3193 healthy children 1-19 years of age. In addition, we performed serotyping of all pneumococcal isolates. Finally, we investigated the correlation between *S. aureus* and *S. pneumoniae* carriage.
Results: Determinants of nasopharyngeal carriage of *S. pneumoniae* (19%) were age (peak incidence: 3 years) and regular day-care visits (OR: 2.14, 95% CI: 1.44-3.18). Risk factors for *S. aureus* carriage (36%) were age (peak incidence: 10 years), male sex (OR: 1.46, 95% CI: 1.25-1.70), large families (≥ 5 members, OR: 1.17, 95% CI: 1.00-1.37) and passive smoking (OR: 1.22, 95% CI: 1.04-1.42), whereas active smoking was inversely related to *S. aureus* carriage (OR: 0.75, 95% CI: 0.53-1.04). Pneumococcal serotype analysis showed 42% vaccine type and 58% non-vaccine type pneumococci. Multivariate regression analysis showed a negative correlation for co-colonisation of *S. aureus* and vaccine-type pneumococci (OR: 0.68, 95% CI: 0.48-0.94), but not for *S. aureus* and non-vaccine serotypes.
Conclusions: These observations suggest the presence of a natural competitive balance between vaccine-type

pneumococci and *S. aureus* during colonisation, which may explain the increase in *S. aureus*-related otitis media events found after vaccination.

G02
Epidemiology of integron-associated resistance in 2 ICUs during a non-outbreak period

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Introduction: Integrons (int), present on plasmids and transposons in Gram-negatives play a role in horizontal transfer (HT) of antibiotic resistance. Little is known about the prevalence, incidence, and HT of int-associated resistance in Enterobacteriaceae in a non-outbreak period in an intensive care unit (ICU).
Methods: During an 8 month period all patients admitted to 2 ICUs were screened for rectal colonization with Enterobacteriaceae with reduced susceptibility to cephalosporins (ERSC) by means of rectal swabs taken on admission and twice weekly thereafter and cultured on agar with cefpodoxime. Isolates (iso) were identified using the VITEK. Two iso of each species/pt were selected for susceptibility testing, int-specific PCR, and AFLP genotyping. Ints were characterized by CS-PCR, RFLP, and DNA sequencing. Demographics and data on antibiotic use were collected.
Results: In total, 456 patients were admitted to these ICUs of which 121 were colonized with ERSC. 61 patients were colonized on admission and 56 acquired colonization in the ICU. 174 iso were selected and 52 iso of 29 patients (24%) carried at least 1 int. The endemic prevalence of ints was 7% (0-33%). Multivariate analysis revealed age as a risk factor for acquisition of int-positive isolates. Characterization revealed 3 groups containing the majority of int-positive iso. Two of these groups were only found in *E. coli* iso collected from ICU-1. The 1st group comprised 11 iso carrying 2 ints with the *aadA2* and the *aadB/catB3* genes. The 2nd group comprised 4 iso carrying an int with the *dfrIa* and *aadA1a* genes. The 3rd group comprised 8 *E. cloacae* iso, 1 *K. oxytoca* iso, and 1 *K. pneumoniae* iso from ICU-2, which carried an int containing the *aadB* gene. Iso carrying identical ints were, with a few exceptions, part of the same AFLP clusters. HT of a complete int-carrying organism (i.e. cross-transmission) was identified 5 times. HT of an int was suspected in 2 patients colonized with genetically different iso of *E. coli*. Tranfer of an int from *E. cloacae* to *K. oxytoca* isolated within a pt was identified once.
Conclusion: Age was a risk factor for acquisition of int-positive isolate. HT of a complete int-carrying organism is more likely than HT of individual ints.

G03
Outbreak of *Serratia marcescens* traced to a health care worker with at least 3 months carriage on the hands

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Introduction: Fifteen patients in a neurosurgical intensive care unit (NSIC) in a Dutch university hospital were infected or colonized with a single strain of *Serratia marcescens* from April 2002 through March 2003 (study period). The objective of the study was to reveal the source of the outbreak.
Methods: Environment and health care workers hands were cultured. A retrospective case-control study was carried out. Fifteen patients in the NSIC with at least one positive culture for the epidemic *S. marcescens* strain from April 2002 through March 2003 were defined as cases. As controls, 30 NSIC patients without the epidemic strain were selected in the study period and matched for both length of stay (LOS) at the NSIC (until first positive culture) (cases 17.7 days vs controls 17.1 days) and mechanical ventilation requirement (13/15 cases vs 24/30 controls). Results: Environmental cultures did not reveal a prominent source of *S. marcescens*. Approximately 200 cultures of the hands of 100 health care workers yielded colonization of a single health care worker with the epidemic strain. Despite instant leave of this health care worker, serial cultures yielded prolonged carriage of the epidemic strain for 3 months. Cultures from other isolation sites of the health care worker revealed predominant colonization of the hands: (no. positive cultures/total no. cultures) hands 34/45, perineum 16/39, elbow 3/34, throat 1/35 and nose 0/34. No additional epidemic cases appeared at the NCIC after leave of the colonized health care worker. Case-control analysis could not confirm the association between *S. marcescens* colonization and the presence of the single colonized health care worker at the ward during a patients' stay as parameter for exposure (14/15 cases vs 28/30 controls, ratio exposed days/LOS cases 0.26 vs controls 0.16).
Conclusions: A single health care worker with the epidemic *S. marcescens* strain on the hands was considered the source of this outbreak. Important rationales in this consideration were the fact that the epidemic ended after leave of the colonized health care worker, and the remarkable tenacious character of the carriage on the hands.

G04
Mannose-binding lectin polymorphisms in Löfgren's syndrome

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Introduction: Mannose-binding lectin (MBL), an opsonin and potent activator of complement, is involved in innate immunity to many micro-organisms. Deficiency of MBL can result from three single nucleotide polymorphisms (SNP) in exon 1 of the MBL gene. This deficiency has been associated with increased susceptibility to infections with several micro-organisms. Sarcoidosis is a chronic and

multisystemic granulomatous disease, thought to result from an environmental antigenic trigger, possibly microbial in origin, in genetically predisposed hosts. A previous study investigated whether genetic variants of MBL predispose to sarcoidosis by increasing the susceptibility to antimicrobial triggers. No correlation was found. However, patients with different clinical presentations were considered. Löfgren's syndrome is a small, but distinct subset of sarcoidosis characterized by an acute presentation of fever, erythema nodosum, bilateral hilar lymphadenopathy and polyarthralgia. Because Löfgren's syndrome has a higher incidence in spring, a microbial trigger needs to be considered especially in this group. Also, it represents a more homogenous subset of sarcoidosis in which genetic associations may better be found. Therefore, we investigated whether MBL variants are associated with Löfgren's syndrome.

Methods: In 46 Dutch Caucasian patients presenting with Löfgren's syndrome between 2000 and 2003 and 225 healthy control subjects MBL exon 1 polymorphisms were detected by denaturating gradient gel electrophoresis. This technique allows all three structural SNPs to be detected in a single PCR product. The found genotypes were grouped as homozygous wild type (AA), heterozygous variant (A0) or homozygous variant (00).

Results: MBL variant frequencies were comparable in patients (56.5% AA, 37.0% A0 and 6.5% 00) and controls (55.1%, 38.1% and 6.8%). There were no significant differences in genotypes of patients presenting in spring (April to June; 51.9% AA, 37% A0 and 11.1% 00) or in other seasons (63.2%, 36.8% and 0%).

Conclusions: In this relatively large cohort of patients MBL gene exon 1 variants were not associated with the susceptibility to Löfgren's syndrome or the moment of disease onset.

G05

An epidemic with a non-MRSA but multiresistant *S. aureus*

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In May 2003, our laboratory extended the set of antibiotics tested for staphylococci. Soon thereafter, 5 surgery patients were found with *S. aureus* resistant to penicillin, ciprofloxacin, erythromycin and clindamycin (CEC strain). The isolates were genetically identical with PCR fingerprinting. Hand hygiene was strengthened and contact isolation for positive patients was started on the ward. In June, new patients were discovered on the same surgical ward but also on 3 other wards. Nursing in single rooms with gloves and gown was implemented. Since several wards were involved we feared transmission during consultations. Physicians were screened but all were negative. Stored strains resistant to penicillin and clindamycin were retested. Surprisingly, 87% (14/16) were also resistant to ciprofloxacin and they had the same genotype as the CEC strain. This made clear that the strain was present in our hospital at least since March 2002. The CEC strain easily lost resistance to erythromycin and clindamycin following subculturing or storage on non-selective agars. Interestingly, also in patients we found strains resistant to only penicillin and ciprofloxacin, which were genetically identical to the CEC strain. Restricted use of clindamycin and ciprofloxacin and screening of all patients (instead of only roommates) and personnel on the wards involved, were started in August. After the introduction of isolation of patients on single rooms, nursing with gloves, gowns, mask and cap, and withdrawal of positive health care workers, no new positive patients have been detected anymore since 3 months. Until January 2004, 56 positive patients have been detected: 48 by positive clinical samples (wounds 87%, sputa 10%, urine 8%, blood culture 4%) and 8 by screening of patients at risk. Eleven health care workers, one partner, a child and a cat, each from different health care workers, were found positive. In January 2004, 5 health care workers and the child are still positive despite 2 to 3 eradication treatments. An important problem during this epidemic with a non-MRSA strain was to find a balance between reducing the risk of transmission and the rights of colonized patients and health care workers.

G06

Epidemiological features of invasive and non-invasive group A streptococcal disease in the Netherlands, 1992-1996

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A prospective, nation-wide, laboratory-based surveillance of invasive Group A Streptococcal (GAS) infections was conducted in the Netherlands from March 1992 through December 1996. Clinical and demographic data were obtained and all isolates were M-typed. From May 1994 through December 1996 all non-invasive GAS isolates were registered. 880 Patients with invasive GAS disease were identified corresponding to an incidence of 4.0 per 100.000 persons. Predominant M-types were M1, 3, 6, 12, 28. Particular age and M-type distribution patterns were observed in different clinical entities. Overall case-fatality rate was 18%, but 59% in TSS. Older age, necrotizing fasciitis, sepsis without focus and pneumonia, infection with M1 or M3 and underlying cardiopulmonary disease were associated with fatality. Furthermore, 10105 patients with non-invasive GAS disease were registered. These differed significantly in age distribution and primary foci from invasive GAS disease cases. Invasive GAS disease is not a reflection of non-invasive streptococcal infections.

H01/02/03

Infectious diseases - a public threat. The organisation of health protection in England

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The threat of infectious diseases for both individuals and populations is again a medical and political priority. Since the pronouncement 30 years ago that infections were conquered, more than 20 new infectious diseases have been recognised, from HIV to SARS, and taking in *C. difficile, E. coli* O157 and *H. pylori,* together with antibiotic resistance and the threat of bioterrorism. Protection of the public health requires the integration of clinical recognition of infections, accurate and prompt laboratory diagnosis, epidemiological expertise providing timely disease surveillance and outbreak investigation, and an effective operational response to treat cases, control spread and implement preventive measures. This requires trained professionals in the infection specialties and an organisational framework that serves both individual patient care and health protection for the population. Until 2003, the PHLS in England and Wales provided public health microbiology from its network of PHLs and its central reference laboratories coupled with surveillance of infectious diseases and outbreak investigation through its Communicable Disease Surveillance Centre. However, the effector component of public health in the community was outside the PHLS. In January 2002, Sir Liam Donaldson (CMO, England) set a new strategy for infectious diseases (*Getting Ahead of the Curve*). The PHLS specialist and reference laboratories and CDSC (central and regional components) were incorporated into a new Health Protection Agency, together with the National Focus for Chemical Incidents and the regional (Chemical Hazards) units and working in partnership with the National Radiological Protection Board, which will become part of the HPA in due course. The HPA brings together the response capability for infections, chemical and radiological threats to public health. This includes the emergency response capability to both new natural hazards (eg, SARS) and acts of deliberate release (bioterrorism). The community public health doctors (Consultants for Communicable Disease Control) were also brought into the HPA to provide the local and regional services together with regional epidemiologists and regional microbiology services. In the laboratory part of this major reorganisation, responsibility for the PHLs that had formed the national network was transferred to their local NHS hospital services, with one regional HPA microbiology laboratory retained with direct HPA management in each region. The former PHLs continue to be responsible for providing local Health Protection support and all microbiology laboratories are expected to contribute to Health Protection as well as to individual patient care. The development of an integrated and cohesive regional and national professional network of accredited microbiology laboratories, with appropriately trained staff working to nationally agreed standards of investigative algorithms, laboratory methods and reporting data for surveillance, is an essential part of Health Protection; this is the aim of the newly created post of Inspector of Microbiology.

H04/05/06
Abstract not available

J01
Using the model organism *Caenorhabditis elegans* to study the pathogenesis of *Cryptococcus neoformans*

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Caenorhabditis elegans, a soil nematode, has been used as a model organism to study several bacterial pathogens (e.g. *Pseudomonas, Yersinia, Salmonella)*. Recently it was shown that *C. elegans* can also be used to study the pathogenesis of the basidiomycetous yeast *Cryptococcus neoformans.*
Cr. neoformans is a free-living organism that can be isolated from pigeon droppings, soil and plants. *Cr. neoformans* causes life-threatening infections, mainly in immunocompromised hosts. Several virulence factors that play a role in cryptococcal infections are known; for example, the formation of capsule and the ability to produce melanin. To test pathogenicity of different *Cr. neoformans* strains, *C. elegans* were transferred from a lawn of *Escherichia coli* (OP50) onto plates inoculated with *Cr. neoformans.* The plates were incubated at 24 °C for 8 days and examined daily for *C. elegans* survival. The absence of an adaptive immune system in *C. elegans* allows the dissection of 'basic' cryptococcal virulence factors in this model system. Differences in virulence between *Cr. neoformans* strains are apparent and we have begun to identify capsule components that modulate virulence independently of their effects on the vertebrate immune system. Together, these data indicate that *C. elegans* will prove a powerful model of the immunocompromised host and may be useful in identifying additional virulence factors not previously described using other model organisms.

J02
Failure of pre-emptive management strategy to detect cerebral aspergillosis early

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In patients treated for haematological malignancy an intensive monitoring program is followed during the period of neutropenia to early detect infectious complications including invasive aspergillosis. For aspergillus infections serum is obtained twice weekly for detection of circulating galactomannan and samples are analyzed once weekly. In addition, HR CT scans of the chest are performed in patients with rising galactomannan titres or those with respiratory symptoms or persistent fever. Patients with high suspicion of invasive aspergillosis (mostly probable and proven diagnosis) are treated promptly. In this management scheme we recently encountered two patients with cerebral aspergillosis. The first presented with sudden loss of consciousness. CT scan of the brain showed a massive intracerebral haemorrhage and the patient died the same day. Retrospectively a rising galactomannan titre was found in the week preceding the event. A second patient complained of headache during an episode of neutropenia and low-grade fever. A CT scan of

the head revealed a single area of infarction. Since the last routinely performed galactomannan titre was 0.9, additional samples were tested immediately and showed a rising titre. Despite immediate treatment with voriconazole, the patient deteriorated rapidly and died 4 days later.
Despite intensive monitoring of high-risk patients we failed to detect cerebral aspergillosis early, which is partly due to the very rapid course of infection. Further intensification of the monitoring strategy might allow earlier identification of patients with cerebral aspergillosis, but this should be weighed against the incidence of the infection. Those involved in the management of these high-risk patients should be aware of the possibility of missing patients with cerebral aspergillosis despite intensive monitoring.

J03
Recognition of *Pseudallescheria boydii* in the clinical laboratory

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Protocols for clinical diagnosis of emerging filamentous agents of mycoses are demonstrated using *Pseudallescheria boydii* (anamorph: *Scedosporium apiospermum*) as an example. Classically this species is known as an agent of mycetoma, joint infections and otitis. The first pulmonary infection was reported 1955, while more recently the number of disseminated mycoses caused by *P. boydii*, often with a marked predilection for the CSF, is growing. Most cases of deep mycoses occur in immunodeficient patients, but also cases in patients after major trauma or near-drowning are observed. *P. boydii* is not as rare as frequently supposed. In the university hospital of Bonn (Germany) our working group found that in 6 out of 11 cases held for Aspergillosis *P. boydii* was proven to be the etiologic agent. Difficulties in routine recognition are at the basis of frequent misdiagnoses. Clinical picture, radiology and histopathology of *Pseudallescheria* may be indistinguishable from that of *Aspergillus fumigatus*. In this presentation an overview is given of steps in the identification of filamentous fungi from clinical samples. The routine identification of *P. boydii* is summarized.

J04
Correlation between *in vitro* MIC and *in vivo* activity of flucytosine

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Introduction: Buffered medium at a pH of 7.0 has been accepted as standard for *in vitro* susceptibility testing of fungi. However, it is not yet clear that this is the optimal pH with regard to clinical outcome. In a patient the pH at the site of a fungal infection may be lower than the natural body pH of 7.4. It may very well be that the *in vitro* activity found at pH 5.0 corresponds better with clinical outcome than the *in vitro* activity found at the standard pH of 7.0. The aim of this study was to determine which medium pH (5.0 or 7.0) gives an *in vitro* activity of flucytosine (5FC), which corresponds best with the *in vivo* efficacy.
Methods: A broth microdilution method according to NCCLS guidelines was used to determine the MIC-0, MIC-1 and MIC-2 of 5FC at pH 5.0 and 7.0 against two *Aspergillus fumigatus* (FASF 8196 and FASF 58) isolates. The *in vivo* activity of 5FC against both FASF isolates was determined by a non-neutropenic murine mouse model. Treatment (100 mg/kg/day every 12h) was begun 2h after infection and was continued for 7 days. Animals were checked daily for mortality until 7 days after treatment.
Results: According to NCCLS breakpoints for yeasts, both FASF isolates were resistant for 5FC at pH 7.0 (MIC ranged from 32 to >1024 ìg/ml, dependent on the isolate tested and the MIC endpoint used). At pH 5.0, FASF 8196 was susceptible for 5FC (MIC ranged from 0.031 to 0.125 ìg/ml, dependent on the MIC endpoint used) and FASF 58 was resistant at MIC-0 and MIC-1 (MIC-0 >1024 ìg/ml and MIC-1 of 512 ìg/ml) but susceptible at MIC-2 (MIC of 0.5 ìg/ml). *In vivo*, we found a significant difference in survival ($p<0.10$) between the treatment and control group for FASF 8196. Four of the ten mice survived in the treatment group, while only one mouse survived in the control group. We did not found a significant difference in survival for FASF 58, all treated and control mice died within 3 days.
Conclusion: We found that the *in vitro* results for 5FC at medium pH 5.0 corresponded better with *in vivo* outcome, than the *in vitro* results found at the standard medium pH of 7.0.

J05
Genotyping by amplified fragment length polymorphism (AFLP), mating type- and serotype-diversity, and susceptibility to fluconazole among Dutch isolates of *Cryptococcus neoformans*

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Introduction: *Cryptococcus neoformans*, an encapsulated basidiomyceteous yeast, is frequently implicated in meningoencephalitis in HIV-positive individuals and other immunocompromised patients. In the Netherlands, *C. neoformans* was found to be implicated in 268 patients with cryptococcosis during 1977-2002. A major increase in the incidence of the disease was observed in 1987, due to the increasing number of AIDS patients. The introduction of highly active antiretroviral therapy (HAART) in 1996 resulted in a significant decrease of patients with cryptococcosis. Here we present data on AFLP genotypes, mating types, and serotypes of these Dutch isolates. In addition, susceptibility to fluconazole was investigated for part of the isolates.
Materials and methods: 172 isolates of *C. neoformans* from the Netherlands were analyzed according to standard AFLP

protocols. Mating- and serotypes were determined by PCR using primer sets derived from the *STE12, STE20 and GPA1* genes. Fluconazole susceptibility of 43 isolates was tested by the Etest system.
Results: AFLP genotypes 1, 1B, 2 and 3 were present with 80, 5, 9 and 5%, respectively. The majority of isolates (63%) had serotype A *MAT*alpha and 12% had serotype D with 75% *MAT*alpha and 25% *MAT*a. Six percent were AD hybrids with two subtypes AalphaDa (83%) and DalphaAa (17%). Ten percent possessed AalphaAa, and 5 percent could not be PCR-typed. All sero- and mating type combinations were observed between 1987 and 1996, whereas fewer were isolated during 1977-1981 or after 1997. Fluconazole resistance was observed in 28% of 43 randomly selected isolates mainly belonging to AFLP genotype 1.
Conclusions: cryptococcosis in the Netherlands is mainly caused by *C. neoformans* var. *grubii*, and to a lesser extent var. *neoformans* and the AD hybrids. The largest variation of mating and serotypes occurred during the peak of AIDS. Fluconazole resistance was observed in almost 30% of the isolates investigated.

J06
A. fumigatus evades immune recognition through loss of TLR4-mediated signal transduction

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To cause infection, *A. fumigatus* has to escape or resist the immune system, and germination of hyphal forms is an important mechanism for initiating and establishing an infection. The exact molecular mechanisms used to evade the host defence during germination are yet unknown. Bearing in mind the differences in the structural components of the cell walls of *A. fumigatus* conidia and *A. fumigatus* hyphae, we hypothesized that TLR stimulation by conidia and hyphae are different and that germination may offer *A. fumigatus* the ability to evade recognition by TLRs. Experiments using macrophages from TLR2-/- mice and those with blocking anti-TLR2 antibodies in human PBMCs demonstrate that TLR2 is important for stimulation of cytokines by both *A. fumigatus* conidia and *A. fumigatus* hyphae. These results were confirmed in hamster 3E10 cells transfected with human TLR2. The most interesting finding of our study is the observation that, upon stimulation with conidia of *AF* but not with hyphae of *A. fumigatus*, macrophages from TLR4-deficient ScCr mice respond with a decreased production of proinflammatory cytokines. Similarly, hamster 3E10 cells, expressing TLR4 but not expressing TLR2, were activated after challenge with conidia of *A. fumigatus* but not after challenge with hyphae of *A. fumigatus*. In addition, *A. fumigatus* hyphae, but not *A. fumigatus* conidia, stimulated production of IL-10 through TLR-2 dependent mechanisms. In conclusion, TLR4-mediated proinflammatory signals, but not TLR2-induced anti-inflammatory signals, are lost upon germination to hyphae. Therefore, phenotypic switching during germination may be an important escape mechanism of *A. fumigatus* that results in counteracting the host defence.

J07
J08
J09
Abstracts not available

K01
Comparison of the NucliSens Magnetic Extraction Reagents to reference extraction methods the isolation of RNA and DNA from various sample types

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Introduction: A new nucleic acid extraction methodology that uses Boom chemistry in combination with magnetic silica particles is developed (NucliSens Magnetic Ectraction Reagents, bioMérieux). This method is generic and can be used for the simultaneous nucleic acid extraction of 12 samples in combination with the NucliSense miniMAG instrument to facilitate the extraction procedure. The objective of this study was to investigate the suitability of this method for the isolation of Enterovirus RNA and *Mycobacterium tuberculosis* DNA from a broad spectrum of clinical specimens.
Methods: The NucliSens Magnetic Extraction procedure was compared to reference extraction procedures, i.e. QIAamp (Qiagen) and Boom, followed by Enterovirus RT-PCR and *M. tuberculosis* PCR, respectively. Several clinical specimens (n=100) were used, a.o. Cerebrospinal Fluid (CSF), faeces, throat swabs, etc.
Results: By performing several extractions (up to 12) of a dilution series of strain Coxacksie B5 in CSF, it was shown that the analytical sensitivity of the Enterovirus RT-PCR was found to be independent of the extraction method used, whereas in very low frequency higher sensitivities were obtained in combination with magnetic extraction. After evaluation of the Enterovirus PCR using CSF and stool samples a 100% correlation between the two extraction methods was found. In addition, using a broad panel of clinical specimens for *M. tuberculosis* PCR, the same samples were identified as positive using the Boom extraction method and magnetic extraction. However, the latter method resulted in less samples having inhibition in PCR.
Conclusion: This new magnetic silica-based nucleic acid extraction method was successfully used to isolate Enterovirus RNA and *M. tuberculosis* DNA from clinical samples. In comparison to the reference methods this procedure performed at least equally well regarding the functional performance and the throughput, but was considered to be more user convenient. For high throughput sample processing this chemistry can be transferred to an automated magnetic extraction device.

K02
DNA recovery of the automated Roche MagNA pure total nucleic acid isolation kit as compared to the manual BOOM extraction method

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Introduction: Direct detection of microbial DNA in clinical samples like blood or cerebrospinal fluid requires a reliable DNA isolation procedure and a sensitive PCR amplification. The use of direct detection is partly troubled by low microbial load in these clinical samples. In this respect a very high recovery of target DNA during extraction is necessary. In this study we compared the performance of DNA recovery of the automated Roche MagNA Pure Total Nucleic Acid Isolation Kit (TNA) and the manual BOOM extraction method (Y/SC).
Methods: For studying the recovery of DNA a known amount of *Hin*dIII digested phage lambda (λ*Hin*dIII) DNA was added to PBS and processed according to the manufacturer's instructions. Manual BOOM extraction was performed as described by Boom et al. (JCM 28:495-503), with 20 µl of size-fractionated silica particles. In addition, DNA was isolated with Y/SC from a fraction of the fluid in which DNA-binding takes place from both methods and from all other washing steps for the TNA. A second elution of the glass particles was performed for the TNA. The recovery of the different fractions was estimated by agarose gel electrophoresis. For both methods eight replicates were run in at least two separate runs.
Results: Y/SC performed with high recoveries (~100%) for both high and low molecular weight (HMW, LMW) DNA and no recognisable loss of DNA. Therefore no second elution and no further study of the other washing steps were conducted. The TNA performed with a recovery of ~20%. Part of the missing DNA was found to be unbound (~10% HMW, ~25% LMW), or not eluted (~35% HMW, ~20% LMW). The remaining DNA (~35%) is probably still bound to the glass matrix, since only about 5% DNA was present in the washing buffers.
Conclusion: The Roche MagNA Pure Total Nucleic Acid Isolation Kit has a substantial lower recovery of λ*Hin*dIII DNA when compared to the manual BOOM extraction method. The reduced recovery may influence the sensitivity of molecular diagnostic methods and may result in false negative results due to low microbial loads. The quality of the DNA from both methods seems identical.

K03
Quantitative analysis of periodontal pathogens by real-time PCR

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Introduction: Periodontitis is a multibacterial chronic inflammatory and destructive disease of the teeth-supporting tissues. Quantitative anaerobic culturing has been used for long time in diagnostics of the periopathogens in plaque samples. The aim of this study is to compare real-time PCR with the anaerobic culture in detection and quantification of several oral bacterial species in both periodontitis and healthy subjects.
Methods: A real-time PCR assay was developed on the 16s rRNA gene of *Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Peptostreptococcus micros (Pm)* and *Fusobacterium*. The PCR was validated on pure cultures of oral and non-oral bacteria. Subsequently subgingival plaque samples from 259 adult patients with periodontitis and 112 healthy persons were analysed with anaerobic culture and real-time PCR. A standard curve was created for each primer-probe set based on CFU equivalents. PCR assay specificities were tested against reference oral strains.
Results: All bacterial species were correctly identified when compared with the reference oral strains used. Bacterial detection was linear over a large range of DNA concentrations. The lower limits of detection were 1-50 CFU depending on the species. There were no cross-reactivities with heterologous DNA of bacterial strains tested. Both in culture and PCR, *Pg* and *Pm* were significantly more prevalent in patients than in healthy controls. The mean CFU of target bacteria were all higher in periodontitis patients (for *Pm* 2.4E+07 by PCR and 6.9E+06 by culture; for *Pg* 4.6E+07 by PCR and 2.5E+07 CFU by culture) compared to healthy persons (for *Pm* 5.0E+04 by PCR and 1.2E+04 CFU by culture; for *Pg* 6.25E+02 by PCR and 0 CFU by culture).
Conclusions: Real-time PCR results show a high resemblance with anaerobic culture with respect to relative species concentrations and actual CFU quantities. This indicates that the PCR assays developed are useful for rapid, species-specific, quantitative identification of oral bacteria real-time PCR may develop to a reliable alternative for diagnostic anaerobe culture.

K04
Identification of specific markers for *Campylobacter fetus* subspecies by amplified fragment length polymorphism

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Campylobacter fetus can be divided into the two subspecies *C. fetus* subspecies *fetus* (*Cff*) and *C. fetus* subspecies *venerealis* (*Cfv*). *Cff* can cause sporadic infections in humans, abortion in cattle and sheep, and can be isolated from humans and several animal species. *Cfv* is the causing agent of bovine genital campylobacteriosis, and can be isolated from the genital tract of cattle. Subspeciation of the two subspecies is important for both clinical and economical reasons. However, phenotypic subtyping of *C. fetus*, which is based on glycine-tolerance, is difficult and can give ambiguous results. Several molecular typing methods have been used for subspeciation, including amplified fragment length polymorphism (AFLP). This method has been proven to be a useful tool for subspeciation of *C. fetus*. Using AFLP, 3 separate groups were observed, namely 1) *Cff*, 2) *Cff* differing from the first group in 1 *Hind*III/*Hha*I band and 3) *Cfv*. The aim of the present study was to identify specific markers for both *Cff* and *Cfv* to be used for diagnostics and pathogenesis studies. Four pools of *C. fetus* strains were composed, each covering a phenotypic or genotypic distinct group. Groups 1, 2 and 3 consisted of the strains mentioned before, whereas group 4 consisted of *Cff* strains that were biochemically aberrant. The four groups were examined by 22 *Dde*I/*Mbo*I primer combinations. Different numbers of unique markers were identified for each group. Amongst them, 6 *Cff* specific and 57 *Cfv* specific AFLP fragments could be deduced and were sequenced, resulting in 56 sequences of interest that were analysed. Homology with known sequences was found for 19/56 sequences, whereas 37/56 showed open reading frames only. Based on the sequences obtained, *Cfv* specific PCRs were developed and tested against a well defined set of *C. fetus* strains. At least 2 PCRs turned out to be useful for subspeciation of *C. fetus*. We conclude that: 1) It is possible to identify species specific markers for closely related subspecies of *C. fetus* by AFLP. 2) Markers specific for *Cfv* and *Cff* were detected. 3) *Cfv* specific PCRs have been developed.

K05
Micro-arrays for the detection of the abundance and distribution of pathogenic protozoa

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Wastewater effluents are used for agricultural irrigation, or released directly on surface waters, the presence of microbial eukaryotes e.g. *Cryptosporidium parvum* and *Giardia intestinalis* might be a serious risk for human health. A rapid detection of these pathogens is therefore of interest for public health control. For the purpose of simultaneous detection of different pathogenic protozoa we chose a combination of PCR and DNA arraying technology.
To obtain DNA *Cryptosporidium sp*. Oocysts and *Giardia sp*. Cysts were disrupted and DNA was isolated and concentrated using a 'Soil DNA isolation kit' (MoBio, USA). Primers to amplify the 18S RNA gene as well as oligo-nucleotide probes were designed via ARB. Colorimetric assays (alkaline phosphatase, NCIP, NBT) were used to check the specificity of DIG labelled probes via slot blot analysis. Amino labelled probes were arrayed on commercial epoxy activated microscopic glass slides. PCR products were fluorescently labelled (Cy5) using a DecaLabel DNA labelling kit (MBI, Germany). Hybridisation results were obtained using a commercial fluorescence slide scanner.
In all cases it was possible to obtain DNA from several *Cryptosporidium* and *Giardia* species. From the 43 primer pairs designed via ARB or taken from literature a majority proved successful in amplifying a major part of the 18S RNA gene of species representing the phylogenetic trees of *Cryptosporidium* and *Giardia*. Low efficiency primers were rejected resulting in 8 useful primer pairs. 280 probes were designed against sub-branches and single species of the *Cryptosporidium* and *Giardia* trees, 25 were selected for slot blot testing covering the pathologically important members. Testing was achieved with positive and negative controls at varying temperatures sorting out unspecific probes and resulting in at least one working probe for each part of the trees. Epoxy activated slides in combination with a marker strategy proved to be best approach for the micro array approach. It could be shown that the combination of PCR and micro array detection can be a powerful tool for the parallel detection of pathogenic protozoa. The experiments will be extended to *Naegleria, Acanthamoeba* and *Entamoeba* species.

K06
Detection of mycobacteria with real-time PCR in children with cervical lymphadenitis

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Introduction: To determine the optimal treatment (surgery or antimicrobial agents) for mycobacterial cervical lymphadenitis in children, a multi*centre*-trial (CHIMED-study) is performed in the Netherlands. Since a rapid diagnosis is required to include patients, a real-time PCR was developed for detection of mycobacteria in fine-needle aspirates or biopsies from affected lymphnodes.
Methods: In total, 67 patients with a suspected diagnosis of mycobacterial disease based on positive skin tests were included. A control group consisted of 50 patients with lymphadenitis caused by *Bartonella* spp. Molecular beacons and Taqman probes were designed in a 175-bp amplicon of the ITS region between the 16S and the 23S gene to recognize the genus *Mycobacterium* and species *M. avium* and *M. tuberculosis*.
Results: The sensitivity of the real-time PCRs was 28 CFU in pus and 2.8 CFU in water. No cross-reactivity was observed with 38 other bacterial genera tested. Of 67 patients included, 31 patients yielded a positive auramine-staining, 27 were culture positive, and 48 had a positive real-time PCR. No positive real-time PCR results were obtained in the control group. Referring to the skin test as golden standard, the real-time PCR had a sensitivity of 72%, a specificity of 100%, a NPV of 73% and a PPV of 100%. The results of real-time PCR showed 38 *M. avium*, 1 *M. tuberculosis* and 8 *Mycobacterium* sp. Sequencing of the produced amplicon in this last group revealed *M. haemophilum* in all 8 cases.
Conclusions: 1) Real-time PCR for identification of mycobacteria in samples of patients with lymphadenitis

is a rapid and specific test. 2) In this study, real-time PCR resulted in 20% more positive diagnoses than obtained with conventional staining and culture techniques. 3) *M. haemophilum* appears to be the second important mycobact erium species as a cause of mycobacterial lymphadenitis.

L01/02/03
Abstract not available

L04/05/06
Genome analysis of environmentally relevant marine bacteria - lessons from the *Pirellula* sp. strain 1 genome
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The technological power of high-throughput sequencing has revolutionized our capabilities to examine the genetic complexity of organisms at the whole genome level. Within the last years more than 150 microbial genomes have been successfully sequenced and many more are currently in progress. Nevertheless a closer look at the origin of these organisms reveal that the majority is of medical or biotechnological interest and environmentally important organisms have not been targeted so far. Since the oceans cover 70% of the Earth's surface and contain an extraordinary diversity of life the Department of Molecular Ecology at the Max Planck Institute for Marine Microbiology initiated a marine environmental genomics initiative – the real environmental genomics-project (REGX) in the year 2000. This project aims at the understanding of the adaptations of marine bacteria to changing environmental conditions. Two sulphate-reducing bacteria (SRB) (*Desulfobacterium autotrophicum, Desulfotalea psychrophila)* and one Planctomycete (*Pirellula* sp. strain 1) were selected for whole genome sequencing, annotation and functional analysis.
In marine habitats *Planctomycetes* were shown as abundant members, involved in important transformations in the global C- and N-cycles. With 7.145 Mb *Pirellula* sp. strain 1 ('Rhodopirellula baltica') has one of the largest circular bacterial genomes sequenced so far. The annotation process identified the standard pathways for heterotrophic bacteria like glycolysis, citrate cycle and oxidative phosphorylation. *Pirellula* sp. strain 1 lacks the glyoxylate bypass and the Entner-Doudoroff pathway but exhibits the pentose phosphate cycle. Unexpected for an aerobic heterotrophic bacterium was the presence of all genes for heterolactic acid fermentation, key genes for the interconversion of C1-compounds and 110 sulphates. With the now available blueprint of life for *Pirellula* sp. strain 1 we were able to predict a certain live-style for this fascinating organism.

L07/08/09
Listeria host pathogen interaction: insights from analysis of evolution and adaptation
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Listeria monocytogenes, an environmental bacterium, is the causative agent of food-borne listeriosis. The clinical features of listeriosis include septicemia, meningitis, abortion, perinatal infections and gastroenteritis. *L. monocytogenes* infects a broad range of vertebrates including mammals, birds and amphibians. *L. ivanovii*, the second pathogenic *Listeria* species has a host-specificity for ruminants. Interestingly, not all strains of *L. monocytogenes* seem to be equally capable of causing disease in humans, as serovar 4b isolates are responsible of all major food-borne outbreaks of listeriosis, and of the majority of sporadic cases.
Evolution within the genus *Listeria* was tracked by different means to understand the specificity of strains causing disease. After sequencing the genome of the laboratory strain EGDe responsible of listeriosis in a laboratory animal house and of an isolate of the closely related non-pathogenic species *L. innocua* we have recently also determined the genome sequence of a *L. monocytogenes* serovar 4b strain responsible of an epidemic event in France and of a *L. ivanovii* strain responsible of several cases of abortion in a yew heard in Spain. Genome comparisons aid for a better characterization of functions required for intracellular multiplication and traits related to the specific epidemiologic feature of each strain. Based on the comparison of *Listeria* sequences we constructed a focused DNA-array, which was used to characterize 113 *Listeria* strains. The correlation of genomic, phylogenetic and epidemiological properties of the strains allowed to identify linage specific marker genes which might be related to the different disease potential.
Listeria monocytogenes according to its different life style in the environment or in interaction with the host will adapt by changing its gene expression program. Using DNA-arrays, we have investigated the effect of the disruption of several regulators in order to evaluate their role in host pathogen interaction. In particular we have characterized the PrfA regulon in two different backgrounds (EGDe, and P14*prfA** a sv 4b strain carrying a constitutively active PrfA variant).
Combination of these evolutionary and physiological analyses allow to draw a global picture of host pathogen interactions for *L. monocytogenes*.

L10/11/12
Analyzing the metabolome of *Trichoderma reesei*
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Metabolomics, the non-biased determination and analysis of the complete metabolite profile of an organism, is an emerging powerful tool to better understand microbial physiology and facilitate metabolic pathway engineering to increase the production of specific proteins and fine chemicals by industrial organisms.
We have developed a very robust and inert metabolomics platform. The detection limit of the analytical methods

that it is comprised out of, is between 0.005 and 0.15 ug/ml depending on the compound analyzed, and the RSD <10% and generally <4% for the more stable metabolites. The (micro)biological and analytical methods have been extensively validated to allow the analysis of metabolome samples that are identical to the metabolome of the cells at the moment when they are harvested ('snapshot'), i.e. preventing the introduction, removal or occurrence of (biotic or a-biotic) changes in the metabolites as present in the sample. To this end, quenching and extraction methods have been set up and validated, and the inertness of the different steps in the procedure have been validated. The biological and analytical reproducibility were found to be very good. These comprehensive methods together allow the detection of 96% of the commercially available *Bacillus subtilis* metabolites, a micro-organism used as a model organism. As the physical properties of metabolites were the basis for setting up the holistic analytical methods, the metabolomics platform could also successfully be applied for studying the metabolomes of *Trichoderma reesei*.

We have applied the metabolomics platform to elucidate the regulation of cellulase production in hyper-secreting variants of the filamentous fungus, *Trichoderma reesei*. Samples of mycelia were collected under inducing and non-inducing fermentation conditions, quenched to halt cellular metabolism, collected and processed. We applied pattern recognition analysis to establish the complete metabolite profiles, and identify the metabolites correlating to cellulase induction and maximum production rate.

M01/02
M03/04
M05/06
Abstracts not available

N01

An outbreak of Lymphogranuloma Venereum proctitis among homosexual males in the Netherlands

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Introduction: Recently, we encountered a solitary case of classical LGV (Sex Transm Infect 2003;79:453-5). A few weeks later, a second case of LGV presented with proctitis. Here we report on our outbreak investigation.

Methods: Antibodies to *C. trachomatis* were determined using a peptide EIA. *C. trachomatis*-positive specimens were genotyped by PCR-RFLP of the MOMP gene. The index patient and all first ring contacts were asked to inform as many contacts as possible. Fifteen patients included in the study were examined by the venereologist, 12 by the gastroenterologist and 2 by the radiologist.

Results: High IgG and IgA titres to *C. trachomatis* were found in 13 patients. PCR was positive in rectal specimens of 12 patients, 8 were confirmed to be serovar L2. The gastro-intestinal symptoms included mucous or purulent discharge in 11, constipation in 10 and blood loss in 8 patients. Twelve men were HIV positive, 5 received HAART. Proctoscopy revealed mucous or purulent exudates in 9, ulcers in 3, blood in 2, and erythematous mucosa in 2 patients. In 9 patients sigmoidoscopy showed proctitis with well demarkated ulceration and a very friable mucosa with easy bleeding. Biopsies demonstrated a chronic and non-specific inflammation, characterized by a granulocytic and lymphohistiocytic infiltrate in the lamina propria, consisting of granulocytes, lymphocytes, plasma cells and histiocytes. Diffuse mucosal wall thickening with submucosal oedema, perirectal infiltration and adenopathy was identified on MRI images of 2 patients. During the study, one patient also seroconverted for HCV. Contacts were reported from the Netherlands (36), Germany (11), Belgium (4), United Kingdom (2) and France (1). Most encounters were at gay parties and gay clubs.

Conclusion: We have revealed a sexual network among homosexual men with a high prevalence of LGV proctitis. In many cases large ulcers were present. Considering these ulcers and the sexual techniques practiced, the risk of transmission of other STIs (like HIV) and blood borne infections (like HCV) appears to be increased. Recognition of this clinical entity is important to advise the appropriate antibiotic therapy (21 days doxycycline twice daily 100 mg).

N02

A case of severe skin infection caused by a panton valentine leucocidin (PVL) producing *Staphylococcus aureus*

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Case: A previously healthy 33-year-old woman presented to the emergency room with an abscess. A painful furuncle had developed above the right scapula, which progressed into a large abscess in just a few days (see picture). Two similar lesions had also developed on the right knee and left inguinal region.

Cultures were taken, the patient was admitted and flucloxacillin intravenously was started. The patient underwent surgery, during which debridement was preformed. The lesions in the knee and inguinal region were drained. *Staphylococcus aureus* was cultured from her wounds. MecA could not be detected. A PCR specific for PVL-genes was performed as previously described (CID 1999;29:1128). PVL-genes were detected in her *S. aureus* isolate. After surgery her further clinical course was unremarkable and she was discharged in good health.

Diagnosis: severe furunculosis caused by a PVL producing *S. aureus*.

Discussion: PVL belongs to the family of synergohymenotropic toxins, which attack the membranes of host defence cells. PVL is produced by approximately 10% of *S. aureus* isolates in the Netherlands (national MRSA-surveillance, period 2000-2003). PVL producing *S. aureus* are usually associated with furunculosis, and occasionally with *S. aureus* pneumonia. More research is needed whether

PVL is associated with other severe *S. aureus* infections, such as mediastinitis following cardiac surgery.

No3

Fever and lymphadenopathy associated with HHV-8 infection in a HIV-positive patient

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A 42-year-old Zambian woman was admitted to our hospital because of recurrent episodes of fever, myalgia, malaise and fatigue. She is known to be HIV-positive since 1998 and is successfully treated with epivir 300 mg/day, efavirenz 400 mg/day and tenofovir 250 mg/day (HIV-RNA <50 copies/mL, CD4 cells 700 x 10^6/L). In the previous 5 months she had 5 episodes of fever, which resolved spontaneously in 14 days. Physical examination revealed a rectal temperature of 40°C and several enlarged cervical and axillair lymph nodes (maximum diameter 3 cm) and a palpable spleen.

Laboratory tests showed: ESR 123 mm, CRP 322 mg/L, Hb 5.1 mmol/L, leukocytes 8.7 x 10^9/L, thrombocytes 48 x 10^9/L, LDH 913 U/L. CD4 cell count had dropped to 450 x 10^6/L. Repeated blood cultures remained negative. Cultures, serology and specific tests for several infectious causes (EBV, CMV, *Mycobacterium* spp, *Bartonella* spp, *Brucella* spp, *Toxoplasma gondii*, lues, malaria, trypanosomiasis among others) were negative. Chest radiography showed bilateral shadowing suggesting interstitial infiltrates. CT-scan of the abdomen confirmed splenomegaly (20 x 6 cm) and revealed enlarged abdominal and retroperitoneal lymph nodes.

Lymph node biopsy revealed a polyclonal plasmocytic infiltration with an intact germinal centre. Castleman's disease was suspected and subsequently additional staining for human herpes virus 8 (HHV8) was performed that was positive. Furthermore HHV8-load in plasma and PBMCs was measured (respectively 1.2 and 3.0 x 10^6 copies/mL).

We concluded multicentric Castleman's disease (MCD) due to HHV8-infection in a HIV-infected patient. Treatment has not been standardized: both antiviral therapy and (combination with) chemotherapeutic agents have been advocated. Currently, she undergoes chemotherapeutic treatment (doxorubicin). The antiretroviral therapy was altered by introducing a protease-inhibiting agent. At present she suffers no more episodes of fever, dyspnoea or fatigue. HHV8 levels are monitored on a regular base during therapy.

No4

Successful treatment of pulmonary mucormycosis and nocardiosis after near-drowning

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Introduction: Infections with *Rhizopus sp.* or *Nocardia sp.* after near-drowning so far never have been reported. We describe a case in which these pathogens were sequentially isolated from bronchoalveolar lavage fluid (BAL) specimens.

Methods: A 22-year old man was admitted to our ICU because of near-drowning due to a car accident and developed pneumonia for which he had to be ventilated. He was initially treated with penicillin and gentamicin. Identification of *Rhizopus sp.* was done by microscopy and of *Nocardia sp.* was done by PCR and sequencing of the 16SrRNA gene. Susceptibility testing was done by E-test.

Results: The patient showed progressive consolidations in both lungs after 48 hours. Sputum cultures revealed *Aeromonas hydrophila* and A. sobria on day 3 after admission, and gentamicin was substituted by ciprofloxacin. At day 8 after admission, the patient developed cavitary lesions. From a BAL specimen on day 9, a *Rhizopus sp.* was grown together with *Aspergillus fumigatus* and several multiresistant Gram-negative rods. Liposomal amfotericin B was added to the treatment and continued for six weeks. Different colonies of branched Gram-positive rods were grown from sputum and BAL specimens obtained on day 16. One of these was identified as belonging to the *Nocardia asteroides* group, sensitive to meropenem, amikacin and co-trimoxazole. The other belonged to the N. farcinica group and was moderately sensitive to meropenem (MIC = 2 µg/ml) and sensitive to amikacin and co-trimoxazole. Initial treatment of these pathogens was meropenem and amikacin, followed by long-term treatment with co-trimoxazole. There were no extrapulmonary sequelae. After two months of treatment the patient was discharged in a satisfying clinical condition.

Conclusions: *Rhizopus sp.* and various *Nocardia sp.* can cause late infections after near-drowning. Early recognition can result in a successful treatment.

No5

Methicillin-resistant *Staphylococcus aureus*: a zoonosis?

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of human infections worldwide. In contrast, there are few reports on infections of animals with MRSA. In 2003, we reported the first isolation of MRSA from animal origin in the Netherlands. This MRSA was cultured from an infected wound in a Dutch dog that underwent surgery abroad. Here we present a case of human-to-animal transmission of an MRSA. Four years earlier, an MRSA was cultured from the urine of a patient in a Dutch nursing home, which is part of a large hospital. This strain (RIVM cluster 35) has reoccurred and spread since then. More than 40 people were identified as MRSA carrier. A nurse was identified as an MRSA-carrier by screening of all patients and nurses of the nursing home during an outbreak with this strain. All carriers were treated for eradication of MRSA

carriership. The nurse initially became MRSA-negative, but later on converted to a carrier again. When screening her environment, we found that she had spread this MRSA strain to her baby and dog, but not to her husband. Pulsed-field gel electrophoresis (PFGE) showed that both MRSAs from the dog and its owner had indistinguishable patterns and that it belonged to RIVM cluster 35, an epidemic human MRSA cluster. Typing of the Staphylococcal Chromosome Cassette *mec* (SCC*mec*) region of both MRSAs revealed that these regions seemed to be identical (no loci except *mecA* and no *ccrA/B*-genes were found). After treatment with doxycycline and rifampicin the dog's MRSA carriership finally was eradicated. In conclusion, MRSA infections have zoonotic potential and investigations aimed at tracing MRSA carriers among contacts should include pet animals.

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R01/02
Oseltamivir reduces transmission, morbidity and mortality of highly pathogenic avian influenza in chickens

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The effect of antivirals on the course and the transmission of infection of chickens with highly pathogenic avian influenza (HPAI) virus was studied as a model for systemic infection in humans. Three groups of ten chickens were either untreated, or treated with the neuraminidase inhibitors zanamivir (intratracheally, 1 mg/kg) or oseltamivir (orally, 120 mg/kg). Per group, five chickens intratracheally inoculated with HPAI A/Chicken/Pennsylvania/1370/83 H5N2 virus, were placed one day post inoculation (p.i.) in one cage with five contact chickens. Inoculated and contact chickens were treated twice daily from one day before inoculation up to day 7 p.i. Then inoculated and contact chickens were separated. The infection chain was monitored daily up to day 8 and at day 14 p.i. by culturing trachea and cloaca samples. All untreated inoculated and contact chickens became infected as shown by positive trachea and cloaca samples. Four of five inoculated, and two of five contact chickens died. Similarly, all of the zanamivir-treated inoculated and contact chickens became infected. Nine had positive trachea samples, and two inoculated and two contact chickens also had positive cloaca samples. All inoculated and four of five contact chickens died. Obviously, locally active zanamivir has no effect. In contrast, although oseltamivir could not prevent tracheal infection of the inoculated chickens, none had positive cloaca samples and only one chicken died. More important, none of the five oseltamivir-treated contact chickens became positive within the treatment period, and none died. After stopping treatment three contact chickens became positive, suggesting limited transmission within or after the treatment period. In conclusion, treatment with systemically active oseltamivir limits to a large extent a severe outcome and chicken-to-chicken transmission of HPAI virus. In addition, our results suggest that oseltamivir might prevent chicken-to-human transmission as occurred recently during the HPAI outbreak with A/H7N7 virus in the Netherlands.

R03
Pre-emptive treatment with rituximab efficiently prevents EBV lymphoproliferative disease (EBV-LPD) in pediatric allogenic stem cell transplantation

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Introduction: EBV reactivation is frequently observed following allogenic stem cell transplantation (SCT). EBV reactivation may proceed to EBV-LPD which is difficult to treat and often fatal. We have previously reported that quantification of EBV-DNA load in plasma is a reliable tool to recognize EBV reactivation in still asymptomatic patients. Using this approach a window-phase is created in which pre-emptive intervention may be more effective compared to therapeutic intervention in a situation of established clinical disease.
Methods: EBV reactivation was prospectively studied in a cohort of 24 pediatric alloSCT recipients by RQ-PCR on weekly plasma samples during 4-6 months postSCT. Based on our previous retrospective study, intervention with a single infusion of CD20 antibodies (rituximab; 375 mg/m^2) was started if EBV DNA load exceeded 1000 cp/ml in two consecutive samples. A second infusion was given in non-responders. Cellular immune reconstitution was analysed by measuring lymphocyte subsets and tetrameric HLA-EBV-peptide complexes during the EBV reactivation episode.
Results: Pre-emptive treatment with rituximab was administered in 8/25 patients. None of these patients developed EBV-LPD. A rapid decrease in EBV-DNA load was seen in 6/8 patients. In 2/8 patients EBV-DNA load declined after a second infusion of rituximab. In 5/8 patients an increase in CD8+ T-cells was observed during the EBV reactivation. The EBV specificity of these CD8+ cells are currently under study. Rituximab treatment resulted in a B lymphopenia that persisted 4-6 months. All patients received IVIG substitution. No treatment-related toxicity was observed.
Conclusions: EBV-LPD in allo-SCT recipients can be

effectively prevented by EBV-DNA load guided pre-emptive therapy with rituximab. The concurrent recovery of CD8+ T cells in some of these patients suggests that this immune response per se may be sufficient to control EBV reactivation. The combined analysis of EBV-DNA load and (EBV-specific) T-cell reconstitution will more accurately define patients at high risk for EBV-LPD. This strategy will now be used to further optimise identification of patients that require pre-emptive therapy.

R04

Immunisation strategy against cervical cancer involving a Semliki Forest virus vector expressing Human papillomavirus 16 E6 and E7

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Introduction: In over 99% of all cervical carcinomas, Human papillomavirus (HPV) DNA can be detected. The transforming potential of these viruses depends on the sustained expression of the early viral genes E6 and E7. In women with cervical cancer, HPV-specific cytotoxic T lymphocyte (CTL) activity is generally low, suggesting a certain degree of immunological tolerance for the HPV-derived antigens. Effective immunisation strategies should, therefore, induce very potent immune responses to overcome this tolerance. For this purpose, we use recombinant Semliki Forest virus vecor expressing HPV 16 E6 and E7 (SFVeE6,7). In the present study, we determined whether SFVeE6,7 is strong enough to break immune tolerance in HPV transgenic mice, tolerant to E6 and E7.
Methods: HPV transgenic and wild-type mice were immunised and boosted with SFVeE6,7 particles with or without SFV expressing interleuking 12 (SFV-IL12). Specific precursor CTLs (pCTLs) induced upon immunisation were determined by tetramer FACS analysis. Specific CTL activity was measured by standard ^{51}Chromium-release assay using HPV-transformed tumour cells as targets. Finally, tumour treatment experiments were performed.
Results: Immunisation of HPV transgenic mice with SFVeE6,7 resulted in the induction of HPV-specific CTLs and thus is able to break immune tolerance in these mice. Moreover tumour outgrowth in transgenic mice was delayed upon immunisation with SFVeE6,7. In both wild-type and transgenic mice, co-administration of SFV-IL12 resulted in a dose-dependent increase of HPV-specific pCTLs and CTL responses.
Conclusions: 1) SFVeE6,7 immunisation resulted in a very strong immune response, as demonstrated by the fact that upon immunisation immune tolerance is broken in a stringent HPV-transgenic mouse model. 2) Co-administration of SFV-IL12 further enhanced CTL induction in both wild-type and transgenic mice. 3) Thus, immunisation with SFVeE6,7 seems very promising for the treatment of HPV-induced (pre)malignant cervical lesions.

R05

Anaerobic incubation of metronidazole-resistant *Helicobacter pylori*: understanding the mechanism of metronidazole resistance

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Introduction: Many *Helicobacter pylori* isolates are resistant to the antibiotic metronidazole, and this resistance affects the efficacy of metronidazole-containing regimens, but surprisingly does not make them completely ineffective. This may be explained by changes in oxygen pressure during infection, as metronidazole-resistant (MtzR) isolates become metronidazole-susceptible after anaerobic incubation. This has been tested by varying the duration of anaerobic incubation required for this reversibility of resistance.
Methods: Eight *H. pylori* MtzR clinical isolates and ATCC reference strain 43504 were incubated under anaerobic conditions with or without metronidazole. At different time intervals the minimal inhibitory concentration (MIC) of metronidazole was determined both in the presence and absence of the bacterial protein synthesis inhibitor chloramphenicol.
Results: Neither incubation with metronidazole, chloramphenicol nor anaerobic growth conditions alone affected the viability of the isolates. For all MtzR isolates, the MICs of metronidazole decreased 10-100 fold after 4 hours of anaerobic incubation. This loss of resistance could not be inhibited by chloramphenicol.
Conclusions: Under anaerobic conditions, MtzR isolates were killed in the presence of metronidazole, but the enzymes required for the activation of metronidazole were already present under microaerophilic conditions. During infection in the somewhat anaerobic gastric mucus, this might result in the activation of metronidazole, and may explain why metronidazole-containing regimens are still of therapeutic value in patients infected with a MtzR isolate.

R06

Analysis from 2208 patients newly diagnosed with HIV from 19 countries show that 10% carry primary drug resistance: the CATCH-study

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Introduction: The reported frequency of transmitted drug resistance varies widely. The CATCH-study (Combined Analysis of resistance Transmission over time of Chronically and acute infected HIV patients in Europe) combines results from antiretroviral naïve patients from 19 European countries with the aim of assessing the prevalence of primary

drug resistance. The CATCH-study is the largest of its kind to date.
Methods: Reverse transcriptase (RT) and protease sequences from 2208 newly diagnosed patients were collected. The prevalence of resistance was assessed over the period 1996-2002 based on the International AIDS Society resistance table (May-June 2002). Genotypic resistance profiles were interpreted using RetroGram and the Stanford drug resistance algorithm.
Results: Primary drug resistant mutations were detected in 10.4% (95% CI: 9.1-11.7%) of antiretroviral-naive patients; 7.6% (6.5-8.7%) of isolates were resistant to nucleoside RT inhibitors (NRTI); 2.9% (2.2-3.6%) of cases were resistant to non-nucleoside RT inhibitors (NNRTI); and resistance to protease inhibitors (PI) was seen in 2.5% (1.8-3.1%) of patients. Reduced susceptibility for NNRTI was predicted in 36% of the patients carrying resistant viruses. For NRTI it ranged from 18% for lamivudine to 65% for zidovudine, and for PI from 11% for boosted saquinavir to 23% for nelfinavir. The prevalence of primary drug resistance mutations among patients with seroconversion in the previous year was 12.4% vs 8.7% in patients who have been infected for over a year; OR: 1.5 (1.1-2.1). The prevalence of resistance was much higher in subtype B (12.9%) than in non-B subtypes (4.8%); OR: 3.0 (2.0-4.4).
Conclusions: We found a prevalence of primary drug resistance of 10%. Drug resistance was predominantly found among patients infected with subtype B. Since there was only a small difference in the prevalence of drug resistant mutations between patients infected for a duration longer or shorter than one year, it seems that mutations frequently persist over time. Baseline sequencing should be considered in newly diagnosed patients who became infected in Europe.

S01
S02
S03/04/05
Abstracts not available

T01
Polymorphisms in the innate immunity genes are associated with the susceptibility to periodontitis

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Periodontitis is a complex infectious disease of the supporting tissues of the teeth. It affects 30% of the adult population, with 10-15% exhibiting severe forms of the disease. The disease is associated with Gram-negative bacteria *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. Smoking contributes significantly to disease severity. Differences in the susceptibility to and severity of infection between subjects infected with the same pathogen are probably the result of genetic variation of the host. The genes involved in the innate immune recognition and the regulation of the subsequent proinflammatory cytokine release may play an important role in pathogenesis of periodontitis. In this study we report on gene polymorphisms involved in the regulation of the innate immune response: *CD14* -260 (previously -159), *TLR4* 299 and 399 and *CARD15* 3020insC, and correlate them with bacterial infection and smoking in patients with periodontitis.
DNA samples were collected from 104 adult patients with severe periodontitis and 97 periodontally healthy controls. All subjects were unrelated Dutch Caucasians. The presence of the two major periodontal pathogens, *P. gingivalis* and *A. actinomycetemcomitans,* was assessed by standard culture techniques. *CD14* -260C>T, *TLR4* 299Asp>Gly and 399Thr>Ile, and *CARD15* 3020insC gene polymorphisms were determinated by PCR-based RFLP analysis.
Frequencies of the rare alleles for *TLR4* and *CARD15* 3020insC were comparable in patients and controls (5.5% and 5.1%; 2.5% and 2.6%, respectively). *CD14*-260 allele T was found more frequently in patients ($p<0.0001$, OR: 1.45, 95% CI: 1.2-1.7). Logistic regression analysis taking into account smoking, gender and age showed a significant association for the *CD14* -260 T/T genotype in patients (55.0%) and controls (23.2%) without *P. gingivalis* and *A. actinomycetemcomitans* ($p=0.008$, OR: 3.4, 95% CI: 1.4-8.3). We conclude that the *CD14* -260T allele contributes to the susceptibility to periodontitis in Dutch Caucasians. The homozygosity of this polymorphism is associated with periodontitis in patients without detectable *P. gingivalis* and *A. actinomycetemcomitans*.

T02
Urease activity in *Helicobacter hepaticus* is nickel-responsive, but acid-independent

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Introduction: Members of the genus Helicobacter are important pathogens of man and animals. One of the essential virulence factors of several Helicobacter species is the urease enzyme. The gastric pathogen *H. pylori* responds to acid shocks by a rapid activation of urease apo-enzyme, whereas growth in acidic conditions leads to increased urease gene transcription. Both these responses depend on the urease cofactor nickel. The aim of this study was to investigate the urease regulation in non-gastric urease positive Helico bacter species, using the murine enterohepatic pathogen *H. hepaticus* as a model.
Methods: *H. hepaticus* ATCC51449 was grown in Brucella broth supplemented with 10% bovine serum. Growth was monitored by OD600. Growth media were supplemented with NiCl2 ranging from 0-100 uM and medium pH was adjusted with HCl. Urease activity was determined by a colorimetric assay. Protein expression was monitored by SDS-PAGE.
Results: Urease activity of *H. hepaticus* was lower than that of *H. pylori*, both in standard and nickel-supplemented media. Acidification of the medium to pH 5.5 did not lead to an increase in urease activity. In contrast, medium supplementation with nickel resulted in a significant increase in urease activity, but this was not accompanied by increased expression of urease enzyme. Nickel-responsive

induction of urease activity was independent of the growth-phase of *H. hepaticus*.
Discussion: Urease activity in *H. hepaticus* seems to be regulated at the enzyme level. This regulation is nickel dependent but pH-independent. However nickel induction takes place only on the enzyme activity level. The absence of a transcriptionally regulated response suggests that *H. hepaticus* is only transiently exposed to low pH, and thus may explain the enterohepatic niche of this murine pathogen.

T03
Identification of polymorphisms in the FCN2 gene encoding the human lectin pathway activator ficolin-2 of innate immunity

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Introduction: Ficolin-2 is a serum protein containing a fibrinogen-like and collagen-like domain that can activate the lectin pathway of complement. The FCN2 gene is located to chromosome 9q34 and contains 8 exons. In both structure and function ficolin-2 bears similarities to the collectin mannose-binding lectin (MBL). To be functional both proteins need a multimeric structure held together by disulfide bonds in the N-terminus. Like the carbohydrate recognition domain of MBL the fibrinogen-like domain of ficolin-2 recognizes carbohydrate patterns on micro-organisms. Subsequently, the collagen-like domain of both proteins can activate MBL-associated serine proteases leading to complement activation. Both proteins also have opsonic properties. This functional resemblance suggests a role for ficolin-2 in innate immunity similar to MBL. Genetic polymorphisms in the MBL gene exon 1 encoding the N-terminus are known to disrupt the multimeric protein structure and thereby affect MBL activity in serum. MBL deficiency as a result of these polymorphisms has been associated with increased susceptibility to several micro-organisms, especially in immunocompromized patients. To study whether such structural polymorphisms affecting multimere formation or complement activation properties are also present in FCN2, we screened exons 1 to 5 of this gene, encoding the 5' untranslated region (5'UTR), a putative signal peptide, the N-terminus and the collagen-like domain for genetic variants.
Methods: To identify polymorphisms in FCN2 we screened exons 1 to 5 in 95 healthy unrelated Caucasians using denaturating gradient gel electrophoresis.
Results: A single nucleotide polymorphism was found in exon 3 (+232 T/C, allele frequency 0.653 and 0.347). This polymorphism does not result in an amino acid substitution. Another SNP was found in the 5'UTR (-4 A/G, 0.724 and 0.276), which precedes the start codon on the mRNA.
Conclusions: These data show that FCN2 is polymorphic, although no structural polymorphisms in the exons encoding the N-terminus and the collagen-like region were found. The polymorphism in the 5'UTR could have consequences for translational control, with a possible effect on biological activity of this protein in innate immunity.

T04
The precence of the *cag* pathogenicity island is associated with increased ROS scavenging by *Helicobacter pylori*

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Helicobacter pylori infection is a risk factor for the development of gastric carcinoma. Reactive oxygen species (ROS) are thought to play an important role in tumour induction, and *H. pylori*-positive patients show increased levels of ROS in the gastric mucosa. *H. pylori* protects itself from the harmful effects of inflammation induced ROS by its ROS scavenging enzymes SOD and catalase. *cag*- positive (*cag*+) *H. pylori* strains induce a stronger inflammatory response than *cag*- negative (*cag*-) strains, and probably require a more active ROS scavenging system. The aim of this study was therefore to compare ROS scavenging capacity between *cag*+ and *cag*- strains.
ROS scavenging activity was determined in the presence of the superoxide-producing xanthine/xanthine oxidase system (XOS) to overnight cultures of *H. pylori*, and superoxide levels were measured on a Bruker electron spin resonance spectrometer in combination with spin-trapping techniques. Decrease in dimethyl-1-pyrroline-N-oxide spin-trapped radical peak surfaces was determined as a measure for ROS scavenging activity. Differences were tested using the Mann Whitney U-test, and considered significant if $p<0.05$.
ROS was only produced when XOS was added to the culture medium, and were characterized by a superoxide radical signal peak surface. Levels of ROS were 68.3 ± 2.3 in medium, and decreased significantly when *cag*- and *cag*+ *H. pylori* strains were added to the medium (47.2 ± 4.0, $p=0.013$ and 33.0 ± 3.8, $p=0.003$, respectively). *cag*+ strains show an higher ROS scavenging capacity than *cag*- strains ($p=0.014$). When *H. pylori* strains were heat or formaldehyde inactivated, no radical scavenging activity was seen.
H. pylori strains actively scavenge ROS, with *cag*+ strains being better radical scavengers than *cag*- strains. This is likely to be an adaptive response of *cag*+ *H. pylori* strains to the increased levels of ROS in the inflamed gastric mucosa, and may play an important role in chronic colonization

T05
Methylation in *Chlamydia trachomatis*: identification of a functional *S*-adenosyl-L-methionine-dependent methyl-transferase encoded by *prmC* (CT024) in *C. trachomatis*

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Methylation of DNA and protein plays an important role in gene expression and activity of enzymes. The reaction is catalyzed by methyl-transferases (MTases) using *S* adenosyl-L-methionine (AdoMet) as methyl donor. The

role of methylation in the regulation of gene expression and protein activity of the obligate intracellular pathogen *Chlamydia trachomatis* is unknown. Genomes of *Chlamydia* spp. apparently lack the homologues of genes encoding well characterized DNA MTases, as well as the gene enabling the synthesis of AdoMet. This raises the question whether methylation events in *Chlamydia* spp. do occur. Recently, PrmC has been demonstrated to act in *E. coli* as an N^5-Glutamine AdoMet dependent MTase of class I peptide release factors (RFs), thereby making PrmC the first N^5-Glutamine MTase identified (Heurgué-Hamard et al EMBO J 2002 (4): 769-78). Using a genetic approach, the putative *prmC* homologue (CT024) of *C. trachomatis* was identified by database searches and functionally analyzed.
Neither RF1 nor RF2 is methylated in *E. coli prmC* knockouts, leading to a global deficiency in termination of translation and hence a deliberated growth. Overexpression of RF2, thereby overwhelming PrmC, also leads to impaired growth. Taking advantage of these phenotypes, potential PrmC function of CT024 was assessed in the genetic background of *E. coli*.
Expression of CT024 in an *E. coli prmC* knockout demonstrated that CT024 restores the growth defect of this strain, suggesting an interaction of CT024 with RFs of *E. coli*. This was substantiated by the observation that overexpression of CT024 suppressed the toxic effect of overproducing *E. coli* RF2. These results together strongly indicate that CT024 encodes a functional PrmC. This makes CT024 the first functionally characterized chlamydial methylase. *In vitro* and *in vivo* studies are now underway to demonstrate methylation of chlamydial RFs by CT024, and to identify the putative chlamydial AdoMet permease.

To6
Three novel complement modulators from *Staphylococcus aureus*

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When staphylococci invade our body they can be destroyed by phagocytes after proper opsonization. Staphylococci lack a thick capsule and their exposed cell wall components are potent complement activators leading to the generation of C5a and deposition of C3b/C3bi. This in turn will attract phagocytes and promote phagocytosis respectively. However Staphylococcus aureus does have a unique set of tools for the counterattack.
S. aureus Pathogenicity Island-5 (SaPI-5), a novel pathogenicity Island located on a phage embedded in the staphylococcal genome encodes three independent complement modulators.
We have cloned, expressed and purified these proteins (*E. coli*) and have studied them in all aspects of innate-immune modulation.
CHIPS (chemotaxis inhibitory protein of staphylococci) is a 14.1 kDa protein that blocks C5a receptors at nanomolar concentration. CHIPS makes neutrophils unresponsive for triggering either chemotaxis or cellular activation to a metabolic burst (J Exp Med in press). CHIPS is also an FPR antagonist. We have pinpointed these two functions to distinct sites within the CHIPS protein.
SAK (staphylokinase) converts human plasminogen to become surface-bound plasmin. This in turn cleaves C3b and C3bi at the staphylococcal surface and is therefore highly anti-opsonic. In addition it cleaves IgG in the hinge region. SAK is a potent inhibitor of phagocytosis.
Lectin pathway inhibitor (LPI) is 9.8 kDa protein and a unique MASP-2 inhibitor, it prevents lectin pathway mediated opsonization specifically as measured by C3b deposition and phagocytosis. All proteins encoded by SaPI-5 turned out to be human-specific, they have very little affinity for mouse (or other) C5aRs, Plasminogen or MASP-2 respectively. They are expressed *in vivo*, since humans have antibodies against these proteins. When acting together, but even as single compounds, they are strongly anti-inflammatory and provide a powerful defence against the human complement system. Furthermore the target-choice and mechanistic strategies that have evolved in these molecules can educate us on the relative importance of different elements in innate immunity. Also these compounds can be considered for anti-inflammatory therapeutic use.

Vo1
Respiration and copper metabolism: checkpoints in morphological development of *Streptomyces*

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Growth conditions were designed that allow complete control over the morphological switch from vegetative to aerial growth in the development of *Streptomyces* without the need for chromosomal mutations. In the absence of Cu2+ (Cu ions removed by the chelator BCDA) development is frozen in the vegetative growth phase but vegetative growth itself is not affected. Upon the addition of Cu2+, development proceeds with aerial growth.
Earlier work has suggested that the key to this copper riddle must be located outside the mycelium. Therefore, the changes in the extracellular proteome as a function of development and the role of Cu-enzymes in development were studied by proteomics. One of the most striking change in the extracellular proteome upon switching to aerial growth is the extensive modification of a number of proteins such as PstS (a phosphate-binding lipoprotein) and GlpQ (glycerolphosphoryl diesterphosphodiesterase). Expression of several proteins is induced by Cu deprivation. A notable example is the strongly elevated expression of a Sco1 homologue. This protein is involved in the maturation of cytochrome *c* oxidase (COX), the respiratory terminal oxidase that contains Cu as co-factor. Sco1 is the copper chaperone of COX and/or acts as a thiol-disulfide oxidoreductase that keeps the cystein ligands of COX available for Cu-binding. Copper starvation triggers the higher expression levels of Sco1 because more chaperone is needed to ensure sufficient maturation of COX. Since vegetative growth is not affected under these conditions, we propose that the absence of mature COX prevents aerial growth. This hypothesis is supported by the strongly reduced COX activity in mycelium stalled in vegetative growth and by the *sco1* knock out strain that displays the predicted phenotype, strongly reduced aerial hyphae formation and very little COX activity.

Alternative respiratory pathways and the potential involvement in development of other Cu enzymes - analysis of the *Streptomyces coelicolor* genome predicts a total of five extracellular Cu enzymes - will be discussed.

V02
Repellents and hydrophobins have a different mode of action

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Repellents of *Ustilago maydis* are cell wall located peptides of 35-53 amino acids that result from cleavage of the precursor protein Rep1 in the endoplasmic reticulum. Repellents were proposed to be analogous to hydrophobins.[1] The best-studied hydrophobin is SC3 of *Schizophyllum commune*. This protein affects cell wall composition, mediates escape of hyphae from an aqueous environment, makes aerial hyphae hydrophobic, and attaches hyphae to hydrophobic surfaces. We here show that repellents do not affect cell wall composition. Levels of water and alkali extractable glucan were almost similar in ΔRep1 and wild-type strains.
In contrast to wild-type strains, ΔRep1 strains did not form aerial hyphae under conditions of high humidity but did produce these hyphae when humidity was low. 80% of the aerial hyphae of the ΔRep1 strains clustered in bundles of 2-6 hyphae. In contrast, most aerial hyphae of the wild-type strains were growing individually. Wetting of the aerial hyphae of the ΔRep1 strains resulted in their collapse, whereas wild-type aerial hyphae were stable. The effect of clustering was not observed in case of wild-type and ΔSC3 strains of *S. commune*.
Hyphae of ΔRep1 strains are not able to grow out of a droplet onto a hydrophobic solid.[1] Addition of the synthetic peptide Rep1-1 partially complemented outgrowth. However, the SC3 hydrophobin of *S. commune* appeared to be more potent to mediate escape of hyphae of ΔRep1 strains from the water droplet. Interestingly, more wild-type hyphae than ΔRep1 hyphae escaped when SC3 was added to the medium.
The results presented show that repellents and hydrophobins have a different mode of action.
Reference: 1. Wösten, et al. EMBO J, 1996;15:4274-81.

V03
Direct detection of methylation in genomic DNA

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The identification of methylated sites on bacterial genomic DNA would be a useful tool to study the three major roles of DNA methylation in prokaryotes: distinction of self and nonself DNA, direction of post replicative mismatch repair, and control of DNA replication and cell cycle. Three types of methylated nucleobases are known: N^6-methyladenine, 5-methylcytosine, and N^4-methylcytosine. The aim of this study was to develop a method to detect all three types of DNA methylation in complete genomic DNA.
It was previously shown that N^6-methyladenine and 5-methylcytosine in plasmid and viral DNA can be detected by intersequence trace comparison of methylated and unmethylated DNA. We extended this method to include N^4-methylcytosine detection in both *in vitro* and *in vivo* methylated DNA. Furthermore, application of intersequence trace comparison to bacterial genomic DNA successfully identified methylated sites. Finally, we showed that intrasequence comparison sufficed to detect methylated sites in genomic DNA.
In conclusion, we present a method to detect all three natural types of DNA methylation total genomic DNA. This provides the possibility to define the complete methylome of a micro-organism.

V04
SC15 of *Schizophyllum commune* mediates formation of aerial hyphae and attachment to hydrophobic surfaces in the absence of SC3

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Formation of aerial hyphae in *S. commune* is used as a model to understand the initial stages of fruiting body formation in basidiomycetes. The SC3 hydrophobin plays a pivotal role in this process. It lowers the surface tension of the medium, enabling aerial growth, and it coats the aerial hyphae rendering them hydrophobic. Moreover, SC3 allows hyphae to attach to hydrophobic surfaces. However, escape of hyphae from the aqueous environment, hydrophobicity of aerial hyphae, and hyphal attachment were not completely abolished in a strain in which the SC3 gene is disrupted (delta *SC3* strain), suggesting that other molecules are also involved in these processes. A 15 kDa protein (SC15) co-purifies with self-assembling SC3. We studied the role of this protein in formation of aerial hyphae and in attachment. SC15 was shown to be secreted into the medium and was also localized in the cell walls of aerial hyphae and the extracellular matrix binding aerial hyphae together. In a strain in which the SC15 gene was deleted, formation of hydrophobic aerial hyphae and hyphal attachment were not affected. In contrast, when *SC15* was deleted in the delta *SC3* background, these processes were almost completely abolished. The absence of aerial hyphae in static liquid cultures of the latter strain is due to the inability to lower the water surface tension of the medium. These data show that SC15 mediates formation of aerial hyphae and attachment in the absence of SC3. A natural isolate obtained from a forest near Wageningen in the Netherlands produced both SC3 and SC15 on minimal medium but only SC15 when grown on wood. From this it is concluded that in nature SC15 may function to enable growth in a non-aqueous environment.

V05

Differential expression of methanogenes in *Methanothermobacter thermautotrophicus*

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Like most methanogenic Archaea, *Methanothermobacter thermautotrophicus* derives its energy for growth from the reduction of carbon dioxide with hydrogen as the electron donor. CO_2 reduction proceeds in a unique pathway consisting of 7 consecutive enzymic steps. Some reactions are brought about by 2 different functional equivalent (iso-)enzymes. Studies in fed-batch systems showed dramatic changes in the transcription levels of most of the 'methanogenes' coding for the methanogenic (iso-)enzymes in response to sudden changes in the hydrogen supply. It has been generally assumed that the shifts in dissolved hydrogen pressure (p_{H_2}) triggers differential gene expression. However, hydrogen concentrations have never been measured in the shift experiments. Moreover, the shifts caused the instantaneous change in growth rates. Studies by us demonstrated that hydrogen changes were accompanied with changes in the energetic parameters as well, in particular intracellular pH. Therefore, it cannot be ruled out that the differential methanogenes expression results from the different factors.
To address the issue of methanogene expression more systematically, *M. thermautotrophicus* was grown in a chemostat at different growth rates, at controlled high (0.2 Bar) and low (0.01 Bar) p_{H_2} values, as well as at neutral pH 7.0 and acidic pH 6.4. Cells collected from the steady state cultures were collected and expression levels of eight methanogenes were quantified by real-time PCR. The analyses showed that p_{H_2} had only a relatively small effect on the expression of most of the genes. However, variation in growth rates played a more prominent role, as did the change in medium pH. Yet, the combination of factors readily explained the expression patterns observed in fed-batch shift experiments. In conclusion, our study suggests that the effect of hydrogen is primarily an indirect one. Hydrogen concentration and supply are determinants for the growth rate and intracellular pH.

V06

False-positive reactivity in *Aspergillus* antigen detection

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Introduction: Galactomannan (GM) can be detected by sandwich ELISA (Platelia *Aspergillus*) where the monoclonal antibody EB-A2 is used as captor and detector. EB-A2 binds the ß(1-5)-linked galactofuranosyl chain of the GM molecule. Circulating GM is an early and specific marker of invasive aspergillosis in adults, but false positive ELISA reactivity is found in up to 83% of neonates. The aim of the study was to find the cause for this false reactivity in neonates.
Methods: The literature was reviewed for micro-organisms that bear epitopes that mimic that of GM. ELISA reactivity was investigated *in vitro* by testing suspensions of the micro-organisms involved including 11 bifidobacterial species and several other Gram-positive and negative bacteria. *In vitro* reactivity was quantified as ELISA OD value correlated to total protein content of the tested cell suspension.
Results: A membrane-associated molecule of *Bifidobacterium bifidum* sp. *pennsylvanicum* mimics the epitope recognized by EB-A2. This lipoteichoic acid (LTA) contains a terminal linear polysaccharide of more than 7 ß(1-5)-linked galactofuranosyl-residues. *Bifidobacterium* sp. are common members of the gut microflora of humans especially neonates, forming up to 91% and 75% of the total microflora in resp. breast-fed and milk formula-fed infants. Cell suspensions of *B. bifidum* sp. *pennsylvanicum* showed reactivity *in vitro*. Also several other bifidobacteria (from human, animal and food) showed cross reactivity in contrast to *B. infantis* and *B. adolescentis*, which have no 1-5 linked galactofuranosyl-residues. Other Gram-positive bacteria known to have LTA without galactose or lacking terminal sugars reacted negative, just as all Gram-negative bacteria.
Conclusions: 1) Bifidobacterial LTA containing galactofuranosyl-residues is a possible cause of false ELISA serum reactivity in neonates by bacterial translocation, i.e. the transmucosal passage of microbes and their by-products. 2) Bifidobacterial LTA in probiotica and diary products can act as a source of ELISA reactivity after gastrointestinal translocation. Moreover, ELISA reactivity of milk products is more likely to come from LTA than from fungal GM originated from bovine aspergillosis.

V07/08/09

Microbial adaptation to soil - what do we know and challenges

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In the last decade enormous progress has been made in moving towards a better understanding of the diversity, structure and functioning of soil microbial communities. These advances have been spurred by the ever-continuing development of novel molecular tools with which microbial communities can be unravelled in greater detail than ever before. At the same time, some of the novel techniques are also revealing their limitations, which affect the way we interpret aspects of soil functioning. Soil microbial communities not only are responsible for large part of the cycling of nutrients, but also are involved in phytopathogen-suppressive processes. In the latter processes, microbial metabolites are often the key. The lecture will show approaches that aim at unravelling the community structure and potential metabolite production of soil microbial communities. Evidence will be presented for the selection of a suite of organisms that are potentially involved in the phytopathogen-suppressiveness of soil. The importance of one microbial metabolite, pyrrolnitrin, will be indicated. Since a large fraction of the soil microbial community is known to be unculturable, the application of approaches

from met6agenomics will also be addressed. From the data obtained, and attempting to link structural and functional data, it is becoming increasingly evident that soil propkaryotes show enormous diversity at the functional level, even within a tightly knit species defined by phylogeny. Hence, a future focus will be to assess how diverse niches in soil drive the adaptation and evolution or organisms belonging to the same species.

P01
Prophage profiling of *Salmonella typhimurium*

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Introduction: An important mechanism in the evolution of bacteria is the acquisition of horizontal transferable genetic elements, like plasmids and prophages. The importance of prophages in *Salmonella*, in relation to virulence characteristics is increasing since genome sequences of several *Salmonella* strains are becoming publicly available. Several phage types within serovar Typhimurium, harbor specific prophages containing virulence associated genes. However such information is lacking about the emerging multiple-antibiotic-resistant *Salmonella* serovar Typhimurium DT104.
Methods: In this work, a genomic subtractive hybridization was performed between *Salmonella* serovar Typhimurium LT2, of which the genome is sequenced, and a DT104 isolate resulting in the identification of 5 possible prophages absent in LT2. The prophages can be annotated using the assembled DNA fragments databases of *Salmonella* serovar Typhimurium DT104, of which the sequence data were produced by the *Salmonella spp.* Sequencing Group at the Sanger Institute and can be obtained from ftp://ftp.sanger.ac.uk/pub/pathogens/Salmonella. Currently the presence of these 5 and other relevant prophages among different *Salmonella* serovar Typhimurium phage types is under investigation using a developed PCR method called prophage profiling.
Results: Prophage profiling resulted in the identification of several groups within serovar Typhimurium. In addition a *Salmonella typhimurium* DT104 unique novel lambdoid prophage containing a novel lipopolysacharide related protein and a probable acid tolerance related transcriptional regulator was identified.
Conclusion: Prophage profiling shows to have potentials for a new molecular-based *Salmonella typhimurium* typing method.

P02
A zoonotic respiratory tract infection

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A 70-year-old man presents with recurrent respiratory tract infections after an episode of pneumonia. Chest X-rays show persistent infiltrates, and bronchiectasis are present on CT. Pulmonary function is normal. Investigations for malignancy and tuberculosis are negative. The patient receives multiple antimicrobial treatments with no effect. Subsequently, *P. multocida* is cultured repeatedly from respiratory specimens. *P. multocida* is a common cause of soft tissue infections following dog and cat bites. It is less known that the second commonest site of human clinical isolates is the respiratory tract. It has been described in cases with sinusitis, bronchitis, pneumonia, lung abscess and empyema, but also as a commensal in the sputum of patients with chronic underlying pulmonary disease. A history of animal exposure is usually positive as was true for the present case.
The chronic productive cough of the patient is probably due to infection with *P. multocida*. However, in the absence of a clinical response to adequate antimicrobial treatment, harmless colonisation with *P. multocida* cannot be ruled out.

P03
Diversity of sulphate-reducing microbes in Dutch riverine floodplains

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During the last decades the river management in the Netherlands has changed from exclusion strategies to the development of more natural riverine systems by removing dams and dikes and allowing the rivers greater access to their floodplains. However, heavily altered hydrological and hydrochemical factors represent important constraints for the sustainable ecological development of typical vegetation types in riverine wetlands. An ongoing project involving areas for water retention and conservation has not led to the intended biodiverse vegetation types. Sulphate-reducing prokaryotes (SRP) are the main producers of the phytotoxic sulphide in sulphate-enriched anaerobic environments, therefore they might play an important role in the restoration process. The aim of this work is to establish whether or not there is a niche differentiation within sulphate-reducing communities inhabiting the river floodplains in the Netherlands. The structure of this microbial guild was investigated in a number of locations with different hydrochemical and hydrological characteristics along the major river systems in the Netherlands. In order to assess the factors that prevent the growth of the desired biodiverse vegetation types, plant species-rich river-influenced areas were compared with species-poor situations. A screening of the sulphate-reducing community was performed by means of a SRP PhyloChip grouping 16S rRNA probes hierarchically targeting all recognized lineages of SRP. The construction of *dsrAB* gene-based clone libraries and a 16S rRNA-based denaturing gradient gel electrophoresis (DGGE) approach were used to confirm the data retrieved from the micro-array assay. The hybridization patterns revealed a high SRP diversity, including members of the Δ-Proteobacteria cluster (genera *Desulfacinum*, *Desulfovirga*, *Desulforhabdus*, *Syntrophobacter*, *Desulfonema*, *Desulfostipes*, *Desulfobacterium*, *Desulfofrigus*, *Desulfofaba*, *Desulfosarcina*, *Desulfomusa*, *Desulfomonile*, *Desulfovibrio*) and low GC Gram-positive bacteria in different soil samples.

P07
Screening for a MRSA that is sensitive for oxacillin in culture

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a treat to hospitals and nursing homes, and major efforts are taken to prevent the spread of this noxious bacterium. The MRSA carry the *mec*A gene that encodes PBP2A protein, a cell wall synthesis enzyme that, in contrast to other PBP's is not inhibited by beta-lactams such as methicillin and oxacillin. Most MRSA strains are multi-resistant and show resistance to several other non-beta-lactam antibiotics. MRSA strains are usually identified by culturing on oxacillin containing plates and a subsequent detection of PBP2A by agglutination. It has been reported that some strains of MRSA are inhibited by oxacillin in culture due to a poor expression of the *mec*A gene. Such 'oxacillin-sensitive' strains will be missed in the MRSA screen. Therefore, we routinely screen all multi-resistant *S. aureus* for the *mec*A gene by PCR. This PCR was performed on the LightCycler and detects in a single run the *mec*A gene and the Martineau gene, a species marker for *S. aureus*. This procedure has two advantages. Firstly, it allows the identification of the 'oxacillin-sensitive' MRSA. Secondly, the method reduces the time of MRSA identification with one or two days, which is of major importance to prevent further spread of the MRSA in or among hospitals. In this report we describe an outbreak of an 'oxacillin-sensitive' MRSA strain in a hospital. This strain carried the *mec*A gene as demonstrated by PCR. The screening of samples from the hospital was a major challenge, since oxacillin could not be used to distinguish the strain from other *S. aureus* or *S. epidermidis* strains. PCR was not suitable, because the large number of strains overwhelmed our PCR facility. It was found that the strain was resistant to gentamycin, an antibiotic that inhibits the growth of most other *S. aureus* or *S. epidermidis* strains. The gentamycin selection reduced the number of strains to be screened by PCR for the final identification of MRSA. The MRSA strain was found to be widely dispersed in the hospital. It was isolated from patients, hospital personnel and equipment from the intensive care unit and two hospital departments.

P08
DNA recovery and PCR detection of a modified BOOM extraction method and three commercial kits for the direct detection of *Salmonella* species in faeces

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Introduction: Previously (NVMM 2003) we showed that direct *Salmonella* PCR detection in faeces was possible in 92% of culture positive samples using a modified BOOM extraction method and a novel *Salmonella* real-time PCR. The aim of this study was to compare four different extraction methods: our modified BOOM method, QIAGEN Stool DNA Mini Kit (QIAGEN), Roche High Pure PCR Template Kit (Roche HPPT) and the automated extractor MagNA Pure LC in combination with Roche MagNA Pure LC DNA III Kit Bacteria (MagNA Pure).
Methods: For the comparison 36 *Salmonella* culture positive and 32 *Salmonella* culture negative faecal samples were randomly selected. Moreover we tested 7 faecal samples that were culture positive but remained PCR negative using the modified BOOM method. DNA for Real-Time PCR detection was extracted from 50% (wt/v) faecal suspensions. The DNA recoveries of the four methods were determined by co-extracting a known amount of *Hin*dIII digested phage lambda (λ*Hin*dIII) DNA from the faecal samples and comparative analysis by gel electrophoresis.
Results: On the PCR analysis of the 36 culture positive faecal samples our modified BOOM method and both Roche methods proved to be slightly better than the QIAGEN method. For the 32 culture negative faecal samples the specificity was 100% for all four methods. No improvement was seen for the 7 culture positive, PCR negative samples with any of the methods. On DNA recovery our modified BOOM method and QIAGEN performed best with an average recovery of more than 80%. Both Roche methods recovered between 50 and 55% of the added λ*Hin*dIII DNA.
Conclusions: 1) Overall our modified BOOM method appears to be the extraction method of choice for the direct PCR detection of *Salmonella* species in faeces. 2) The experience that 8% of the culture positive fecal samples are PCR negative in direct detection remains a challenge.

P09
Detection of mycoplasma in blood samples eight years after the Bijlmer crash

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Introduction: In 1992 an airplane crash occurred in the Bijlmer area in Amsterdam. In the following years people affected by this disaster expressed health complaints. In 2000 a controlled epidemiological study was started to examine long-term health effects among occupationally involved subjects. In this epidemiological study fire fighters, police officers and hangar workers who were occupationally involved in this disaster were compared to reference groups of non-involved colleagues. One of the health outcomes was the detection of *Mycoplasma fermentans (incognito)*.
Methods: Immediately after collection of the blood, white blood cells were isolated and frozen. Automated DNA isolation was performed with the MagNA Pure. Mycoplasma DNA was detected by real-time PCR after thorough validation of the method. Each test included a DNA isolation control and a PCR inhibition control. Two PCRs were used. All samples were tested with a mycoplasma species-specific PCR; positive samples were confirmed with a PCR specific for *M. fermentans* (performed at the National Institute of Public Health and the Environment). In addition, random samples (10% of total sample number) were analyzed by the *M. fermentans*-specific PCR.

Results: A total of 2576 blood samples from occupationally involved and reference workers were collected and analyzed for the presence of mycoplasma DNA. Of these, 2499 were included in the statistical analysis. All samples of occupationally involved and reference fire fighters (n=528), police officers (n=1468) and hangar workers (n=503) were negative, both in the species PCR and, if tested so, also in the *M. fermentans*-specific PCR.
Conclusions: Using this validated technique for Mycoplasma DNA detection not a single blood sample was tested positive for mycoplasma DNA in a large community sample of fire fighters, police officers and hangar workers. Accordingly, health complaints of these workers cannot be attributed to Mycoplasma species.

P10
Phase 1 evaluation of a new, random access serological robot

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Introduction: A new serological robot, employing chemiluminescence technology, became available (Centaur, Bayer). This random access machine features a maximum throughput of 240 tests/hour and refrigerated on-board storage of 30 assays. We tested the performance of the machine in the daily routine of the virology department of our hospital.
Methods: During a discrete period, all samples submitted for HBsAg, anti-HBcore, anti-HBs, anti-HCV, Rubella-IgG and Rubella-IgM testing, were tested in the Centaur and AxSym system (Abbott Labs): 1565 tests were performed on 829 samples.
Results: 1) Operation and maintenance of the Centaur and AxSym machine were without difficulties.
2) HBsAg (409 samples), anti-HBcore (270 samples) and anti-HCV (360 samples) test results:

Centaur:	HBsAg		anti-HB-core		anti-HCV		
	pos	neg	pos	neg	pos/ind		neg
AxSym:	pos	34	3	33	3	9	5
	neg	0	372	0	234	1	345

Additional testing suggests that all samples, positive for HBsAg or anti-HCV only by AxSym (n=8), or positive for anti-HCV only by Centaur (n=1), are false-positive.
3) Quantitative anti-HBs test results (337 samples):

Centaur: anti-HBs				
	<10	10-100	>100 IU/L	
	<10	199	6	0
AxSym	10-100	5	25	1
	>100	0	5	96

Quantitative results as generated by AxSym and Centaur show moderate agreement: 24% of anti-HBs and 25% of anti-Rubella levels show more than two-fold difference. Linear regression confirms poor concordance: the X-coefficient for anti-HBs is 0.81, R2=0.58; the X-coefficient for Rubella is good (1.03), but the R2 is only 0.23.
Conclusions: 1) In a daily routine setting the Centaur performed well. 2) This phase 1 study suggests superior specificity of Centaur serology as compared to AxSym. However, a follow-up study is needed to corroborate this result (eg. to exclude superior sensitivity of AxSym). 3) The interpretation of Centaur test results, as prescribed by the manufacturer, deserves consideration. An 'equivocal' range in anti-HCV testing seems undesirable, as equivocal results must be confirmed like positive test results. A cut-off value of 7.5 IU/L for anti-HBs is contrary to general practice, which considers 10 IU/L or more indicative of presence of anti-HBs antibodies.

P11
Reproducible identification and typing of vegetative *Clostridium* species and spores with amplified fragment length polymorphism

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Typing of *Clostridium* species - especially *Clostridium difficile* - with molecular techniques is still under evaluation. One of the burdens is the inter-test reproducibility, probably due to DNA degradation. We tested the reproducibility of typing with amplified fragment length polymorphism (AFLP).
A total of 26 isolates from 4 different species were used: *C. difficile, C. bifermentans, C .innocuum* and *C. perfringens.* DNA was isolated from fresh cultured bacteria and from spores. Different DNA isolation techniques were used and several AFLP primers were compared. AFLP was carried out on an automated sequencer (ABI3100).
The best DNA isolation procedure in our system was pre-treatment according to Pituch et al. and DNA isolation with a QiaAmp kit. The results did not differ between cultured bacteria and spores. The different *Clostridium* species could be easily identified by AFLP using Pst-0 and Mse-C (cut-off at 40% homology). The reproducibility of the AFLP pattern per strain varied between 93 and 98%.
The AFLP patterns from vegetative bacteria and spores were identical. The AFLP is a promising technique for identifying and typing *Clostridium* species. It therefore may be used in outbreaks of *Clostridium difficile*. Moreover, the level of reproducibility makes the procedure suited for database construction of typing results.

P12
The implementation of *Hepatitis B* vaccination and other follow-up recommendations after accidental exposure to blood borne viruses

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Introduction: To determine whether the recommendations for follow-up that were given by a regional centre for postexposure management 'Prikinfo', to casualties reporting accidental exposure to blood borne viruses, were implemented.
Methods: All reported cases who received advice on postexposure follow-up such as Hepatitis B virus vaccination,

subsequent serology testing for Hepatitis C virus or human immunodeficiency virus (HIV), postexposure prophylaxis (PEP) for HIV, or to check their Hepatitis B virus surface antibody titre (anti-HBs), were contacted. Structured interviews were conducted with 53 responders.
Results: The number of low-risk accidents was 51 (96.2%), the remaining two were high-risk accidents. Accidental blood exposure with a need for follow-up happened in 47 (88.7%) cases outside a hospital setting. Hepatitis B virus vaccination was recommended to 46 casualties and started in 26 (56.6%) cases. The main reasons reported for not starting Hepatitis B virus vaccination were unwillingness of employers to bear costs 7/20 (35%), low-risk perception 5/20 (25%) and failure to remember 5/20 (25%). Subsequent serology testing was advised twice, both cases had forgotten to do so. PEP was recommended and started once, but stopped after two days because of lower risk perception. Nine healthcare workers were instructed to test their anti-HBs titre, 6 (66.7%) of them were able to report the result.
Conclusions: 1) Most accidental blood exposures, that need follow-up, happen to be unvaccinated healthcare workers outside the hospital setting. 2) Employers, unwilling to pay, seem to be an important reason not to start Hepatitis B virus vaccination. 3) Management and implementation of postexposure follow-up involves many partners. An active, co-ordinating role by 'Prikinfo' could enhance the quality of their service.

P13
Dressing changes under laminar flow conditions to prevent *Staphylococcus aureus* wound colonization in patients admitted to a burn centre

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Introduction: *Staphylococcus aureus* colonization and infection of burn wounds increases morbidity and delays wound healing of patients suffering from burn wounds. A vulnerable moment in the route of transmission of *S. aureus* is the exposed burn wound during dressing changes. The aim of this study was to evaluate the effect of dressing changes under laminar flow conditions on burn wound patients with regard to *S. aureus* colonization.
Method: From the first of February 2003 to the first of June 2003, 25 patients were included in this study at our 10-bed burn centre. In 12 of these patients the dressings were changed under laminar flow conditions. All patients were frequently screened for *S. aureus* in nose, throat and burn wounds. All *S. aureus* isolates were genotyped with pulsed field gel electrophoresis.
Results: At admission 17/25 (68%) patients had no *S. aureus* colonization in their nose, throat or burn wounds. Dressing changes under laminar flow conditions were carried out on 8/17 patients of this group. However, 6/8 (75%) patients acquired burn wound colonization with *S. aureus* during their stay at the centre. Six out of 9 patients (67%) whose dressing changes were not carried out under laminar flow conditions acquired burn wound colonization with *S. aureus*. A total of 12 patients acquired burn wound colonization; these patients also acquired carriage of *S. aureus* in nose or throat. The same type of *S. aureus* was carried in the nose or throat as well as the burn wounds in 7 (58%) of these patients.
Conclusion: The results of this study suggest firstly that dressing changes under laminar flow conditions does not prevent colonization of burn wounds with *S. aureus*, and secondly, that the exogenous route plays an important role in the transmission dynamics of this pathogen in burn wound centres. Future research should be focused on this mode of transmission of *S. aureus* in this special circumstance.

P15
The role of pets in human campylobacteriosis: the bacteriological point of view

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From epidemiological studies, it is clear that direct contact with (pet) animals is a risk factor for human campylobacteriosis. There are some reports with strong indications that direct contact with (young) dogs or cats have led to human Campylobacter infection, and a few casuistic reports in which the correlation between infected pets and human illness is supported or proven by typing of the Campylobacter isolates. From April 2002 till April 2003, a study was performed in the Netherlands, to identify cases in which the Campylobacter isolates from owner and pet show homologous genotypes. The study was part of a gastroenteritis case control study for the identification of risk factors, carried out by the National Institute of Public Health and the Environment. Gastroenteritis patients (proven infection by isolation of Campylobacter) received a questionnaire. If the patient had stated any contact with pet animals, and if they were willing to co-operate in further research, they were supplied with a short questionnaire about the health status of their animal(s), and with material to collect animal faeces. Five separate colonies per pet and the human isolate were speciated and genotyped by AFLP. Campylobacter was isolated from 20% of a total of 676 animal samples, resulting in 490 pet strains for comparison with 58 human strains. So far, 37 cases were analyzed. In one case (2.7%), the pet and human isolates showed an identical genotype. In 36 cases there was no similarity. Twenty-one cases are still under investigation, at least 10 of these are suspected (*C. jejuni* in both human and pet). In contrast with former studies, the percentage of *C. jejuni* of total Campylobacter isolates from the pets was low (16%). We conclude that in this study, a similarity between human and pet animal isolates was found relatively rare.

P16

Gene expression profiling of a *Salmonella typhimurium* DT104 quorum sensing knockout mutant during stress

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Introduction: During the last decades, the incidence of food borne diseases has increased and new pathogens, like the multiple-antibiotic-resistant *Salmonella typhimurium* DT104, have been identified. *S. typhimurium* DT104 has the potential to adapt to food preservation related stress conditions, i.e. starvation and acid, which can result in an increased survival in the GI-tract. Bacterial communication, quorum sensing, can be involved in stress adaptation and survival. Therefore, our objective is to analyze gene expression of a quorum sensing (*luxS*) knockout mutant of *S. typhimurium* DT104 (kindly provided by J. Kieboom) compared to wild type to gain insight in the molecular pathways involved in stress survival in relation to quorum sensing.
Methods: A thematic micro-array was constructed for *S. typhimurium* DT104. Cells of the *luxS* mutant and wild type were harvested at 4 time points spanning from mid-log to the stationary growth phase (starvation stress adaptation). The obtained RNA samples were hybridized to the micro-array and analyzed.
Results: Micro-array data analysis showed an increased expression of stress related genes, in the course of time for both strains, probably due to starvation stress. When bacteria grow, the resources are getting depleted and e.g. stress proteins have to protect the cells against damage. Lipopolysacharide (LPS)-associated genes were down regulated in the course of time in both strains. Bacterial growth requires a vast amount of LPS production. When the growth rate decreases due to starvation, less LPS is needed. Differences in gene expression between both strains were that the LPS genes were down regulated and the stress genes were up regulated at a later time point in the mutant. The lack of the quorum sensing communication system can be the reason of this delay. In the wild-type, quorum sensing activated the expression of the general stress regulator *rpoS* earlier as in the mutant**.** Nevertheless at a later time point *rpoS* is activated also in the mutant by other mechanisms, which resulted in a later activation of stress related genes.
Conclusion: Quorum sensing affects the expression of stress related genes in *S. typhimurium* DT104.

P17

New DGGE strategies for the analyses of methanotrophic microbial communities using different combinations of existing 16S rRNA-based primers

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Submerged wetland soils (e.g. swamps, bogs, rice paddies) are regarded as the most important source of atmospheric methane. Non-flooded upland soils (e.g. forests, grassland and arable) are regarded to be the only biological sink of atmospheric methane. In both wetland and upland soils, obligate aerobic methanotrophic bacteria (MB) use molecular oxygen to oxidise methane to CO_2 and cell carbon. To elucidate the distribution and diversity of MB in various habitats molecular biological techniques (e.g. DGGE; denaturing gradient gel electrophoresis) have been widely used in the past decade. We faced problems in applying the as yet described 16S rRNA-based DGGE protocols for the analyses of MB communities. Hence, the purpose of this study was to develop new DGGE assays for the analyses of MB communities. Using existing 16S rRNA-based primers we developed several direct and nested PCR-DGGE protocols and applied these primers on MB from culture collections, isolates and environmental samples. In soil cores retrieved from a Glyceria maxima stand in a freshwater marsh in the Netherlands, MB belonging to both alpha (i.e. type II MB) and gamma (i.e. type I MB) proteobacteria were detected. The type II MB community consisted of *Methylocystis spp*. However, enrichment and subsequent isolation of strains also indicated the presence of species belonging to the genus Methylosinus that escaped the detection by PCR-DGGE probably because they did not reach the detection limit of the assay. The type I DGGE bands retrieved were most closely related to the genera Methylomicrobium and Methylobacter. Depth profiles of the soil cores showed no differences of the community composition. However, PLFA (polar lipid derived fatty acids) analyses displayed a dominance of type II MB with increasing depth. The overall potential activity decreased strongly with depth suggesting that type I MB contributed substantially to methane consumption potential in these wetland soils. In conclusion we can say that the newly developed DGGE assays are a substantial improvement of the existing 16S rRNA-based PCR-DGGE assays for MB. These assays offer flexibility in the detection limit due to the direct and nested designs.

P18

The formation of the rodlet layer of streptomycetes is the result of an interplay between rodlins and chaplins

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Streptomycetes form hydrophobic aerial hyphae after a submerged feeding mycelium has been established. These hyphae, that will eventually septate into spores, are hydrophobic and possess a typical surface layer called the rodlet layer. We here present evidence that rodlet formation is conserved in the streptomycetes. The formation of the rodlet layer is the result of an interplay between rodlins and chaplins. A strain of *Streptomyces coelicolor* in which

the rodlin gene *rdlA* and/or *rdlB* was deleted did no longer form the rodlet layer. Deletion of all eight chaplin genes also resulted in the absence of the rodlet layer at surfaces of aerial structures. Apart from forming the rodlet layer, chaplins are involved in the escape of hyphae into the air, as was shown by the strong reduction of the number of aerial hyphae in the strain lacking all chaplins. The decrease in the number of aerial hyphae correlated with a lower expression of the *rdl* genes. This indicates that expression of the *rdl* genes is initiated after the hypha has sensed that it has grown into the air.

P19
Metabolic diversity of newly isolated *Magnetospirillum* species

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The genus *Magnetospirillum* is best known for the production of magnetite in the cell. Magnetite crystals are arranged in chains of 10 to 20 crystals and cause the cell to move in a north-south orientation along magnetic fieldlines. Considerable interest exists in the mechanism of magnetite formation and the commercial applications of magnetite production. From a geological point of view interest lies in the magnetization of minerals and the potential of fossil magnetite as a tracer for biological activity. Less attention has been paid to the ecological role of magnetospirilla in the environment. Magnetospirilla are fastidious organisms mostly because of the requirement of microaerobic growth conditions and only a limited number of species is present in pure culture. For these species isolation was based on the magnetic property.
We have used growth systems with opposing gradients of electron donor and oxygen to create microaerobic growth conditions. Freshwater sediment was used as inoculum. Substrates used for isolation were FeS, Na_2S, $FeCl_2$ and ferrihydrite. Repeated transfers and serial dilutions yielded 3 new strains of *Magnetospirillum*, which have been characterised using molecular and physiological properties. 16S rDNA sequences of *Magnetospirillum* species can be divided into two branches and the sequences of all 3 new strains clustered in one branch also containing *M. gryphiswaldense*. Although the similarity between 16S rDNA sequences was very high, DNA-DNA hybridisation results showed considerable differences. In addition, substrate utilization showed differences between the strains, indicating new species. Substrate utilization revealed that *Magnetospirillum* species are involved in Fe reduction, oxidation of sulphur compounds and denitrification.

P20
Diversity analysis of microphytobenthos along a salinity gradient in the Westerschelde

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PCR-DGGE of 18S rRNA gene fragments was used to study the diversity of microphytobenthos along a salinity gradient in the Westerschelde. Samples were taken from the upper 2 mm of the sediment at 3 different sites in the Westerschelde and at two different time points, i.e. April and May 2002. The results indicated that the number and intensity of the bands varied both spatially and temporarily. Comparative sequence analysis of excised bands showed the presence of diatoms affiliated to members of the genera *Cylindrotheca, Goomphonema, Amphora, Cylindrotheca, Nitszchia* and *Navicula*. Apart from these sequences, however, also sequences of other eukaryotes were found. So for a better understanding of the (micro)diversity and niche differentiation of these diatoms, specific primers for the 18S rRNA of diatoms as well as primers for functional genes, such as the rbcL, needs to be developed.

P21
The anammoxosome: an intracytoplasmic compartment in anammox bacteria

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Planctomycetes form a distinct phylum and order of the domain Bacteria. They have many interesting properties, one of which is the presence of intracytoplasmic membranes. Various types of cellular compartmentalization occur. One of its members, *Candidatus* 'Brocadia anammoxidans', contains an inner ribosome-free compartment, called the anammoxosome, bounded by a single membrane. The anammoxosome membrane lipids contain unique linearly concatenated cyclobutane rings. The anammoxosome contains at least one enzyme (hydroxylamine oxidoreductase) involved in the anammox catabolism. Anammox (anaerobic ammonium oxidation) is the anaerobic conversion of ammonium with nitrite (as the electron acceptor) to dinitrogen gas. This project addresses the functionality of the anammoxosome. The hypothesis is that the closed cyclic system of the anammox catabolism is connected to the anammoxosome membrane and could lead to the build-up of a proton motive force (PMF) across this membrane allowing ATP-synthesis. A separate membrane dedicated to anammox catabolism could be advantageous to the organism because: 1) the anammoxosome enables total control of the physical chemistry of the postulated PMF and thus more efficient energy generation. 2) The ladderane lipids in the anammoxosome membrane give rise to an exceptionally dense membrane, a tight barrier against diffusion, leading to a lower loss of valuable catabolic intermediates. 3) Membrane specialization gives more freedom to the organism. The anammoxosome membrane could be used to generate a PMF and to keep toxic intermediates of the anammox catabolism away from the rest of the cell. The outer membrane could be used for homeostasis and thus would have to be relatively permeable. By dividing these tasks, the cell can overcome

the problem of needing a single membrane to be both impermeable and permeable.

P22
GTF180: a glucosyltransferase producing a glucan with anti-corrosive properties

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Glucansucrases or glucosyltransferases (GTFs, EC 2.4.1.5) of lactic acid bacteria are large extracellular enzymes that use sucrose to synthesize glucans with molecular masses ranging from 101 to 104 kDa. They consist of a signal peptide followed by a variable region, a catalytic domain and a glucan-binding domain with a number of repeats at the C-terminus. Little is known about the glucansucrases of Lactobacillus species. Only recently, the first glucansucrase from Lactobacillus reuteri strain 121 has been described (Kralj et al., 2002) that synthesizes a glucan with α-(1→4) and α1→6) bonds.
This work reports the characterization of a glucansucrase from Lactobacillus reuteri 180 (GTF180), that forms a branched glucan with α-(1→3) and α-(1→6) bonds. A truncated GTF180 (GTF180-ΔN-Chis), lacking the N-terminal signal sequence and the variable region, was purified from *E. coli* and characterized. The hydrolytic activity of GTF180-ΔN-Chis on sucrose as substrate is maximal at 45°C while optimal polymerisation occurs at 50°C. Interestingly, the polymer produced has anti-corrosive properties. In an EET-funded project entitled 'Biopolymers as anticorrosion additives for heavy-duty coatings' we are currently studying how this glucan interferes with corrosion. For this purpose, the GTF180 gene is subjected to rational and random mutagenesis to analyse structure-function relationships of the GTF180 enzyme. The variety of glucans produced by these mutant GTF180 enzymes will be characterized (Mw, glycosidic bonds, degree of branching, etc), amongst others with respect to anti-corrosive properties.

P23
Functional biodiversity and environmental genomics of marine and fresh water anammox bacteria

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The freshwater anaerobic ammonium-oxidizing bacteria (AAOB) *Brocadia anammoxidans* and *Kuenenia stutgartiensis* and the marine AAOB *Scalindua sorokinii* were discovered and assigned on basis of 16S rRNA and morphological data to the order Planctomycetales. Environmental samples, which harbour AAOB, were almost exclusively studied by a planctomycete-specific full cycle rRNA approach. This approach indicated a worldwide distribution of AAOBs. They occur not only in wastewater treatment, but also in many fresh water and marine ecosystems. Furthermore, the actual AAOBs diversity was revealed and visualised by fluorescence in situ hybridisation (FISH). Microautoradiography and FISH as well as the combination of immunofluorescence and FISH-linked structural and functional analysis in AAOB communities. Since none of these methods could display the full metabolic potential of AAOBs, an AAOB environmental genomics project for *Kuenenia stuttgartiensis* was launched. In this context we present the first results on several catabolic enzymes involved in the nitrogen conversion and electron transport.

P24
Microbial interactions with heavy metals

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Many of the sediments in coastal waters are contaminated with various metals. Micro-organisms within these sediments can exert great influence on the bioavailability of these metal compounds. The transformations of heavy metals in marine sediments depend on the biological activity affecting decomposition of organic matter, reduction and re-oxidation of metal oxides, and the formation of sulphides. The objectives of this research are 1) to characterise bacterial communities from both pristine and contaminated marine sediments, 2) to isolate species involved in heavy metal transformations and 3) to assess the changes in microbial communities upon heavy metal stress. Samples were collected from four sites with different metal contaminations, i.e. Bremerhaven (G), Rotterdam (NL), Helsingör (DK) and the isle of Sylt (G), in order of decreasing metal concentrations.
To gain more insight in the complex microbial interactions with heavy metals, both molecular tools and culture methods have been applied. Different enrichment and isolation strategies were employed, such as serial dilutions for sulphate-, Fe(III)- and Mn(IV)-reducing bacteria. Additionally, systems with opposing gradients have been inoculated, in an attempt to isolate both autotrophic and heterotrophic bacteria growing at the oxic/anoxic interface utilising either Fe(II), Mn(II) or thiosulphate as electron donor. Molecular techniques, such as PCR-DGGE (polymerase chain reaction - denaturing gradient gel electrophoresis) were used to monitor the progress of the enrichment cultures as well as for community analysis of environmental samples. Combined with primer sets specific for bacteria, archaea or SRB subdivisions this technique provides detailed information on the presence of these particular metabolic groups of micro-organisms.
Sequences retrieved from the microbial cultures and from the environmental samples have been used to construct a phylogenetic tree that shows the clustering of the different species. The sequence database generated can serve as a starting point for more elaborate community analyses in order to investigate the interactions between heavy metals and microbes in a marine environment.

P26

Genogrouping of *Campylobacter lari* based on amplified fragment length polymorphism correlates with nalidixic acid susceptibility

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Campylobacter lari is a potential human pathogen that is phenotypically and genotypically very diverse. Classical *C. lari* strains are resistant to the quinolone nalidixic acid and urease negative. Variants can be distinguished based on nalidixic acid susceptibility and urease production. The aim of the present study is to establish the presence of *C. lari* lineages and to study nalidixic acid susceptibility within these lineages.

A total of 250 strains from different sources (humans, mammals, wild and tropical birds, shellfish, surface water) were analysed by amplified fragment length polymorphism (AFLP), and tested for nalidixic acid susceptibility and urease production. AFLP analysis identified 11 genogroups of which 8 groups were susceptible to nalidixic acid and 3 groups were resistant. Urease activity was restricted to 4 nalidixic acid susceptible genogroups. The phylogenetic clustering of nalidixic acid-resistant strains suggests that resistance may have been acquired prior to other events that affected the bacterial genome. The same holds true for the acquisition of urease production.

To identify the molecular basis of nalidixic acid resistance, a subset of 48 strains representing all genogroups was subjected to antibiotic resistance typing. MICs for other quinolones (ciprofloxacin, flumequine) showed a similar distribution of resistance as nalidixic acid. None of the strains showed multidrug resistance, which suggests that a multidrug efflux pump does not contribute to nalidixic acid resistance in *C. lari*. Sequence analysis of the quinolone resistance-determining region of the gyrA gene showed that resistance correlates with an amino acid substitution at position 86.

P27

Culture negative endocarditis: the value of 16S rDNA PCR and sequencing

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Introduction: An essential element in the diagnosis of bacterial endocarditis is the finding of positive bloodcultures. In patients with endocarditis successive *negative* bloodcultures can be misleading and finally at best, by exclusion, the inadequate diagnosis of a so-called 'culture negative endocarditis' is made. In these special cases 16S rDNA PCR and sequencing of a blood sample can be of great value as is demonstrated in the following case history.

Methods: A 64-year-old man presented himself at our hospital with complaints of fatigue, fever and weight loss since a couple of weeks. Eight years ago he had received an aortic valve bioprosthesis. Under the working diagnosis of a bacterial endocarditis several blood- and a bone marrow samples were taken for culture. Incubation time was extended, however, all cultures remained negative. Since trans-esophageal-echoscopy of the valves could not confirm the diagnosis of an endocarditis, uncertainty concerning the diagnosis was growing.

Finally, a 3 ml EDTA blood sample was taken for 16S rDNA PCR and sequencing. DNA was extracted from the sample by a bead-beating/silica/guanidinium thiocyanate procedure. Amplification was performed with universal bacterial primers. A positive signal was found and subsequent sequencing revealed a Bartonella species. A positive Bartonella serology (IgG titre 500) confirmed our 16S rDNA PCR finding and the diagnosis of a Bartonella endocarditis was made. At present patient is treated with a combination of antibiotics.

Conclusion: Direct detection of bacterial DNA in an EDTA blood sample using broad-range PCR and subsequent DNA sequencing is an important diagnostic tool, particularly helpful in cases where bacteraemia is suspected but cultures nevertheless remain negative.

P28

Genomic diversity of the *Cryptococcus neoformans* species complex

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Cryptococcus neoformans is a basidiomycetous yeast causing life-threatening infections, mainly in immunocompromised hosts.

The existence of the two varieties *C. neoformans* var. *neoformans* (serotype A, AD, D) and *C. neoformans* var. *gattii* (serotype B, C) has long been recognized. A third variety: *C. neoformans* var. *grubii* (serotype A) was described a few years ago. The observation of mating resulted in the description of a separate genus *Filobasidiella*.

Molecular studies on the intergenic spacer (IGS) and internal transcribed spacer (ITS) of the rDNA, mitochondrial large ribosomal subunit RNA (mtLrRNA), orotidine monophosphate pyrophosphorylase (URA5), diphenol oxidase (LAC) and phospholipase B (PLB1) genes showed that the three varieties belong to different phylogenetic lineages and may represent species. This result was supported by amplified fragment length polymorphism (AFLP) data and PCR fingerprinting. Recently var. *gattii* has been raised to the species level as *C. gattii*.

To gain a better insight into the phylogeny of the species complex 160 strains from different origin (clinical vs environmental, different geographic regions etc.) are being analysed for 11 regions of DNA. Here we present the results of the mitochondrial genes ATP6 and mtLrRNA, a gene associated with laccase production (LAC) and of three different regions of rDNA, namely ITS, large subunit rDNA (LSU) and small subunit rDNA (SSU).

Besides these regions elongation factor 1 alpha (EF1alpha), the largest and second largest subunit of the RNA polymerase II gene (RPB1 and RPB2) and two mating type specific genes

encoding protein kinases (STE12 and STE20) are also being studied.
Results obtained so far show that six main phylogenetic groups can be distinguished within the *C. neoformans* species complex.

P29
Validation of AFLP fingerprinting to investigate the taxonomy and epidemiology of bacteria of the genus *Acinetobacter*

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Introduction: AFLP fingerprinting is a robust method to assess the genomic relatedness of micro-organisms. The aim of the present study was to document its value in the study of the taxonomy and epidemiology of *Acinetobacter* strains.
Methods: AFLP was done with *Eco*RI and *Mse*I as restriction enzymes and with Cy5-labelled *Eco*RI+A and *Mse*I+C (A, C = selective nucleotides) as primers for amplification. Pattern analysis was done with BioNumerics vs 2 software (Applied Maths, St. Martens-Latem, Belgium) using the Pearson correlation coefficient as similarity measure and UPGMA for clustering. Four sets of strains were used. Set I comprised 273 reference strains of 22 described (genomic) species. Set II included 49 *Acinetobacter baumannii* strains belonging to the previously described European clones I and II. Set III included 28 *A. baumannii* isolates from 10 patients, and set IV comprised 46 isolates from 10 nosocomial outbreaks.
Results: Cluster analysis of (taxonomic) set I, showed that isolates of the same species were linked at >=50%. At 83%, strains of European clone I and II (set II) were found in two respective clusters. Multiple isolates of single patients were linked >= 90%. This >=90% level was also seen for isolates from the same outbreak. Thus, different grouping levels can be used to identify strains, species, and to assess a possible clonal relationship. Currently, a database of >1000 fingerprints is available for identification of isolates at different levels.
Conclusions: It is concluded that AFLP analysis is a powerful tool in the study of the taxonomy and epidemiology of *Acinetobacter*. Due to its robustness it can be used to generate databases as libraries for species identification, to monitor the spread of strains, and to identify cross-infections.

P30
The role of panton-valentine leukocidin (PVL) producing *Staphylococcus aureus* in mediastinitis following cardiac surgery

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Introduction: PVL is an extracellular product of *Staphylococcus aureus* and is a virulence factor associated with primary skin infections such as furunculosis, and severe necrotising pneumonia. The frequency of PVL in *S. aureus* isolates in the Dutch population is unknown, but is around 10% among methicillin-resistant *S. aureus* (MRSA) isolates collected in the Netherlands between 2000-2003.
Recently we observed two patients with serious skin infections caused by PVL producing *S. aureus* in our hospital. We addressed the question whether PVL is associated with other severe *S. aureus* infections, such as mediastinitis following cardiac surgery.
At the Department of Cardio-Thoracic Surgery at the St. Antonius Hospital, 1750 patients undergo cardiac surgery each year. Severe infections are infrequent, with postoperative mediastinitis in less than 1% of all patients undergoing median sternotomy. However postoperative mediastinitis is associated with a relatively high mortality and prolonged hospitalisation. Therefore we have retrospectively investigated whether PVL producing *S. aureus* was prevalent among mediastinitis causing *S. aureus*.
Methods: A total of 24 isolates from patients with mediastinitis were analysed, 8 from 1997, 9 from 2001 and 7 isolates from 2003. The 24 isolates were previously shown to be not clonally related. A PCR specific for PVL was performed as previously described (CID 29:1128). A PCR specific for *S. aureus* and MecA was also performed.
Results: All 24 isolates were confirmed to be methicillin-susceptible *S. aureus* (MSSA) isolates. None of the 24 *S. aureus* isolates of patients with mediastinitis were positive in the PVL PCR.
Conclusions: PVL producing *S. aureus* can cause severe skin infections but was not associated with mediastinitis caused by MSSA in a Dutch thoracic surgery centre.

P31
CD1 does not play a role in the murine immune response to pneumococcal capsular polysaccharides

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Introduction: *Streptococcus pneumoniae* is a micro-organism that frequently cause serious infections. T lymphocytes play

an important role in the antibody response to pneumococcal capsular polysaccharides. It is an unresolved question how T lymphocyes become activated by caps-PS. CD1 is expressed on antigen presenting cells and is required for the presentation of glycolipids to T lymphocytes. Our aim was to study whether CD1 plays a role in the murine antibody response to pneumococcal capsular polysaccharides (caps-PS).
Methods: Six to eight week old balb/c mice were treated either with a monoclonal blocking antibody to CD1 (20H2) or with ratIgG and were immunized with caps-PS (Pneumovax®). Fourteen days after immunization, blood was drawn by heart puncture and anti-caps-PS antibodies were measured using ELISA. Furthermore, CD1 KO mice were immunized with Pneumovax® and after 14 days anti-caps-PS were measured using ELISA. To study the effect of CD1 on stimulatory effect of CD4(+) T lymphocytes and the inhibitory effect of CD8(+) T lymphocytes on the anti-caps-PS antibody response, *SCID/SCID* mice were injected with spleen cells from balb/c mice from which either CD4(+) or CD8(+) T lymphocytes were depleted, with or without 20H2 and immunized with Pneumovax®. Fourteen days after immunization, blood was drawn by heart puncture and anti-caps-PS antibodies were measured using ELISA.
Results: There was no difference in the immune response to caps-PS between mice that were treated with 20H2 or with ratIgG. Furthermore, the anti-caps-PS antibody response in CD1 KO mice was comparable to that in WT mice. Administration of 20H2 to *SCID/SCID* mice did not affect the stimulatory effect of CD4(+) T lymphocytes, or the inhibitory effect of CD8(+) T lymphocytes on the anti-caps-PS antibody response.
Conclusions: CD1 does not play a role in the murine immune response to casp-PS.

P32
A quantitative molecular analysis of the human gut microflora

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Introduction: Real-time quantitative PCR is a valuable tool to study the gastrointestinal microflora that adheres to the colonic mucosa. We developed primers and probes, based on 16S ribosomal DNA gene sequences, for the most prevalent groups of gut bacteria. Also a universal assay was developed for the estimation of the total bacterial load and to calculate the percentage of each bacterial species contributing to this total bacterial load.
Material and methods: Total DNA was isolated from 25 gut biopsy specimens and quantified by the developed assays. DNA was extracted with the DNeasy(tm) Tissue Kit. All primers and probes were based on 16S rDNA sequences. Specificity of the different assays was tested with representative bacterial species and closely related species. The probes were labelled at the 5'-end with FAM (6-carboxyfluorescein) as the reporter dye and with TAMRA (6-carboxytetramethylrhodamine) as the quencher dye at the 3'-end. Primers and probes were used in a quantitative PCR on an ABI7700.
Results: The assays showed a high sensitivity (1-50 CFU) and specificity. The total bacterial population consisted of approximately 14.4% *B. vulgatus*, 5.4% streptococci, 3.6% bifidobacteria, 1.0% *E. coli*, and 0.4% propionibacteria. In contrast to what is usually found by culture, fusobacteria were hardly detected in the gut samples.
Conclusions: Real-time PCR is applicable to study the gastrointestinal microflora. Molecular analysis is a promising alternative approach to study the microflora that adheres to the intestinal mucosa. Real-time PCR CFU equivalents may lead to new insights in the actual ratio's of the different bacterial species present in the gut. This in turn can lead to a better understanding of the effects of antibiotics and probiotics on the gut microflora.

P33
Molecular identification of *Malassezia* species

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Introduction: *Malassezia* yeasts are associated with several dermatological disorders. Identification of *Malassezia* yeasts by phenotypic characteristics is complicated and time consuming, and epidemiological surveys based on culture methods are difficult to interpret.
Methods: We compared AFLP and rDNA sequencing with traditional method of identification of isolates belonging to *Malassezia* yeasts. We also searched for genetic diversity in clinical isolates of *Malassezia* species from the Ontario region of Canada and compared them with those that had been isolated from other parts of the world.
Results: Clustering of AFLP fingerprints and D1/D2 and ITS sequences of the rDNA were fully concordant. All species can be differentiated using these methods. Considerable genetic divergence was observed in the species *M. furfur*. A few discrepancies were noted when the conventional method of identification was compared with these molecular methods. This is also the first study that demonstrates the presence of a hybrid genotype in *Malassezia furfur.*
Conclusions: 1) AFLP and rDNA sequences can reliably be used for the identification of isolates of *Malassezia*. 2) Physiological identifications turned out to be less reliable. 3) A considerable genetic divergence is present in *M. furfur*, which correlate to some extent with clinical origin. 4) A diploid hybrid genotype is detected in the putative asexual species *M. furfur*.

P35
Hand hygiene in medical microbiology laboratory personnel

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Objective: To measure the compliance of laboratory personnel with different components of hand hygiene.

Design: Prospective study.
Setting: Laboratories in the Department of Medical Microbiology of the University Medical Centre St Radboud, Nijmegen, the Netherlands.
Methods: The study consisted of three components: 1. Observations (the use of hand hygiene methods and compliance with a no-jewellery policy). 2. Microbiological cultures from hands and wrists of laboratory personnel (with and without jewellery), and 3. Interventions (feedback, posters, e-mail messages). The compliance with the no-jewellery policy was re-observed a week and one month after the intervention.
Results: Compliance of laboratory technicians with hand hygiene was 100% at the end of their duty. Of 49 laboratory workers, 36.7% wore a ring, 46.9% a watch and 6.1% a bracelet. After the interventions the compliance with the no-jewellery policy among laboratory personnel improved. The attributable colonization with commensal flora due to watches and rings was 1.2 and 0.7 grades, respectively in 19 technicians wearing jewellery. Potentially pathogenic micro-organisms were exclusively found on hands of laboratory personnel that wore jewellery (37%). Colonization with pathogenic micro-organisms was found in 1.0% (1/10) after disinfection, 2.2% (2/9) after washing only and 80% (4/5) after washing followed by disinfection.
Conclusion: Rings and watches reduce the effectiveness of hand-hygiene leaving hands of laboratory technicians with higher remains of commensal flora. Furthermore, colonization with pathogenic micro-organisms was exclusively found in laboratory technicians wearing jewellery. Our results clearly show that efforts to increase hand hygiene practices were primarily directed at HCWs in patient care. Infection control should direct some of their attention towards education and behavioural changes of laboratory personnel and their hand hygiene.

P36
Risk and outcome of nosocomial *staphylococcus aureus* bacteremia in nasal carriers vs non-carriers

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Introduction: *Staphylococcus aureus* nasal carriage is a major risk factor for acquiring nosocomial *S. aureus* infection, but a reliable risk estimate is still unknown.
Methods: To estimate the risk for acquiring nosocomial *Staphylococcus aureus* bacteremia in *S. aureus* nasal carriers vs non-carriers, we screened 14,014 non-surgical patients for *S. aureus* nasal carriage at admission, and assessed them for the development of bacteremia. Relevant clinical data of those with bacteremia were collected. Nasal and bacteremic strains were genotyped by PFGE and interpreted according to standard criteria.
Results: Nosocomial *S. aureus* bacteremia developed in 40/3,420 (1.2%) *S. aureus* carriers and in 41/10,588 (0.4%) non-carriers (RR: 3.0, 95% CI: 2.0-4.7). Non-carriers with bacteremia were older and had higher in-hospital mortality rates (all causes) than *S. aureus* carriers with bacteremia. Genotyping by PFGE revealed that 80% of the nosocomial bacteremia in carriers were of endogenous origin.
Conclusion: These findings stress the importance of developing differential prophylactic strategies against nosocomial *S. aureus* infections in nasal carriers and non-carriers. In the future, molecular characterisation of nasal *S. aureus* strains may predict invasive infection.

P37
Comparison of quantitative culture and real-time PCR for *Tannerella forsythensis*, in plaque samples from patients with periodontitis

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Introduction: *Tannerella forsythensis* (formerly *Bacteroides forsythus)* is a fastidious, Gram-negative, obligate anaerobe bacterium associated with a etiological role in adult periodontitis. Complex media are required for optimal growth and there is no selective medium available. Multiple subcultures and biochemical analysis are required for isolation and identification. Despite this, quantitative anaerobic culturing is used to diagnose a possible role of this bacterium in periodontitis. The use of real-time PCR would be of great benefit for identification and quantification of *T. forsythensis* and to study its relation to periodontitis. In this study we compared real-time PCR with conventional anaerobic culture in detection and quantification of *T. forsythensis*.
Methods: Specificity and sensitivity of the real-time PCR test was evaluated. Plaque samples from periodontal pockets were collected from 259 patients with periodontitis. Equal aliquots were evaluated by real-time PCR and quantitative anaerobic culture.
Results: The PCR specificity test against several other oral bacteria showed a high specificity for *T. forsythensis*. The detection limit was 1 CFU equivalent. *T. forsythensis* was detected in 215 subgingival plaque samples by culture and in 230 samples by PCR. Nineteen samples were culture negative/PCR positive. Four samples were culture positive/PCR negative. A total of 211 samples were positive with both methods. Comparison of the quantitative results showed that 73% had a less than 10-fold difference, 22% showed a 10-100-fold and only 5% showed a >100 fold difference between culture and PCR.
Conclusions: *T. forsythensis* was identified in more than 80% of periodontal plaques. Only 9% of the samples showed differences between the real-time PCR and culture.
Based on the positive quantitative results of both methods the performance of the real-time PCR is very promising and seems to be a useful alternative for rapid, specific, quantitative identification of *T. forsythensis* in plaque samples.

P38

M. tuberculosis PCR performed directly on clinical samples: a four years survey

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Introduction: In order to shorten time needed for laboratory diagnosis of tuberculosis a directly performed *M. tuberculosis* PCR (DPMT) was introduced. Based on the good results obtained in a previous study, in which we validated this PCR for respiratory specimens by comparing it with culture, we implemented a new policy for the use of this PCR directly for respiratory specimens from non-immunocompromised hosts.

Methods: Ziehl-Neelsen (ZN) staining, DPMT on clinical specimens and culture (BACTEC) were carried out on a routine basis on each extrarespiratory specimen and on respiratory specimens of immunocompromised patients. Sputum, broncho- and bronchoalveolar lavages of non-immunocompromised patients were firstly processed for microscopy and DPMT; if one or both of these tests was positive or inconclusive, the sample was further cultured, if both test were negative no further culture was performed. Initially DPMT was carried out with a conventional in-house PCR, in 2002 this was replaced by an in-house real-time PCR.

Results: Between 2000 and 2003, 5031 samples have been processed. Forty-three respiratory tract specimens were found positive with one of the three methods. DPMT and ZN were carried out on 40 of these samples: 15/40:ZN -/DPMT+;1/40: ZN -/DPMT inconclusive(i); 22/40 ZN +/ DPMT+; 1/40 ZN +/ DPMT i; 1/40 ZN -/ DPMT -. Nine of the 15 samples with ZN -/DPMT +, were positive in culture.

A total of 56 extrarespiratory specimens, were positive with one of the three methods. DPMT and ZN were carried out on 44 of these specimens: 24/44 ZN -/ DPMT +; 13/44 ZN +/DPMT +; 7/44 ZN -/DPMT -. These last 7 samples were all positive in culture. Distribution of percentages of positive respiratory specimens over the years: 2000: 1.45%, 2001: 1.39%, 2002: 0.78%, 2003: 4.38%. Conclusion: Overall DPMT performed much better than microscopy. A good correlation was found between DPMT and culture. There are no significant differences in percentages of positive respiratory specimens per year since we skipped the culture of these specimens in case of a negative ZN and DPMT, compared to the period before (1.8%).

P39

Comparison of two commercial diagnostic tests for tuberculosis with two different extraction protocols

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In a previous study it was shown that the sensitivity of the amplified *Mycobacterium tuberculosis* direct test (aMTD, Gen-Probe), when tested in spiked sputum samples, could be enhanced by replacing the lysis protocol included in the kit with the DNA-extraction protocol as described by Boom, et al. (Boom-extraction). To further test the value of this extraction protocol we collected 254 respiratory samples from 225 hospital patients, which were sent to the microbiology laboratatory for diagnosis of tuberculosis. Among these samples, 18 (7%) were positive by microscopy and/or culture for *Mycobacterium tuberculosis* complex. The samples were analyzed with the aMTD and the COBAS Amplicor *Mycobacterium tuberculosis* test (Roche), with and without replacement of the original lysis-extraction protocol. The sensitivity and specificity of the aMTD were 100% and 97.9%, and 83.3% and 99.6% when Boom-extraction was applied, respectively. The specificity increased to 100%, both without and with Boom-extraction, when a higher cut-off value of 150.000 was used instead of 30.000 as recommended by the manufacturer. However, the sensitivity without Boom-extraction decreased to 88.9%. The sensitivity and specificity of the Roche test were 100% and 100%, and 66.7% and 100% when Boom-extraction was applied. From these results it was concluded that there is no additional value of Boom-extraction in microbiological laboratory diagnosis of tuberculosis. Furthermore, on this set of respiratory samples, the Roche test was superior.

P40

Development of an internally controlled Taqman-based PCR assay for the detection of *Chlamydia pneumoniae* in the Lightcycler 2.0 system

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Chlamydia pneumoniae is an intracellular micro-organism causing community acquired pneumonia. Currently available tests to diagnose *C. pneumoniae* infections are hampered by low sensitivity (culture), lack of reproducibility (MIF) and specificity (ELISA). Therefore, a specific and sensitive test is urgently needed for accurate diagnosis. Here we describe an internally controlled real-time PCR assay to detect *C. pneumoniae* in clinical samples.

C. pneumoniae specific primers, amplifying an 80 bp fragment of the 16s rRNA gene (target) were developed. An internal control (IC) was constructed using 2 oligonucleotides containing above mentioned primer sites but a shuffled probe site compared to the target. PCR products of both the target and IC were cloned and plasmid DNA was used for dilution experiments. Target amplimer and IC were detected by Taqman probes labelled with 5'FAM-3'TAMRA and 5'VIC-3'TAMRA respectively.

Elementary Bodies (EBs) (5.5 10^5IFU/ul) (kindly provided by Dr. F. Stassen) were used as input for DNA extraction efficiency experiments. Extraction of DNA was performed by either the silica-guanidiniumthiocyanate procedure (Boom-extraction) or the MagNaPure (Total NA kit) procedure. PCR was performed in the Lightcycler 2.0 system.

The detection limit of the PCR was assessed by spiking of bronchoalveolar fluid (BAL) with limiting amounts of target plasmid DNA and 80 copies of IC. After spiking, DNA was isolated using Boom-extraction and subjected to PCR. This way, a lower limit of detection of 5 copies of target plasmid DNA per µl of PCR input was reached. This corresponds to a sensitivity of 1250 target copies per milliliter clinical sample.

Using 10-fold dilutions of EBs to spike, we were able to detect as few as 1 IFU/µl of PCR input, irrespective which DNA extraction method was used.
The specificity of the PCR was tested using 13 commonly encountered bacteria in respiratory samples. All samples remained PCR negative while the IC amplified correctly. We have developed a highly specific and sensitive internally controlled PCR to detect *C. pneumoniae* using the Lightcycler 2.0 system with two dual-coloured Taqman probes. This PCR is currently being evaluated in a clinical setting.

P41
A novel dual-colour semi-quantitative real-time *Mycobacterium tuberculosis complex* assay with internal amplification control using hybridization probes on the LightCycler™ system

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Introduction: Detection of *Mycobacterium tuberculosis* complex in clinical samples by PCR or other molecular amplification assays using home-brew or commercial detection systems is routine practice in many microbiological laboratories. We developed a novel dual-colour semi-quantitative real-time PCR assay based on a hybridization probe format for the LightCycler system containing an internal amplification control.
Methods: Target for this novel PCR is the IS6110 element. This element is present as either low-or multicopy element in more than 99.9% of *M. tuberculosis* complex isolates. A modified version of this element (containing an artificial DNA insertion) inserted as single copy element in the genome of *Mycobacterium smegmatis* 1008 (Kolk AHJ, et al. 1994) was used as internal amplification control target. Both DNA targets can be amplified with the same PCR primer combination. The amplification performance of six different PCR primer combinations was evaluated. The performance of the entire assay was also compared to the performance of a commercially available *M. tuberculosis* complex assay on a collection of more than 70 clinical samples.
Results: The amplification performance of this novel assay was evaluated on the modified IS6110 element from *M. smegmatis*. A linear correlation was obtained on serial dilutions spanning a 6-log range of target copies. The detection limit of this assay reached down to the single copy level. A comparison between the performance of this novel assay vs a commercially available *M. tuberculosis* complex assay (LCX) on a clinical sample collection showed that our novel assay was at least equally sensitive and suffered less from inhibition. Moreover, it has successfully been used on a variety of non-respiratory samples as well.
Conclusion: Our novel *M. tuberculosis* complex assay with excellent performance characteristics makes it an attractive in-house assay for routine identification of *M. tuberculosis* complex in clinical samples.

P42
Detection of bacteria in platelet concentrates: comparison of broad-range real-time 16S rDNA PCR and automated culturing

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Introduction: Platelet concentrates (PCs) are frequently associated with transfusion-transmitted infections. To assess the presence of bacteria in PCs a rapid and dependable method is needed. Based on real-time PCR technology, a broad-range 16S rDNA assay was developed. After optimization, the assay was validated and its performance compared to an automated culture system to determine its usefulness for routine screening of PCs.
Methods: The validation was performed with fresh PCs derived from pools of 5 individual blood donors each. A total of 3140 specimens were sampled. The presence of bacteria in these PCs was routinely assessed in an automated culture system (BacT/Alert). Culturing was conducted until a positive signal is detected or for up to seven days when remaining negative. The PCR assay was performed with DNA extracted from the same cultured samples. DNA extraction was done with a fully automated method (MagNA Pure). PCR amplification was performed with a set of universal primers and probe targeting *eubacterial* 16S rDNA. PCR results were compared with those obtained by culturing.
Results: A total of 1366 samples were tested by both the PCR assay and culture system. Three specimens were found to be contaminated by both methods. Identified micro-organisms included *Micrococcus* spp, *Staphylococcus epidermidis* and *Propionibacterium* spp. One sample generated an amplification signal in the PCR but gave no positive signal in the BacT/Alert. This discordance is probably due to the detection of DNA from a non-viable bacterium by PCR that can not be detected by culture.
Conclusion: These preliminary resutls indicate that the PCR assay enables accurate and sensitive detection of bacterial DNA in PCs. Hence this method may be a valuable improvement in monitoring bacterial contamination of PCs.

P43
Comparison of DNA extraction efficiency of three nucleic acid extraction methods

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The quality of nucleic acid amplification techniques for detection of micro-organisms in a routine laboratory is dependent on pre-treatment of a sample and extraction of nucleic acids (NA). The silica-based Boom method is used in many laboratories because it has a high efficiency and works best for NA extraction from mycobacteria, which are difficult to lyse. However, with increasing numbers of samples, automation is needed to save time and to avoid cross contamination between samples. We compared the

extraction efficiency and DNA quality of two small NA extraction machines, the MagnaPureCompact (MPC-Roche) and the Qiagen Robot EZ1 (Westburg) with the Boom method. We prepared 24 samples for duplicate extractions with the three methods. Sixteen samples with different clinical material, sputum, pleural fluid, tissue or CSF containing *M. tuberculosis* complex, *N. meningitidis* or phocid herpes virus. Efficiency of DNA extraction was estimated with specific real-time PCR tests, comparing the Ct (ABI7000) or Cp (LightCycler) values. For eight samples colonies of *S. aureus* or *P. aeruginosa* were extracted and DNA concentrations, A260-280 ratio's and PCR fingerprint patterns were compared. For extraction of *M. tuberculosis* DNA from sputum samples, the Boom method was superior and resulted in the lowest Ct-values. However, with an extra lysis incubation on pleural fluid and tissue extraction the MPC was equal to the Boom method. The EZ1 showed slightly more variation and higher CTs. No differences were found between Boom, MPC and EZ1 in extraction efficiency of *N. meningitis* DNA and phocid herpes virus and in inhibition of the PCRs. The ratio of Boom extracted DNA from colonies was very low (1.1-1.3) in comparison to the MPC and EZ1 (both 1.7-1.8). Eight and six samples are extracted in 25 and 20 minutes on the MPC and EZ1, respectively. However, the extraction volumes differ and the MPC has extra facilities such as droplet-catch, cloth-detection and an UV-lamp. Both machines are easy to use and suitable for application in a laboratory for molecular diagnosis where flexibility is needed for daily handling of small numbers of different samples for different tests.

P44
Evaluation of a TaqMan real-time PCR-test to detect *Neisseria gonorrhoea*

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Gonorrhoea is caused by *Neisseria gonorrhoeae*, a micro-organism that is transmitted perinatally or by sexual contact. Diagnosis of *NG* infection is important because - if left untreated - it may lead to neonatal conjunctivitis, female infertility or acute epididymitis. Several diagnostic tests based on DNA amplification have been described to detect *N. gonorrhoeae*, however, false-positive results (Amplicor *N. gonorrhoeae* PCR), or false negative results (*cppB* gene-tests) pose a major problem. *Neisseria spp.* possess Opa proteins, a family of variable outer membrane proteins that mediate various host cell interactions. To investigate whether the Opa-genes are suitable targets for *N. gonorrhoeae* detection, we designed Opa-primers and probe, and analysed a panel of 500 samples consisting of 450 well defined clinical *N. gonorrhoeae* cultures (diluted DNA samples), and -distributed randomly among these - 50 samples containing either water/buffer or DNA from other micro organisms. No cross reactivity was observed with *Neisseria lactamica*, *Neisseria meningitidis* (10 strains tested) and *Neisseria mucosa*. Of the 450 *N. gonorrhoeae* strains only 424 generated a positive fluorescent signal in the TaqMan-PCR. However, when analysing the 26 PCR products of the *N. gonorrhoeae* strains that were negative in the TaqMan PCR on agarose gel, 24 showed ample PCR products of the expected size. Apparently in 24 cases a PCR product is generated that is not detected by the Opa-probe. The other 2 (negative fluorescent signal and no PCR product) were also found negative in all the other laboratories that analysed the panel, probably due to a low concentration of *NG*-DNA. We currently determine the sequence of the Opa PCR product of the 24 *N. gonorrhoeae* strains with the aim to design an Opa-probe (mixture) capable of detecting all 450 *N. gonorrhoeae* strains.
We conclude that the Opa-genes might be suitable target sequences for a PCR-based *N. gonorrhoeae* test. However, adjustment of the DNA-sequence of the probe used in this study is required for real-time *N. gonorrhoeae* detection.

P45
Enrichment of isolated septal pore caps of the plant pathogen *Rhizoctonia solani*

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The septal pore caps (SPCs) are situated at both sides of the dolipore septum within hyphae of many Basidiomycetous fungi. Though ultrastructural studies on SPCs demonstrated different SPC morphologies (tubular, nonperforate and perforate), the function of the SPC is still not well understood. The goal of this study is to isolate the SPC and to characterize the proteins to understand its function in the fungal cell.
We used the plant pathogen *Rhizoctonia solani* as a model organism for its large perforate SPCs (diameter about 1.6-2.0 ìm). To characterize the SPC we used three different approaches. Firstly, we used a number of fluorescent biomarkers (among others Calcofluor white, WGA-alexa488 and ER-tracker) to visualize the SPC. Secondly, we used French pressure cell press fractionation, followed by isopycnic and differential centrifugation for the isolation of the SPC. Fractions were processed and analyzed with a transmission electron microscope and the proteins were analyzed by SDS-PAGE. Thirdly, we used laser microdissection to isolate the dolipore septum region from 1 micron sections of hyphae.
The use of the different fluorescent biomarkers resulted in different patterns of staining of the SPC region, illustrating the different biochemical properties of the SPC. These biomarkers can be used to monitor the fate of the SPC during the isolation methods. Fraction analysis showed that not only the SPC was enriched, but also the plugging material, that remained attached to the SPC via tubular material. Furthermore, we were able to isolate dolipore septum regions with laser microdissection, showing that this is a useful tool to isolate fungal structures. Protein analysis showed protein bands exclusively for the fractions with the enriched SPCs. Further analysis of the proteins of the enriched SPC fraction and the microdissected regions, e.g. N-terminal sequencing and 2D gel electrophoresis, will give us more insight in the protein content of the SPC and the plugging material. Once these proteins are known, a better understanding of the SPC function will be achieved.

P46
Species diagnostics in *Fonsecaea*

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The internal transcribed spacer rDNA domain is frequently used in taxonomy and diagnostics of filamentous fungi. In black yeasts and relatives it is even the gold standard of taxonomy, since it is the only marker with sufficient resolution and of which ample sequences for comparison are available. However, the widespread occurrence of paralogues with different signatures in the same ribosomal repeat has shed doubt on the general applicability of this marker. We intend to investigate this problem using a model species of black yeast-like fungi, the genus *Fonsecaea*.
We noted the existence of ITS-based groups, which do not seem to coincide with phenotypic characters. Either these are cryptic species, or alternatively, coincidental amplification of only one of several possible repeats is concerned. We tried to separate the two groups found by the use of selective primers of each of the signatures, as done by O'Donnell et al. with paralogues in *Fusarium*. Using SSCP only single repeats were revealed after amplification with selective as well as with universal primers; the patterns differed between the two pre-established cryptic species. However, in some strains amplification was ambiguous, showing thin bands located at both positions characteristic for each of the cryptic species. We therefore need to establish whether repeats are different, bearing both signatures, in some strains of this species. To this aim, the species complex is investigated by cloning the ribosomal spacer domain and sequencing the clones harbouring the two ITS types.

P47
Cladophialophora agents of human chromoblastomycosis have a natural reservoir in cactus plants

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Cladophialophora carrionii is one of the four known etiologic agents of human chromoblastomycosis, a skin disease characterized by emerging, cauliflower-like lesions. In the arid climate of Venezuela the infection is supposed to be caused by traumatic inoculation of cactus spines carrying fungal cells.
A molecular study (rDNA ITS, EF1alpha) of a collection of strains from cactus material revealed the existence of a second species, that is introduced as *C. yegresii* sp. nov. The two species probably share the same ecology. Artificial inoculation of cactus plants grown from seeds in the greenhouse showed that the fungus grows with hyphae in cactus tissue, but when reaching the spines it produces cells that are morphologically very similar to muriform cells, the invasive form of human chromoblastomycosis. The muriform cell thus can be regarded as the extremotolerant survival phase of the organism. The phylogenetic affinity of *Cladophialophora* to the black yeast family Herpotrichiellaceae, which contains a very large number of human opportunists. The possible reasons why this is the case are explored.

P48
Successful treatment of *Fusarium* keratitis with cornea transplantation and topical and systemic voriconazole

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A previously healthy male patient presented with a painful keratitis of the right eye, which developed in one week. This patient had been diving in the port of Curaçao and used daily wear disposable contact lenses. The cornea showed an ulcer with an infiltrate. Treatment was initially started with ciprofloxacin, amphotericin B and acyclovir but was changed to topical amphotericin B and systemic itraconazole when scrapings and culture of the affected area showed mycelia and *Fusarium solani* respectively. Debris from the anterior chamber was also positive for *Fusarium* indicating invasive growth through Descemete's membrane. However, the corneal infiltration expanded, consistent with the finding that this *Fusarium* strain was resistant to itraconazole. A debulking penetrating keratoplasty was performed and antibiotic treatment was changed to topical voriconazole 1% and systemic voriconazole (8 mg/kg/day). The corneal infiltrates did not return in the donor cornea and disappeared slowly from the recipient cornea. A whitish mass in the anterior chamber also disappeared slowly. Two weeks after transplantation the voriconazole titre in the aqueous of the anterior chamber and plasma was 3.2 mg/l and 2 mg/l respectively. Systemic voriconazole combined with topical application resulted in much higher concentrations in the anterior chamber compared to systemic voriconazole alone (160% of the plasma concentration vs 53%, respectively). The MIC for the *Fusarium* isolate was 1 mg/l. After four months the cornea was still clear and no adverse effects of the topical application were observed. In conclusion: a previously healthy male patient with a *Fusarium* keratitis of the right eye was successfully treated with a cornea transplantation and topical and systemic voriconazole. The combination of systemic and topical voriconazole resulted in high concentrations in the anterior chamber.

P49
Structure and function of the septal pore cap (SPC) in basidiomycetes

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The ascomycetes and basidiomycetes comprise most of the species of filamentous fungi. The hyphae of these fungi are capable of forming complex, well-organised structures such as mycelia and mushrooms. Fungal hyphae are made up of cytoplasmic compartments that are separated by perforated septa, generating a continuous system. Septa are crucial for important processes such as differentiation (e.g. sporulation)

and damage control (pore-plugging) but they may have other functions as well. Ascomycetes are characterized by relatively simple structured septa, while those of basidiomycetes can be very complex. Basidiomycete septa contain a characteristic rim around their central pore, the dolipore, which in higher basidiomycetes is often covered by a septal pore cap (SPC). Although SPCs were already observed in 1958, their composition and function is still unknown.
To understand the function of the SPC of the model basidiomycete *Schizophyllum commune*, we will determine its composition and identify genes involved in the formation of the structure. The SPC of *S. commune* is a membranous, multi layered, regularly perforated structure of approximately 550 nm. The pores appear to be penta- to heptagonal as was shown by electron microscopy. We have enriched the SPC from a homogenate of mycelium, which was obtained using a dismembrator. The homogenate was fractionated by isopycnic and differential centrifugation. TEM analysis showed that SPCs accumulated at 48%-50% {w/w} sucrose (buoyant density +/- 1.22 g cm<-3>). They could be pelleted by centrifugation at 10.000 x g for 2 h. In this fraction membrane vesicles were also abundantly present. Extraction of the 45-50% sucrose interface fraction with Triton-X-100 (1%) removed most of the membrane vesicles.
These results show that the SPC is a highly dense and rigid structure that possesses resistance towards a membrane dissolving detergent. These properties will be used to further purify the SPC of *S. commune*, which will enable us to determine its composition.

P50
In situ hybridization of RNA in filamentous fungi using peptide nucleic acid probes

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DNA probes do not diffuse through the fungal cell wall. Therefore, *in situ* hybridisations of RNA in yeast are done with protoplasts. However, in case of filamentous fungi the hyphal morphology is lost upon removal of the cell wall. This abolishes the possibility to use *in situ* hybridisation to localize gene expression at the level of the hypha and the colony. Therefore, we attempted to make the cell wall of *Aspergillus niger* and *Schizophyllum commune* permeable by freeze/thawing and by short treatments with lysing enzymes. In contrast to hybridisations with protoplasts, hybridisations with permeabilised hyphae using ALEXA594 labelled 18S rDNA probes were highly irreproducible.
Peptide nucleic acids (PNA) probes are synthetic DNA mimics developed in the 1990's.[1,2] These probes do diffuse through the *Saccharomyces cerevisae* cell wall and have been used in *in situ* hybridisations to detect 18S rRNA. We used PNA probes for *in situ* hybridisations in *S. commune* and *A. niger*. Highly reproducible fluorescence was observed after hybridising the fluorescein labelled 18S PNA probe. The signal was absent when the hyphae were pre-treated with RNAse. *In situ* hybridisation was also successful using a PNA probe hybridising to *SC3* mRNA of *S. commune*. Hybridisation was absent in 2- and 4-day old colonies of a strain in which the *SC3* gene has been deleted. In contrast, high fluorescence was observed in a 4-day old colony of a wild-type strain, while signals were lower in 2-day old colonies. The fluorescence intensity correlated with accumulation of *SC3* mRNA as determined by Northern analysis. Thus, PNA probes are an excellent tool to study gene expression in filamentous fungi.
References: 1. Egholm, et al. Nature 1993;365:556-68.
2. Nielsen, et al. Bioconjugate 1994;Chem 5:3-7.

P51
Is Hepatitis E virus endemic in the Netherlands?

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Hepatitis E virus infections are recognised as an imported disease, related to travel in well-known endemic regions. However, increasing evidence suggests that Hepatitis E virus infection may also occur in developed countries and that swine may act as a possible reservoir. To investigate the possibility of indigenous transmission of Hepatitis E virus in the Netherlands, we investigated serum samples from unexplained acute hepatitis patients with with no travel history to a Hepatitis E virus -endemic region. A total of 500 serum samples from hepatitis patients were screened with a commercially available ELISA for IgG and IgM Hepatitis E virus -specific antibodies. All positive results were confirmed with immunoblot. Serological evidence of Hepatitis E virus infection was detected in 9% of these patients, and 1.4% of these cases were confirmed by a positive PCR. In 6 out of 7 cases, we found sequences that were related to virus strains detected in pigs (93 to 97% homology). The detection of swine-like Hepatitis E virus in hepatitis patients without a recent travel history is strong evidence that Hepatitis E virus is endemic in the Netherlands. It is therefore recommended to test patients with acute hepatitis for Hepatitis E virus infection.

P52
Cell-ELISA for antiviral susceptibility testing of influenza virus: performance depends on the compatibility of virus strain and type of MDCK cells

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Increased use of antiviral drugs to manage influenza requires, ideally, universal methods to determine the susceptibility of influenza virus strains for these drugs. For this purpose, a Cell-ELISA assay (cELISA), which can be used with the adamantanes and neuraminidase inhibitors was developed and characterized. The assay is based on detection of influenza virus nucleoprotein 18 hours post

inoculation of MDCK cells. *Influenza A viruses* A/Chicken/Pennsylvania/21525/83 (H5N2), A/Chicken/Saoudi-Arabia/569017/00 (H9N2), and A/PR/8/34 (H1N1) were used. Because reproducible virus spreading is a prerequisite for a good performing cELISA, the heterogeneous MDCK cell line was cloned. Two clones, MDCK-T CB4 and MDCK-I BD5, were selected that best supported virus spreading. For amantadine, the 50% inhibitory concentrations (IC_{50}'s) were similar with both clones. The A/PR/8/34 virus was resistant for amantadine. The IC_{50}'s of the avian viruses for oseltamivir and zanamivir using clone MDCK-T CB4 paralleled those estimated using a fetuin-based neuraminidase inhibition (NI) assay. For A/PR/8/34 virus, the IC_{50}'s estimated using the cELISA were up to 70-fold higher than in the NI-assay. In contrast, using MDCK-I BD5 cells, the IC_{50}'s for the neuraminidase inhibitors could not be estimated, or only with higher IC_{50}'s and larger confidence intervals. This is probably caused by extensive neuraminidase-induced pile up of virus on the membranes of infected cells. In conclusion, since the cELISA performed well with three very distinct influenza viruses and three different antiviral drugs our results suggest that the cELISA using MDCK-T CB4 cells can be universally used. However, since adjustment of the cELISA appeared to be virus- and cell-specific this approach is only recommended in a research setting. Because results between the cELISA and the NI-assay may differ for particular strains, further work should indicate which method gives the best prediction of the *in vivo* situation.

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