NEDERLANDS TIJDSCHRIFT VOOR

MEDISCHE MICROBIOLOGIE

SUPPLEMENT BIJ DERTIENDE JAARGANG, APRIL 2005

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, 11 - 13 april 2005

De voorjaarsbijeenkomst van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) vindt plaats op dinsdag 12 en woensdag 13 april 2005 te Papendal.

Zoals vorig jaar reeds opgemerkt zijn de locatie, Papendal, en de formule van onze tweedaagse bijeenkomst inmiddels traditie geworden. We beginnen met een algemeen symposium op dinsdagochtend met als thema: 'Microbes in a changing world'. De gevolgen van globalisering, wereldwijd reizen en klimaatveranderingen zijn niet alleen voelbaar voor ons mensen, maar ook voor de micro-organismen die ons allen interesseren. Wie hier meer over wil horen mag de dinsdagochtendsessie 2005 zeker niet missen! Tradities staan geen vernieuwingen in de weg. De Voorbereidingscommissie heeft nieuwe ideeën wat betreft het invullen van de thematische sessies. Vanaf 2006 willen wij graag dat IEDER lid van de verenigingen die participeren in de Voorjaarsvergaderingen de mogelijkheid krijgt om zelf een thematische sessie (of zo u wilt: een minisymposium) te bedenken en een voorstel te doen voor de invulling ervan. Dit naar analogie van de sessies bij de General Meeting van de ASM, waar ieder lid als 'convener' een sessie mag voorstellen. Uiteraard zal de Voorbereidingscommissie keuzes moeten maken uit de grote aantallen ingediende voorstellen, maar voor die keuzes zullen criteria worden opgesteld die van tevoren bekend gemaakt zullen worden. Als voorproefje voor deze nieuwe aanpak zullen dit voorjaar een aantal sessies volgens het bovenstaande principe door leden van de Voorbereidingscommissie worden ingediend.

Mist u een onderwerp op de Voorjaarsvergadering? Heeft u ideeën met betrekking tot onderwerpen die u graag anders belicht zou zien? Denk hier tijdens deze Voorjaarsvergadering alvast over na. Wij zullen u zo spoedig mogelijk berichten wanneer de ideeënbus open gaat!
Tot slot nog een vernieuwing: voorafgaand aan de tweedaagse Voorjaarsvergadering zullen op maandagmiddag alle arts-assistenten in opleiding tot arts-microbioloog deelnemen aan de eerste landelijke toets in het kader van de opleiding. Tevens is er een aantal educatieve lezingen voorzien.
Wij wensen alle geledingen binnen de microbiologie in Nederland twee vruchtbare dagen in Papendal toe.

Voorbereidingscommissie
Prof. dr. C.M.J.E. Vandenbroucke-Grauls, voorzitter
Dr. T. Boekhout
Dr. C.H.E. Boel
Prof. dr. S. Brul
Mw. dr. B. Duim
Prof. dr. L. Dijkhuizen
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
Prof. dr. P.E. Verweij
Prof. dr. W. de Vos
Dr. H.A.B. Wösten
Prof. dr. M. Zwietering

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 Typering
Werkgroepen Oost en West Medische Microbiologie
Nederlandse Werkgroep Klinische Virologie
Stichting Kwaliteitsbewaking Medische Microbiologie

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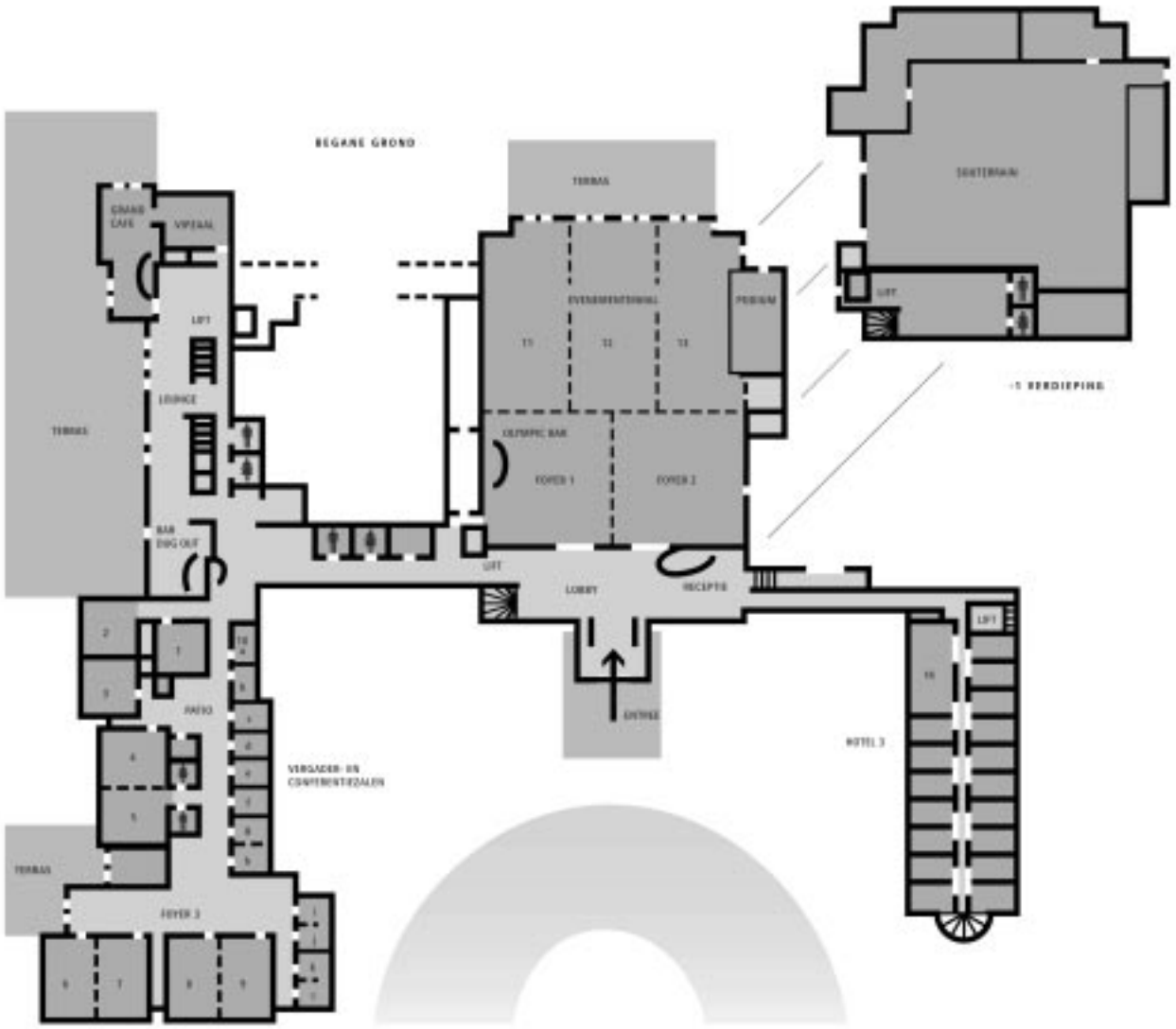

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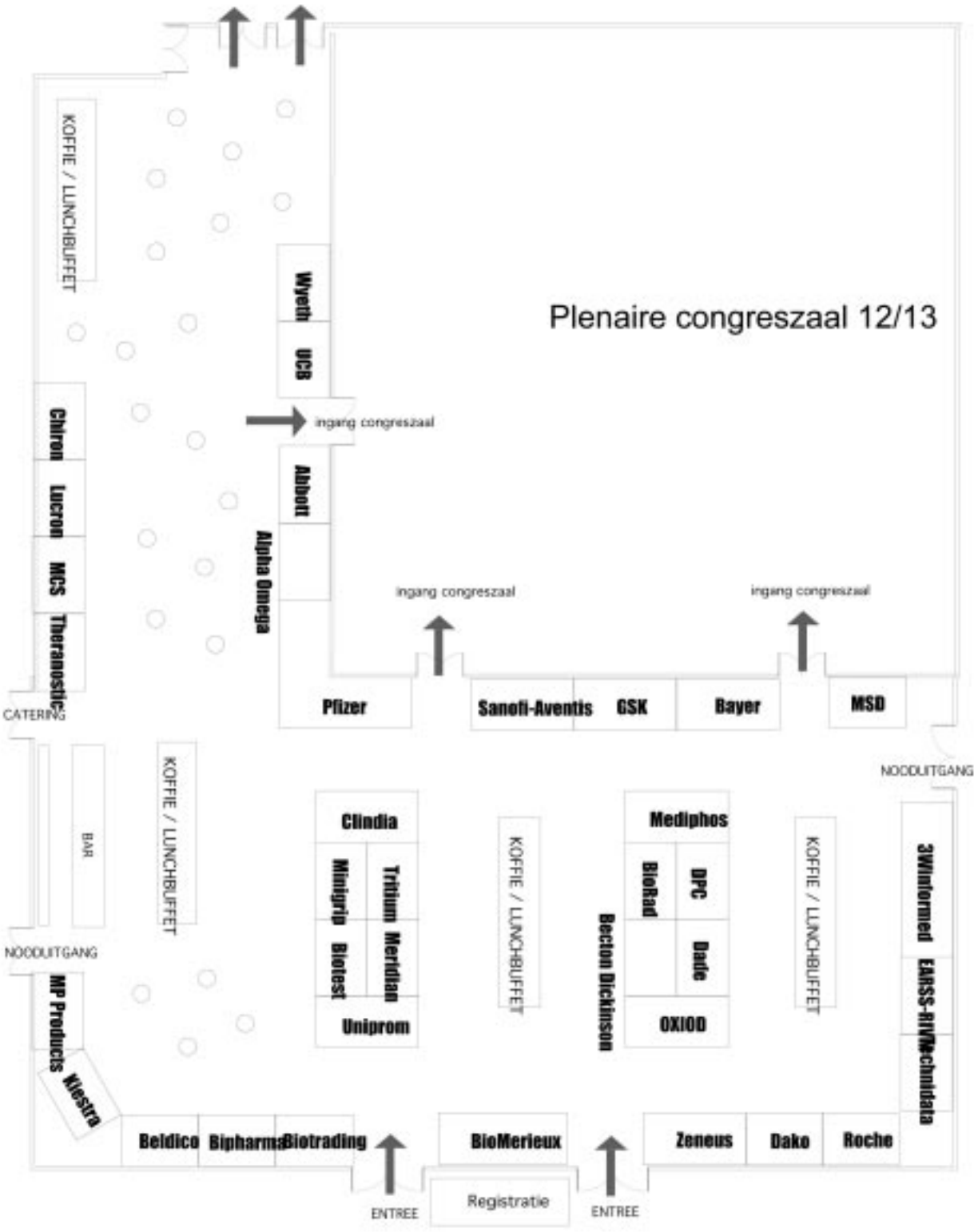
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Maandag 11 april 2005

12.30	Registratie en koffie/thee	Zaal 6/7
13.00	Landelijke toets voor arts-assistenten in opleiding tot arts-microbioloog	
16.00	Koffie/thee	
16.30	Lezingen voor arts-assistenten in opleiding tot arts-microbioloog	
18.30	Diner	
20.30	Lezingen voor arts-assistenten in opleiding tot arts-microbioloog	
21.30	Sluiting	

Dinsdag 12 april 2005

09.00	Registratie en koffie/thee		
09.30	Plenary session 'Microbes in a changing world'	A	Zaal 12/13
11.00	Koffie/thee		
11.15	Plenary session (continued)		
12.45	Lunch en BBC-MMO-vergadering		Zaal 2
14.00	Working group Epidemiological typing	B	Zaal 4/5
	Sectie onderwijs NVvM (NL-talig)	C	Zaal 3
	Pathogenesis - Genes and regulation	D	Zaal Sydney
	Multidisciplinaire sessie - Zoönoses	E	Zaal 8/9
	Progress in Microbiology 1 - Molecular Ecology	F	Zaal 12/13
	Werkgroep Oost en West NVMM - MRSA	G	Zaal 2
15.15	Koffie/thee Working group leaders microbial pathogenesis		Zaal 6/7
15.45	Working group Epidemiological typing (continued)	B	Zaal 4/5
	Pathogenesis - Evasion	D	Zaal Sydney
	Multidisciplinaire sessie - Zoönoses (continued)	E	Zaal 8/9
	Progress in Microbiology (continued)	F	Zaal 12/13
	Werkgroep Oost en West NVMM - MRSA (continued)	G	Zaal 2
	Diagnostics	H	Zaal 3
17.15	Plenaire sessie Uitreiking Kiemprijs Het Centrum Infectieziekten en de medisch microbiologen R. Coutinho (RIVM)	A	Zaal 12/13
18.30	Diner		
20.00	Postersessie		
22.00	Uitreiking Yakult-posterprijzen		

Woensdag 13 april 2005

09.00	Epidemiology	L	Zaal 3
	Medical mycology	M	Zaal Sydney
	Multidisciplinary session - Tuberculosis: New insights in an old disease	N	Zaal 8/9
	NVvM - Microbial diversity and typing	Q	Zaal 12/13
	NWKV - Enteroviruses in clinical practice	R	Zaal 4/5
10.30	Koffie/thee		
11.00	Medical mycology (continued)	M	Zaal Sydney
	Multidisciplinary session - Tuberculosis: New insights in an old disease	N	Zaal 8/9
	NVvM - Microbial diversity and typing (continued)	Q	Zaal 12/13
	NWKV - Tumor virology in clinical practice	R	Zaal 4/5
	Case presentations	S	Zaal 2
	WOGIZ: Infectieziekten en de openbare gezondheidszorg (NL-talig)	T	Zaal 3
12.15	Business meeting NVvM		
13.00	Lunch		
14.00	EPD: Integratie of communicatie (NL-talig)	V	Zaal 8/9
	WMDI Clinical relevance of molecular diagnostics	W	Zaal 4/5
	Pathogenesis, General	X	Zaal Sydney
	NVvM - Progress in Microbiology	Y	Zaal 12/13
15.30	Koffie/thee		
16.00	Business meeting NVMM		Zaal 12/13
18.00	Sluiting		

Maandag 11 april 2005

Zaal 6/7

12.30	Registratie en koffie/thee
13.00	Landelijke toets voor arts-assistenten in opleiding tot arts-microbioloog
16.00	Koffie/thee
16.30	Lezingen voor arts-assistenten in opleiding tot arts-microbioloog
18.30	Diner
20.30	Lezingen voor arts-assistenten in opleiding tot arts-microbioloog
21.30	Sluiting

Dinsdag 12 april 2005

A Zaal 12/13 **Plenary session 'Microbes in a changing world'**

Voorzitters: T. Boekhout, W. Spaan

09.30 - 10.15	S.C. Weaver (Galveston, USA) **Transmission cycles, host range, evolution and emergence of arboviral disease**	A01
10.15 - 11.00	M. Garbelotto (Berkeley, USA) **Phytophthora ramorum and sudden oak death**	A02
11.00 - 11.15	Koffie/thee	
11.15 - 12.00	K.P. Klugman (Atlanta, USA) ***Streptococcus pneumoniae* infections: resistance, therapy and prevention**	A03
12.00 - 12.45	J. Fuhrman (Los Angeles, USA) **Evolving ideas on marine microbial systems, from the microbial loop and viruses to genomics, biogeography and global change**	A04

B Zaal 4/5 **Working group Epidemiological typing**

Voorzitter: P.H.M. Savelkoul

14.00 - 14.30	T.L. Pitt **Pathotyping: added value of virulence gene profiling to inform the clinical significance of bacterial strain types in disease**	B01/02
14.30 - 14.45	L.M. Schouls **State of the art of microbial typing in the Netherlands**	B03
14.45 - 15.15	**WET Plenary Discussion** Topics to address are: When is typing needed and what is the purpose for typing? Is there a difference between epidemiological typing and clinical typing? What methods are available? Costs? Band-based methods versus binary data?	B04/05
15.15 - 15.45	Koffie/thee	

B Zaal 4/5 **Working group Epidemiological typing (continued)**

Voorzitters: A. van Belkum, B. Duim

15.45 - 16.00 M.A.P. van Bergen, K.E. Dingle, M.C. Maiden, L. van der Graaf-van Bloois, J.A. Wagenaar B06
Multi locus sequence typing, a suitable tool for epidemiology and subspeciation of *Campylobacter fetus*

16.00 - 16.15 Nog niet bekend B07

16.15 - 16.30 J.L.W. Rademaker, M.J.C. Starrenburg, H. Herbet, D. Molenaar, J.E.T. van Hylckama Vlieg B08
Genomic and phenotypic diversity *Lactococcus lactis* from dairy and non-dairy origin

16.30 - 16.45 L. Dijkshoorn, L. Dolzani, R. Bressan, T.J.K. van der Reijden, E. van Strijen, D. Stefanik, H. Heersma, H. Seifert B09
Standardization and inter-laboratory reproducibility assessment of Pulsed Field Gel Electrophoresis for the generation of fingerprints of *Acinetobacter baumannii*

16.45 - 17.00 H.J.A. de Valk, I.M. Curfs, J.W. Mouton, J.F.G.M. Meis, C.H.W. Klaassen B10
Exact and high resolution fingerprinting of *Aspergillus fumigatus* isolates using a Novel Multicolor Multiplex STR Assay

17.00 - 17.15 F. Hagen, D.J.C. Gerits, E.E. Kuramae, W. Meyer, T. Boekhout B11
A detailed AFLP analysis on the *Cryptococcus gattii* Vancouver Island outbreak isolates

C Zaal 3 **Sectie onderwijs NVvM - Onderwijs in de microbiologie, problemen en uitdagingen (Nederlandstalige sessie)**

Voorzitter: L. van Alphen

14.00 - 14.15 L. van Alphen C01
Inleiding en voorstellen van de sectie door de voorzitter

14.15 - 14.30 K. Eijkemans C02
Competenties in het hoger beroepsonderwijs, uitdagingen en kansen tot vernieuwing

14.30 - 14.45 J. Jacobs C03
PGO in de medische microbiologie binnen de faculteit geneeskunde

14.45 - 15.00 H. Noordergraaf C04
Hoe onzichtbaar is microbiologie in het onderwijs? Waar zijn biotechnologen?

D Zaal Sydney **Pathogenesis - Genes and regulation**

Voorzitter: S.A.J. Zaat

14.00 - 14.30 J. Van Eldere D01/02
Staphylococcal foreign body infections

14.30 - 14.45 C.A.N. Broekhuizen, L. de Boer, K. Schipper, C.D. Jones, S. Quadir, R.G. Feldman, C.M.J.E. Vandenbroucke-Grauls, S.A.J. Zaat D03
Antibodies increase adherence of *Staphylococcus epidermidis* to biomaterials in an *in vivo* murine model of biomaterial-associated infection

14.45 - 15.00	F.D. Ernst, J.G. Kusters, R. Sarwari, A. Heijens, J. Stoof, C. Belzer, E.J. Kuipers, A.H.M. van Vliet **The NikR protein mediates nickel-responsive induction of *Helicobacter pylori* urease via binding to the ureA promoter**	D04
15.00 - 15.15	R.G.J. Pot, E.J. Kuipers, A.H.M. van Vliet, J.G. Kusters **UreA2B2: a second urease system in the gastric pathogen *Helicobacter felis***	D05
15.15 - 15.45	Koffie/thee	

D Zaal Sydney — Pathogenesis - Evasion

Voorzitter: J.A.G. van Strijp

15.45 - 16.00	M.P. Bergman, A. Engering, H.H. Smits, S.J. van Vliet, A.A. van Bodegraven, H.P. Wirth, M.L. Kapsenberg, C.M.J.E. Vandenbroucke-Grauls, Y. van Kooyk, B.J. Appelmelk ***Helicobacter pylori* modulates the Th1/Th2 balance through phase variable interaction between lipopolysaccharide and the dendritic cell lectin DC-SIGN**	D06
16.00 - 16.15	B.J. Appelmelk, T. Lowary, E.J. Brown, P. Willemsen, P. van der Ley, C.M.J.E. Vandenbroucke-Grauls, W. Bitter **Mannose cap-lacking mutants in mycobacterial lipoarabinomannan**	D07
16.15 - 16.30	P.J. Haas, C.J.C. de Haas, M.J.J.C. Poppelier, K.P.M. van Kessel, J.A.G. van Strijp, K. Dijkstra, R.M. Scheek, H. Fan, J.A.W. Kruijtzer, R.M.J. Liskamp, J. Kemmink **Chemotaxis Inhibitory Protein of *Staphylococcus aureus* defines a versatile structural fold**	D08
16.30 - 16.45	S.H.M. Rooijakkers, M. Ruyken, A. Roos, M.R. Daha, J.S. Presanis, R.B. Sim, T. van Steeg, W.J.B. van Wamel, K.P.M. van Kessel, J.A.G. van Strijp **Staphylococcal Complement Inhibitor (SCIN) prevents complement activation via all three pathways**	D09
16.45 - 17.00	A.P. Heikema, P.C.R. Godschalk, M. Gilbert, C.W. Ang, J. Glerum, N. Yuki, B.C. Jacobs, T. Komagamine, A. van Belkum, H.P.H. Endtz **The crucial role of *Campylobacter jejuni* genes in autoimmune antibody induction**	D10
17.00 - 17.15	A.M. van der Sar, A.H. Meijer, E. Salas-Vidal, H.P. Spaink, F.J. Verbeek, C.M.J.E. Vandenbroucke-Grauls and W. Bitter **The *Mycobacterium marinum*-zebrafish infection model: host transcriptome profiling**	D11

E Zaal 8/9 — Multidisciplinaire sessie Zoönoses (Nederlandstalige interactieve sessie)

Voorzitters: J.M.D. Galama, L.M. Kortbeek

14.00 - 14.25	J. Galama **Een kind met een huidafwijking**
14.25 - 14.50	J. Tolboom **Een kind met diarree**
14.50 - 15:15	B. Mulder, J. van der Giessen **Kalveren met blaaswormen**
15.15 - 15.45	Koffie/thee

15.45 - 16.10 R. van Oosterom en een arts-microbioloog
Luchtwegaandoeningen

De sessie wordt afgesloten met een algemene discussie.

F Zaal 12/13 Progress in Microbiology 1 - Molecular Ecology

Voorzitter: M.S.M. Jetten

14.00 - 14.15 M.J. Foti, D.Y. Sorokin, S. Ma, J.L.W. Rademaker, J.G. Kuenen, G. Muyzer F01
Ecology of halo-alkaliphilic sulphur oxidizing bacteria

14.15 - 14.30 M. Strous, I. Cirpus, L. van Niftrik, H.R. Harhangi, H.J.M. Op den Camp, D. Le Paslier, J. Weissenbach, M. Wagner, M.S.M. Jetten F02
Analysis of the genome and proteome of the anammox bacterium *Kuenenia stuttgartiensis*

14.30 - 14.45 I. Cirpus, H.J.M. Op den Camp, J.G. Kuenen, M. Strous, D. Le Paslier, W. Pluk, E. Lasonder, J. Allen, M.S.M. Jetten F03
Importance of cytochromes in the metabolism of the anammox bacterium *Kuenenia stuttgartiensis*

14.45 - 15.15 H. Bolhuis F04/05
'*Haloquadratum walsbyi*'; isolation and preliminary insight in its genome

15.15 - 15.45 Koffie/thee

F Zaal 12/13 Progress in Microbiology (continued)

Voorzitter: H.A.B. Wösten

15.45 - 16.00 S.J.C.M. Oomes, J.O. Hehenkamp, A.C.M. van Zuijen, S. Brul F06
Genomics tools used in food production

16.00 - 16.15 B.J.F. Keijser, S.J. Oomes, H. van der Spek, S. Brul F07
Genetic analysis of spore germination and the effects of thermal spore injury

16.15 - 16.30 L.M. Hornstra, Y.P. de Vries, M.H.J. Wells-Bennik, W.M. de Vos, T. Abee F08
Characterization of the germination receptors of *Bacillus cereus* ATCC 14579

16.30 - 16.45 R. Kort, A.C. O' Brien, I.H.M. van Stokkum, S.J.C.M. Oomes, W. Crielaard, K.J. Hellingwerf, S. Brul F09
Assessment of heat resistance of bacterial spores from food product isolates by fluorescent monitoring of dipicolinic acid release

16.45 - 17.00 A. S. Ter Beek, C. Blohmke, B. Keijser, S. Brul F10
Weak Acid Stress in *Bacillus subtilis*; the responses of *Bacillus subtilis* towards Sorbic Acid

17.00 - 17.15 P.L.E. Bodelier, S.R. Mohanty, V. Floris H.J. Laanbroek, R. Conrad F11
Differential effects of nitrogenous fertilizers on methane consuming microbes: 'Microbial diversity matters to global fluxes!?'

G Zaal 2 — Werkgroep Oost en West NVMM - MRSA

Voorzitter: J.A. Kaan

14.00 - 14.30	A.C.A.P. Leenders **Screening op MRSA in het routine-laboratorium**	G01/02
14.30 - 14.50	R. Hendrix **Experimentele MRSA-diagnostiek**	G03
14.50 - 15.15	E.A.N.M. Mooi-Kokenberg, T. Koster **Eradication of carriage of methicillin-resistant *Staphylococcus aureus* in medical personnel**	G04/05
15.15 - 15.45	Koffie/thee	

G Zaal 2 — Werkgroep Oost en West NVMM - MRSA (continued)

Voorzitter: R. Vreede

15.45 - 16.15	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* in companion animals**	G06/07
16.15 - 16.45	W.J.B. Wannet, M.E.O.C. Heck, G.N. Pluister, E. Spalburg, M.G. van Santen, X.W. Huijsdens, E. Tiemersma, D. Beaujean, A.J. de Neeling **Panton-Valentine leukocidin positive MRSA: the Dutch situation**	G08/09
16.45 - 17.00	**Uitslag van de 'Enquête Werkgroepen Oost-West'**	

H Zaal 3 — Diagnostics

Voorzitter: J.E. Degener

15.45 - 16.00	R.J. van den Berg, E.S. Bruijnesteijn van Coppenraet, H.J. Gerritsen, H.P. Endtz, E.R. van der Vorm, E.J. Kuijper **Rapid detection of *Clostridium difficile*-associated diarrhea in a prospective multi-center study, using a new immunoassay and real-time PCR**	H01
16.00 - 16.15	J.D.F. de Groot-Mijnes, A. Rothova, A.M. Loon, M. Schuller, B. Benaissa, et al **The contribution of PCR and analysis of intraocular antibody production to the diagnosis of infectious uveitis**	H02
16.15 - 16.30	J. Gooskens, K.E. Templeton, E.C.J. Claas, V.T.H.B.M. Smit, A.C.M. Kroes **Real-time quantitative detection of herpes simplex virus DNA in the lower respiratory tract**	H03
16.30 - 16.45	K.E. Templeton, S.A. Scheltinga, W.C.J.F.M. van den Eeden, A.W. Graffelman, P.J. van den Broek, E.C.J. Claas **Comparison of real-time PCR and conventional methods to determine etiology of community-acquired pneumonia**	H04
16.45 - 17.00	R.P.H. Peters, P.H.M. Savelkoul, A.M. Simoons-Smit, S.A. Danner, C.M.J.E. Vandenbroucke-Grauls, M.A. van Agtmael **Shorter time to identification of pathogens in positive blood cultures by FISH in routine practice**	H05

17.00 - 17.15	M. Bovers, M. Diaz, J. Fell, T. Boekhout **Luminex xMAP technology: a new reliable method to detect *Cryptococcus neoformans* and *Cryptococcus gattii***	H06

A Zaal 12/13	**Plenary session**	
	Voorzitter: G.J.H.M. Ruijs	
17.15 - 18.00	**Uitreiking Kiemprijs**	
18.00 - 18.30	R. Coutinho **Het Centrum Infectieziekten en de medisch microbiologen**	A06

P	**Postersessie en uitreiking Yakult posterprijzen**	
20.00 - 21.00	**Posterpresentatie oneven posternummers**	
21.00 - 22.00	**Posterpresentatie even posternummers**	
22.00	**Uitreiking posterprijzen**	

Woensdag 13 april 2005

L Zaal 3 — Epidemiology

Voorzitter: E.R. van der Vorm

09.00 - 09.15 — M.E.A. de Kraker, E.W. Tiemersma, A.J. de Neeling, N. Bruinsma, J.C.M. Monen, H. Grundmann — L01
Antimicrobial resistance in *Escherichia coli* in the Netherlands and Europe: results from the European Antimicrobial Resistance Surveillance System (EARSSS)

09.15 - 09.30 — L.M. Kortbeek, T.G. Mank — L02
***Giardia* and *Cryptosporidium* in the Netherlands**

09.30 - 09.45 — M.J. Mooij, I. Schouten G. Vos, A. Van Belkum, C.M.J.E. Vandenbroucke-Grauls, P.H.M. Savelkoul, C. Schultsz — L03
Association between ciprofloxacin resistance *Escherichia coli* and integron class 1

09.45 - 10.00 — C.S. de Brouwer, E.C.J. Claas, E.P.A. de Klerk, A.C. Lankester, C. Malipaard, M.J.D. van Tol, A.C.M. Kroes — L04
Sequential emergence of multiple adenovirus serotypes after pediatric stem cell transplantation

10.00 - 10.15 — B. Zwart, C. Visser, J. Kok, C.M.J.E. Vandenbroucke-Grauls — L05
Outbreak of *B. pertussis* on a neonatal intensive care unit (NICU) and the role of macrolide prophylaxis

10.15 - 10.30 — J. Top, R.J.L. Willems, A. Troelstra, H. Blok, M.J.M. Bonten — L06
Molecular epidemiology of Ampicillin resistant *Enterococcus faecium* in the UMC-U hospital

M Zaal Sydney — Medical mycology

Voorzitters: G.S. de Hoog, P.E. Verweij

09.00 - 09.15 — D. Delfino, M. Benecchi, F. Fanti, S. Galatioto, G. Manti, G.S. de Hoog, V. Cusumano — M01
Recurrent brain abscess caused by *Cladophialophora bantiana* in a drug abuser: case report

09.15 - 09.30 — D. Shu-wen, G.S. Bulmer, H. Yan — M02
Identification of Dermatophytes isolated from tinea capitis in western China using ITS sequencing

09.30 - 09.45 — B.L. Rottier, S. van der Heide, H. Hovenga, H.F. Kauffman — M03
A case of a child with cystic fibrosis and infection with *Aspergillus fumigatus* and a *Pseudoallescheria boydii*: clinical parameters and serology

09.45 - 10.00 — E. Fréalle, F. Soula, C. Noël, N. Nolard, F. Symoens, E. Dei-Cas, D. Camus, E. Viscogliosi, L. Delhaes — M04
Fungal manganese superoxide dismutase (MnSOD) genes: from phylogeny to the molecular diagnosis of invasive mycoses

10.00 - 10.15 — J.S. Zeng, D.A. Sutton, G.S. de Hoog — M05
Identification and pathogenicity of clinical isolates of genus *Exophiala* from the USA

10.15 - 10.30 — G.S. de Hoog, P. Zalar, A.H.G. Gerrits van den Ende, N. Gunde-Cimerman — M06
Relation of halotolerance to human-pathogenicity in the fungal Tree of Life: an overview of ecology and evolution under stress

10.30 - 11.00	Koffie/thee	

M Zaal Sydney **Medical mycology (continued)**

11.00 - 11.15	K.E. Templeton, J. Gooskens, E.C.J. Claas, E.J. Kuijper **Rapid diagnosis of PCP and resistance to co-trimoxazole using real-time PCR**	M07
11.15 - 11.30	M. Bovers, F. Hagen, B. Theelen, E. Kuramae, T. Boekhout **Multi-locus sequencing raises new questions in the *Cryptococcus neoformans* species complex**	M08
11.30 - 11.45	R.R. Klont, W. van der Velden, N.M.A. Blijlevens, J.P. Donnelly, P.E. Verweij **Primary hepatic invasive aspergillosis (IA) in a hematopoietic stem cell transplant (HSCT) recipient**	M09
11.45 - 12.00	R.R. Klont, N.M.A. Blijlevens, J.P. Donnelly, P.E. Verweij **Failure of caspofungin (CAS) as primary treatment of proven invasive aspergillosis (IA) in a hematopoietic stem cell (HSCT) transplant recipient**	M10

N Zaal 8/9 **Multidisciplinary session - Tuberculosis: New insights in an old disease**

Voorzitter: B.J. Appelmelk

09.00 - 09.30	M. Borgdorff **Epidemiology of tuberculosis, worldwide and in the Netherlands**	N01/02
09.30 - 10.00	W. Bitter **Virulence factors of *Mycobacterium tuberculosis***	N03/04
10.00 - 10.30	T.H.M. Ottenhoff **Immunogenetics of tuberculosis**	N05/06
10.30 - 11.00	Koffie/thee	

N Zaal 8/9 **Multidisciplinary session - Tuberculosis: New insights in an old disease (continued)**

11.00 - 11.45	S.M. Arend **Tuberculosis as an old acquaintance with new faces: Clinical manifestations, the risk of TNF-alpha blockade, and specific immunodiagnosis**	N07/09
11.45 - 12.00	P.H.M. Savelkoul **Molecular diagnosis of tuberculosis**	N10
12.00 - 12.15	H.R. van Doorn **Resistance in *Mycobacterium tuberculosis*: epidemiology and molecular detection**	N11

Q Zaal 12/13 **NVvM - Microbial diversity and typing**

Voorzitter: A. van Belkum

09.00 - 09.30	J.Wells **Mechanisms of genetic variability in foodborne bacterial pathogens**	Q01/02
09.30 - 09.45	E.J. Kuijper, J.S. Kalpoe, C.H.W. Klaassen, K.E. Templeton, A.M. Horrevorts, H. Endtz **Phenotypical characterization, antimicrobial resistance and molecular typing of 23 clinical isolates of *Nocardia farcinica* in the Netherlands**	Q03

09.45 - 10.00 C.C.G.M. Booijink, E.G. Zoetendal, H. Smidt, M. Kleerebezem, W.M. de Vos Q04
Functional microbiomics: elucidation of the functionality of the human GI-tract microbiota

10.00 - 10.15 E. van Zanten, T. Schuurman, A.M.D. Kooistra-Smid, A.A. van Zwet Q05
A cost-effectiveness study comparing real-time PCR with traditional culture for detection of *Salmonella* spp. and *Campylobacter jejuni* in feces

10.15 - 10.30 E.J. Gaasbeek, F.J. van der Wal, J.A. Wagenaar, J.P.M. van Putten Q06
Clonal *Campylobacter jejuni* strains are deficient in DNA competence

10.30 - 11.00 Koffie/thee

Q Zaal 12/13 NVvM - Microbial diversity and typing (continued)

Voorzitter: S. Brul

11.00 - 11.15 J. van de Vossenberg, M. Schmid, M. Kuypers, J. Sinninghe Damste, N. Risgaard-Petersen, M. Jetten, M. Strous Q07
Marine anaerobic ammonium oxidizing bacteria

11.15 - 11.30 D. van Soolingen Q08
Improvements in the secundary laboratory diagnosis of tuberculosis

11.30 - 11.45 F.J. van der Wal, J.R. Dijkstra, E.A.E. Geerts, J.A. Frost, J. Waldenstrom, W.F. Jacobs-Reitsma, J.A. Wagenaar Q09
Nalidixic acid resistance in *Campylobacter lari*

11.45 - 12.00 P.J.M. Steenbakkers, S. Mattijssen, M.S.M. Jetten, J.T.M. Keltjens Q10
Identification of proteins binding to the pseudomurein cell wall of *Methanothermobacter thermautotrophicus*

12.00 - 12.15 S.M. Bialek, B.J.F. Keijser, M. Machczynski, G.W. Canters, R. van der Heijden, E. Vijgenboom Q11
A proteomics approach to study the copper homeostasis - development relation in *Streptomyces lividans*

R Zaal 4/5 NWKV - Enteroviruses in clinical practice

Voorzitter: J.M.D. Galama R

09.00 - 09.15 P. van den Broek, H. Shimizu, J. Maas, M. Luken, G. Koen, C. Li, A. Utama, T. Miyamura, M. Beld, H. Zaaijer, B. Berkhout, L. van der Hoek and R. Mang R01
A novel human enterovirus in faecal samples from HIV1 infected persons and patients with acute flaccid paralysis

09.15 - 09.30 C.M.A. Swanink, H. Vennema, H.G. van der Avoort, M.P.G. Koopmans R02
A newly identified parechovirus causing sepsis-like syndrome in neonates

09.30 - 09.45 W. Melchers, S. Strijbosch, J. Bakkers, J. Galama R03
Molecular diagnosis of Enteroviruses

09.45 - 10.00 A.M. van Loon R04
External quality assessment of nucleic acid amplification techniques for the detection of Enteroviruses

10.00 - 10.15	J. Galama, M. de Bruijni, M. Kramer A. Boot, C. Rongen-Westerlaken, W. Melchers, G. Adema, F. van Kuppeveld **Enteroviruses and type 1 diabetes mellitus (T1D)**	R05
10.15 - 10.30	H.G.A.M. van der Avoort, also on behalf of Dutch Working Group on Clinical Virology (NWKV) **Enterovirus surveillance: 1996-2003**	R06
10.30 - 11.00	Koffie/thee	

R Zaal 4/5 NWKV - Tumor virology in clinical practice

Voorzitter: A.C.M. Kroes

11.00 - 11.15	A.C.M. Kroes **Introduction tumor virology in clinical practice**	R07
11.15 - 11.30	J. ter Schegget, J.N. Bouwes Bavinck, M.C.W. Feltkamp **Human papillomavirus (HPV) in cervical and cutaneous tumors**	R08
11.30 - 11.45	M. Cornelissen, A. Polstra, R. van den Burg, F. Zorgdrager, B. Berkhout, T. van der Kuyl **Human herpes virus 8: virology and disease**	R09
11.45 - 12.00	J.S. Kalpoe, P.B. Douwes Dekker, J.H.J.M. van Krieken, R.J. Baatenburg de Jong, A.C.M. Kroes **Epstein-Barr virus and nasopharyngeal carcinoma: practical role of viral DNA detection**	R10
12.00 - 12.15	G.J. Boland **Hepatocellular carcinoma and hepatitis B and C virus**	R11

S Zaal 2 Case presentations

Voorzitter: J. Verhoef

11.00 - 11.15	P.C.A.M. Buijtels, P.L.C. Petit, A. van Belkum, D. van Soolingen **Isolation of clinically relevant nontuberculous mycobacteria in Zambia; eight case reports**	S01
11.15 - 11.30	N. Vaessen, C. van Nieuwkoop, Y.W.J. Sijpkens, A.C.M. Kroes **Recurrent chickenpox in renal transplant recipients**	S02
11.30 - 11.45	T. van der Brugge, E. Gomez-Sanchez, F.J.E.M. Blomjous, M. Tersmette, V.A.M. Duurkens **Pulmonary *Enterobius vermicularis* infection, a case report**	S03
11.45 - 12.00	L.M. Kortbeek, J. Jager, A.A. van Zwet, J.W.B. van der Giessen **Endemic Echinoccocosis in the Netherlands?**	S04
12.00 - 12.15	J.W.B. van der Giessen, M. Fonville, I. Briels, A. de Vries, P. Teunis, E. Pozio **Genetic diversity of encapsulated and non-encapsulated *Trichinella* by studying the 5S rDNA tandemly repeated intergenic region and isolation of the first *T. pseudospiralis* in the Netherlands**	S05

T Zaal 3 — **WOGIZ: Infectieziekten en de openbare gezondheidszorg (Nederlandstalige sessie)**

Voorzitters: P.M. Schneeberger, B. Mulder

11.00 - 11.15	P. Schneeberger **WOGIZ, COGIZ en Centrum Infectieziekten**	T01
11.15 - 11.30	A. Timen, B. Mulder **LCI-richtlijnen en de arts-microbioloog**	T02
11.30 - 11.45	H. Wertheim **LCI-richtlijn community onset MRSA**	T03
11.45 - 12.00	A.M. Horrevorts, A. Bosman **ISIS: nieuwe ontwikkelingen**	T04
12.15 - 14.00	Lunch	

V Zaal 8/9 — **EPD: Integratie of communicatie (Nederlandstalige sessie)**

Voorzitter: M. Tersmette

14.00 - 14.30	B.L. Kabbes **Introductie HL7-standaard**	V01/02
14.30 - 15.00	W. Kalis **Informatie- en berichtenstromen toegespitst op de medische microbiologie m.b.v. Hl7-berichten**	V03/04
15.00 - 15.30	J.H.H. Houben **IHE: Integrating the Healthcare Enterprise**	V05/06

W Zaal 4/5 — **WMDI Clinical relevance of molecular diagnostics**

Voorzitters: P.H.M. Savelkoul, R. Schuurman

14.00 - 14.30	J.W.A. Rossen, J.J. Oosterheert, R. Schuurman, G. Nossen, A. Hoepelman, M. Bonten, A.M. van Loon **Cost-effectiveness of routine real-time PCR for the aetiological diagnosis in adults hospitalised with lower respiratory tract infections**	W01/02
14.30 - 14.45	J.S. Kalpoe, E.F. Schippers, Y. Eling, Y.W. Sijpkens, J.W. de Fijter, A.C.M. Kroes **Similar reduction of cytomegalovirus DNA load by oral valganciclovir and intravenous ganciclovir on pre-emptive therapy after renal and pancreas transplantation**	W03
14.45 - 15.00	T. Mohamadi, H.W. Reesink, C.M.J.E. Vandenbroucke-Grauls, P.H.M. Savelkoul **Quantitation of 16S ribosomal DNA and RNA as a new approach to monitor the presence and state of viability of bacteria**	W04
15.00 - 15.15	S.R. Konstantinov, H. Smidt, P. Bosi, M. de Vos **Representational difference analysis and real-time PCR for strain-specific quantification of porcine commensals closely related to *Lactobacillus amylovorus***	W05
15.15 - 15.30	C.F.M. Linssen, J.A. Jacobs, P. Beckers, K.E. Templeton, J. Bakkers, E.J. Kuijpers, W.J.G. Melchers, M. Drent, C. Vink **Inter-laboratory agreement of three real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples**	W06

X Zaal Sydney — Pathogenesis, General

Voorzitter: P. Hermans

14.00 - 14.15 — J.J.E. Bijlsma, E.A. Groisman — X01
The PhoP/PhoQ system controls expression of the intramacrophage type three secretion system of *Salmonella enterica*

14.15 - 14.30 — A.M. Abdallah, T. Verboom, C.M.J.E. Vandenbroucke-Grauls, J. Luirink, W. Bitter — X02
PPE protein Rv2430c is secreted by pathogenic mycobacteria

14.30 - 14.45 — P.J. Burghout, T.G. Kloosterman, J.J.E. Bijlsma, H.J. Bootsma, P.W.M. Hermans, O.P. Kuipers — X03
Development of genomic array footprinting to identify conditionally essential genes in *Streptococcus pneumoniae*

14.45 - 15.00 — A. Bart, M.M. Feller, A. van der Ende — X04
Different roles of the *Neisseria meningitidis* outer membrane export proteins in susceptibility to antimicrobial agents

15.00 - 15.15 — H.J. Bootsma, C.A. Cummings, D.A. Relman, J.F. Miller — X05
Comparative analysis of the BvgAS transcriptional regulon in *B. pertussis* and *B. bronchiseptica*

15.15 - 15.30 — S. Ouburg, J.M. Lyons, J. Land, J.B.A. Crusius, J. Pleijster, J.I. Ito, A.S. Peña, S.A. Morré — X06
The role of the bacterial CpG sensing Toll-like receptor 9 in *Chlamydia trachomatis* female genital tract infection: the knockout mouse and human candidate gene approaches

Y Zaal 12/13 — NVvM - Progress in Microbiology

Voorzitter: H.A.B. Wösten

14.00 - 14.15 — J. Dijksterhuis, R. Samson, H. Wösten, L. Lugones — Y01
PLAY, an abundant ascospore cell wall protein in *Talaromyces macrosporus*

14.15 - 14.30 — F.E.J. Coenjaerts, A.I.M. Hoepelman, J. Scharringa, M. Aerts, P.M. Ellerbroek, L. Bevaert, J.A.G. van Strijp, G. Janbon — Y02
Stress-response regulation in *Cryptococcus neoformans*

14.30 - 14.45 — A. Vinck, M. Terlou, W.R. Pestman, E.P. Martens, A.F. Ram, C.A.M.J.J. van den Hondel, H.A.B. Wösten. — Y03
Fungi deploy specialized hyphae for waste processing

14.45 - 15.00 — K.G.A. van Driel, A.F. van Peer, H.A.B. Wösten, A.J. Verkleij, W.H. Müller, T. Boekhout — Y04
Isolation of septal pore caps from basidiomycetous fungi

15.00 - 15.30 — E.E. Kuramae, V. Robert, B. Snel, M. Weiß, T. Boekhout — Y05/06
Analysis of shared proteins: a promising method to resolve the eukaryotic Tree of Life

A01
Transmission cycles, host range, evolution and emergence of arboviral disease

S.C. Weaver
Center for Biodefense and Emerging Infectious Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA

Most arthropod-borne RNA viruses use one or more of 3 basic mechanisms to cause human disease: 1) direct spillover from zoonotic transmission cycles involving arthropod vectors and wild animal reservoir hosts; 2) secondary amplification in domestic animals, leading to increased levels of circulation and enhanced spillover to humans, and 3) adaptation to humans as amplification and/or reservoir hosts. Examples of each mechanism will be reviewed, with emphasis on host range changes in the alphavirus Venezuelan equine encephalitis virus (VEEV) and the flaviviruses, dengue viruses (DENV). Phylogenetic studies of VEEV strains indicate that epidemics arise when enzootic strains, which normally circulate in sylvatic habitats among rodent hosts, mutate and adapt to amplify in equines via high titer viremia. Reverse genetic studies indicate that the epizootic (equine amplification-competent) phenotype is determined by only 1-2 mutations in the E2 envelope glycoprotein. Similar mutations also adapt VEEV for more efficient infection of mosquitoes that transmit in agricultural settings. The 4 serotypes of DENV, which have their origins in sylvatic transmission cycles involving nonhuman primate reservoir hosts and arboreal mosquito vectors, emerged independently by adapting to more efficiently infect the peridomestic mosquito vectors *Aedes albopictus* and *Ae. aegypti*. Finally, experimental model systems for studying evolutionary constraints on host range changes by arboviruses will be discussed.

A02
Phytophthora ramorum and sudden oak death

M. Garbelotto
Forest Pathology and Mycology, Extension Specialist & Adjunct Professor, Department of Environmental Science, Policy and Management, Ecosystem Sciences Division, 151 Hilgard Hall, University of California, Berkeley, CA 94720, USA

Phytophthora ramorum is a recently described plant pathogen. Originally isolated in European nurseries from Rhododendron and Viburnum, it was found to be the causal agent of an extremely serious emergent forest disease in California. The disease, known as Sudden Oak Death, has killed tens of thousands of oaks and tanoaka in coastal forests of California around the San Francisco Bay Area, and has received significant attention by the public, the media, and the governments of several countries. Although oaks are killed by girdling canker stems, the disease does not sporulate on oaks. Alternative hosts are necessary for the spread of the disease which, first among forest Phytophthoras of the temperate world, is aerial in nature. Leaves of bay laurels in California forests and leaves of rhododendrond and camellias are excellent substrates for sporulation.
While bay laurels play a key epidemiological role in the natural spread of the disease, the lesions caused by this pathogen are strictly confined to the leaves with minimal effects on the overall health of teh infected trees. Rhododendrons and camellias instead, suffer a significant die-back disease when infected by *P. ramorum*. Despite the presence of obvious symptoms, the nursery trade, with its sanitation efforts and treatment regimes has spread the disease among nurseries across the world via plants with masked symptoms: e.g. across Europe, from California and Oregon to the East Coast of the United States and from Europe to the North American Pacific Northwest. Molecular analyses have shown that the original SOD epidemics was not caused by a strain imported from Europe, as the European and the US lineages are clearly distinct from one another, and have highlighted and extreme narrow genetic diversity in both continents.
Despite this genetic diversity we have observed a huge phenotypic variability across different isolates of the pathogen, even within the same genotype. Recent analyses have uncovered a third lineage that contains some alleles of both Euroepan and US populations. This lineage is maybe representative of the broader P. ramorum population from its native environment. All the information points to an exotic origin of this pathogen for both Europe and North America. Molecular analyses have also been useful to set up one of the largest DNA-based diagnostixc programs in the USA.

A03
Streptococcus pneumoniae infections: resistance, therapy, prevention

K.P. Klugman
Professor of Global Health, Department of Global Health, Rollins School of Public Health, Professor of Medicine, Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, Georgia; Director, Respiratory and Meningeal Pathogens Research Unit of the NICD/MRC/Witwatersrand University, Johannesburg, South Africa

High levels of drug resistance in the pneumococcus remain a global problem with considerable evidence pointing to inappropriate antibiotic use as a major driver of resistance. In this regard, the Netherlands has contained resistance to amongst the lowest levels in the developed world. New trends in antimicrobial resistance in the pneumococcus include the emergence of combined erm and mef resistant clones, non-mef macrolide resistance, increasing evidence of first-step fluoroquinolone resistance that is not identified by routine diagnostic testing, and the first linezolid-resistant pneumococci have recently been described. While there are few oral antimicrobial agents available for the management of highly-penicillin resistant pneumococcal otitis media, there is growing consensus that high dose intravenous penicillin remains the drug of choice for pneumococcal

pneumonia. Conflicting data suggest that there may be an advantage to combination therapy for the treatment of severely ill patients with pneumococcal bacteremia, but the biological basis for this observation is undefined. Pneumococcal conjugate vaccine has played a major role in reducing the burden of invasive pneumococcal disease in both children and adults (through herd immunity) in the United States. There has been a dramatic impact on antibiotic resistance in blood isolates, but there is recent evidence of the emergence of increasing antibiotic resistance in non-vaccine types causing upper respiratory tract infections. The use of conjugate vaccine as a probe has identified pneumococcal superinfection as a major reason for hospitalization of children infected with respiratory viruses including influenza and RSV.

A04
Evolving ideas on marine microbial systems, from the microbial loop and viruses to genomics, biogeography and global change

J. Fuhrman
University of Southern California, Los Angeles, CA 90089, USA

Two decades ago, the 'Microbial Loop' was recognized as critical to global flux of carbon and nutrients via DOM cycling. Subsequent studies showed viruses are 10 times as abundant as bacteria and influence matter & energy flux and community composition. With 16S rRNA-based analysis, we learned that deep communities are ~40% archaea, which recent work suggests may be mixotrophs, combining chemosynthetic and heterotrophic lifestyles. New studies show phototrophy is unexpectedly functionally diverse, with bacteriochlorophyll a and proteorhodopsin-based solar energy capture common – yet these organisms are probably also mixotrophs, surviving without light. Some reports on bacteriochlorophyll a-containing bacteria appear to have exaggerated their numbers, but proteorhodopsin may be very common, as Venter's shotgun sequencing study of the Sargasso Sea found > 780 proteorhodopsin genes in 13 subfamilies.

Early 16S work showed about 10 major divisions of marine prokaryotes worldwide; newer studies are showing remarkable 'microdiversity' with hundreds or thousands of close relatives coexisting. Whole genome sequences from cyanobacteria show even close relatives can have surprisingly different ecological niches, and suggest viruses play a key role in maintaining diversity. Biogeographic studies in our lab show remarkable geographic structure in community composition, even in the oligotrophic central gyres and deep sea. Our time series studies show microbial communities change over weeks but reassemble themselves annually. New ideas on biogeochemical processes demonstrate the significance of Fe and maybe other metals in airborne dust, linking to nitrogen fixation and global change. Soon we may integrate all these aspects into a unified picture.

B01/02
Pathotyping: added value of virulence gene profiling to inform the clinical significance of bacterial strain types in disease

T.L. Pitt
Laboratory of HealthCare Associated Infection, Health Protection Agency, Colindale, London NW9 5HT, United Kingdom

Since the time of Pasteur microbiologists have attempted to group bacterial isolates according to their properties and characteristics. In the early years, species were most often subdivided into serotypes and phage susceptibility types and these groups occasionally had biological and clinical significance. For example, some serotypes and phage types were clearly associated with the more invasive disease (*Escherichia coli* K1 and neonatal meningitis) or infection of a specific body site (phage group II strains and skin infections due to *Staphylococcus aureus*). Over time many of these classical systems were replaced by gel electrophoretic patterns and much of the associations between strain 'type' and disease were progressively lost.

However, the introduction of sequence based methods (MLST, binary type, VNTR etc) which give portable and relatively unambiguous type designations has begun to allow the reestablishment of type and disease association. More recently we have seen the introduction of pathotyping which seeks to relate gene complements of strains to their pathogenic potential. Using chip type formats we now have the ability to seek heterogeneity in house keeping genes (core genome) to define strain or clonal types and this can be combined with screens of the accessory genome to identify pathogenicity related genes. The challenge facing microbiologists today is how to use this increase in information to inform epidemiological studies and contribute to the control or possible reduction of the burden of bacterial disease. Some of these issues will be discussed in the context of epidemiological studies of *S. aureus* in the community and *Pseudomonas aeruginosa* in cystic fibrosis patients.

B06
Multi locus sequence typing, a suitable tool for epidemiology and subspeciation of *Campylobacter fetus*

M.A.P. van Bergen[1], K.E. Dingle[2], M.C. Maiden[3], L. van der Graaf-van Bloois[1], J.A. Wagenaar[1], J.A. Wagenaar[4]
[1]Animal Sciences Group, Division of Infectious Diseases, Lelystad, the Netherlands, [2]Nuffield Department of Clinical Sciences, University of Oxford, John Radcliffe Hospital, Department of Microbiology, Oxford, United Kingdom, [3]University of Oxford, The Peter Medawar Building for Pathogen Research and Department of Zoology, Oxford, United Kingdom, [4]Faculty of Veterinary Medicine, Utrecht University, Department of Infectious Diseases and Immunology, Utrecht, the Netherlands

Campylobacter fetus can be divided into subspecies *C. fetus* subsp. *fetus* (Cff) and *C. fetus* subsp. *venerealis* (Cfv). Cff can cause sporadic infections in humans, abortion in cattle and sheep, and can be isolated from a variety of sites in different hosts. In contrast, Cfv is very host restricted and isolated mainly from the genital tract of cattle, being the causative agent of bovine genital campylobacteriosis. Despite these clinical differences, subspeciation using the only available

phenotypic assay (glycine tolerance) has proven difficult. However, this test is still used as a gold standard. Several molecular methods including polymerase chain reaction (PCR), Pulsed Field Gel Electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) have proven useful for subspeciation, but sometimes give contradictory results. In our hands AFLP gives the best results, but independent confirmation of these results is required since subspeciation is economically and epidemiologically important. Multi locus sequence typing (MLST) has proven useful for studying the epidemiology and population genetics of many bacterial species. Therefore, we have developed a MLST scheme for *C. fetus* by specific amplification and sequencing of the loci *aspA, glnA, gltA, glyA, tkt, pgm,* and *unc*A (http://pubmlst.org/cfetus). MLST was performed on chromosomal DNA of 108 reference and field Cff and Cfv isolates typed previously by AFLP. A total of 14 different sequence types (ST) were identified. A very high level of sequence identity was found among the isolates with only 23 variable sites in 3312bp (0.7%). However, all the Cfv strains examined were ST 4, but differed by only one nucleotide from some of the Cff strains. The Cff isolates were more diverse in terms of ST and ST correlated with epidemiological relationships. For example, isolates from previously identified outbreaks had the same ST. We conclude that MLST is a useful tool for 1) subspeciation and 2) epidemiological studies of *C. fetus.*

B08

Genomic and phenotypic diversity *Lactococcus lactis* from dairy and non-dairy origin

J.L.W. Rademaker[1], M.J.C. Starrenburg[2], H. Herbet[1], D. Molenaar[2], J.E.T. van Hylckama Vlieg[2]
[1]*NIZO food research, Health & Safety, Ede, the Netherlands,* [2]*NIZO food research, Flavor, Ede, the Netherlands*

Lactococcus lactis is the primary constituent of many starter cultures used for the manufacturing of fermented dairy products. Over the last decades numerous industrial and private research programmes have resulted in detailed knowledge of the molecular biology and physiology of this organism. At this moment there are three whole genome sequences available and together with the availability of a vast molecular toolbox. *L. lactis* has gained a strong position as a model organism for low-GC Gram-positive micro-organisms.

The model strains that are used in most studies almost exclusively originate from dairy fermentations. In recent years there has been growing interest in isolates from (fermented) plant material. Plant isolates have only been poorly characterised but several examples have been reported that indicate that they may have phenotypes of industrial interest such as a unique flavour forming potential or the production of bacteriocins with a broad mode of action.

Genomics and high-throughput technologies provide the possibility to systematically analyse the phenotypic diversity and relate these to diversity at the genome level. In the present study we report a systematic evaluation of the molecular and functional diversity present in a highly diverse set of 92 *L. lactis* strains from plant and dairy origin. The molecular diversity was studied using repetitive sequence based PCR fingerprinting, 16S rDNA sequencing and a novel Multi Locus Sequence Analysis (MLSA) scheme targeting house-keeping and -functional genes. MLSA showed that plant isolates represent some unique gene sequence types within the species. Phenotypic analysis showed that plant isolates are characterized by their ability to ferment various additional sugars, tolerance to elevated temperature and resistance to high salt and nisin concentrations as compared to dairy isolates. Moreover, the results clearly showed that genetically diverse dairy isolates were phenotypically very similar. This is probably caused by the selection pressure imposed by the dairy environment.

B09

Standardization and inter-laboratory reproducibility assessment of Pulsed Field Gel Electrophoresis for the generation of fingerprints of *Acinetobacter baumannii*

L. Dijkshoorn[1], L. Dolzani[2], R. Bressan[2], T.J.K. van der Reijden[1], E. van Strijen[1], D. Stefanik[3], H. Heersma[4], H. Seifert[3]
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Introduction. A standard procedure for Pulsed Field Gel Electrophoresis (PFGE) of macrorestriction fragments was set up for *Acinetobacter baumannii* and validated for its interlaboratory reproducibility and its potential to construct an internet-based database for regional and international monitoring of epidemic strains.

Methods. PFGE fingerprints of strains were generated at three different laboratories with *Apa*I as restriction enzyme using a rigorously standardized procedure. Digitized fingerprints were centrally analysed by computer-assisted analysis using the Dice coefficient as a similarity measure and UPGMA as a clustering algorithm.

Results. First, 20 *A. baumannii* strains including three isolates from three hospital outbreaks each, and 11 sporadic strains were investigated blindly in each participating laboratory. Central analysis showed 87% matching of corresponding strains if processed at different laboratories. Next, 30 *A. baumannii* isolates representing ten hospital outbreaks at different locations in Europe (three isolates per outbreak) were blindly distributed to the three laboratories so that each participant investigated ten epidemiologically unrelated isolates. Central analysis correctly identified the isolates to their corresponding outbreak at a 87% threshold.

Conclusion. (1) The grouping level at 87% of identical strains and isolates from the same outbreak if processed at different locations indicates that this level can be used to identify epidemiologically related strains.

(2) This finding indicates that an electronic database of fingerprints to monitor the geographic spread of epidemic strains is feasible.

B10

Exact and high resolution fingerprinting of *Aspergillus fumigatus* isolates using a Novel Multicolor Multiplex STR Assay

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Introduction. For assessing genetic and epidemiological relationships between environmental and clinical *Aspergillus fumigatus* isolates, it is important to have reproducible and reliable fingerprinting techniques. Short tandem repeats (STRs) fulfill these criteria and are increasingly being used for micro-organisms. We developed a novel STR fingerprinting assay for *A. fumigatus*.

Methods. Genomic sequences produced by the *A. fumigatus* Sequencing Group at the Sanger Institute (ftp://ftp.sanger.ac.uk/pub/pathogens/A_fumigatus/) were analysed for the presence of short tandem repeats (STRs) using tandem repeats finder. Three perfect di-, tri- and tetranucleotide repeats (AG_{18}, CA_{18}, GA_{26}, TCT_{46}, TAG_{23}, AAG_{20}, $TTCT_{11}$, $CTAT_{10}$ and $ATGT_{8}$) were selected for further analysis. Three multicolour multiplex PCR reactions were developed to simultaneously amplify and label all three di-, tri- or tetranucleotide repeats. The nine STR loci were used to genotype 100 assumedly unrelated isolates recovered from different patients from several hospitals. Amplicons were analysed on a capillary DNA analysis platform (MegaBACE 500). To determine the exact number of repeats in the obtained PCR products, a selected number of fragments were sequenced.

Results. In this population of isolates, the number of alleles varied between 11 and 37 for all loci, resulting in 96 different fingerprints of all 100 isolates. One isolate displayed a mixture of two different *A. fumigatus* strains. The combination of all nine markers yielded a diversity index of 0.9994, indicative of the very high discriminatory power of the technique. In theory, this panel of 9 markers is able to distinguish between more than $2{,}7.10^{10}$ different combinations.

Conclusion. We report a novel exact high resolution fingerprinting assay for *A. fumigatus*. The exact nature of the assay and the high discriminatory power make it a extremely suitable tool for large scale epidemiological studies.

B11

A detailed AFLP analysis on the *Cryptococcus gattii* Vancouver Island outbreak isolates

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The pathogenic basidiomycetous yeast *Cryptococcus gattii* causes a life-threatening disease of the central nervous system, lungs and skin in humans and animals. *C. gattii* can be found mainly in tropical and sub-tropical regions of South America, Asia and Australia where it is endemic. Recently, a cryptococcosis outbreak in both humans and animals occurred on Vancouver Island (British Columbia, Canada).[1] Using different molecular biological tools we found that this outbreak was caused by a rare genotype of *C. gattii* (AFLP 6 or RAPD VGII). The main objective was to know the origin of the outbreak.

All outbreak related strains (n=98) were analyzed by standard Amplified Fragment Length Polymorphism analysis (AFLP). Based on this AFLP analysis, thirty-four outbreak isolates were selected, together with forty additional strains. AFLP with seven different selective primer combinations was used to further analyzed these strains. This analysis was carried out in two-fold and phylogenetic analysis was performed. Reproducible marker fragments were used for population genetic analysis.

All outbreak isolates were identified with the standard AFLP analysis as the rare genotype AFLP 6, two sub genotypes could be distinguished (6A and 6B) with an overall similarity of 91%. The AFLP analysis with seven different selective primer sets revealed the same two clusters: a cluster which contained almost all strains originating from Vancouver Island (6A) and a cluster which contained most of the additional global isolates (6B). Remarkably the clinical isolates from HIV patients are all genotype 6B isolates. The use of seven different selective primer combinations for AFLP analysis resulted in a total of 4810 marker fragments (presence or absence). Most of them are specific for one of the AFLP sub genotypes. Analysis of these marker fragments will give information about the origin of the Vancouver Island outbreak.

References

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D03

Antibodies increase adherence of *Staphylococcus epidermidis* to biomaterials in an *in vivo* murine model of biomaterial-associated infection

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Introduction. The pathogenesis of biomaterial-associated infection (BAI) due to *Staphylococcus epidermidis* involves biofilm formation by the bacteria. Monoclonal antibodies (mAbs) against polysaccharide antigens have been shown to increase phagocytic activity and inhibit adherence of these bacteria to plastic surfaces. We aimed to raise antibodies against major surface protein antigens of *S. epidermidis*, and to assess their possible protective activity in experimental BAI.

Methods. Monoclonal antibodies were raised against immunodominant antigens from mice immunized with a cell wall protein preparation of *S. epidermidis* clinical isolate AMC5. Since LTA is a well known cell wall cell surface-exposed component, anti-LTA monoclonal antibodies (QED Biosciences, UK) were also tested. Two polyvinylpyrrolidone-

coated silicon elastomer (SEpvp) biomaterial segments (BM) were implanted s.c. in mice (C57Bl/6). Mice (9/group) were then injected with different concentrations of mAbs or saline, and challenged 30 min later with 10E7 cfu of *S. epidermidis* AMC5. Mice were sacrificed after 8 days. BM and peri-BM tissue were processed and cultured on blood agar plates and in Brewer Tween liquid medium.

Results. Two major antigens of immunized mice were recognized, which were identified as Accumulation Associated Protein (AAP) and Serine-aspartate repeat protein F (SdrF). AAP was the most immunodominant protein. Anti-AAP and anit-LTA mAbs were used for passive immunization of C57Bl/6 mice. Neither of the two antibodies showed any protective effect. In contrast, bacterial adherence to the biomaterial segments was significantly increased in the group treated with 80mg of anti-LTA. Anti-AAP also increased bacterial adherence to the biomaterial segments, but this effect was not significant. In all infection sites, the tissue biopsies were more often culture positive than the corresponding biomaterial segments.

Conclusions. Antibodies against *S. epidermidis* LTA or AAP did not protect mice against biomaterial-associated infection. Anti-LTA even increased bacterial adherence to the biomaterial. Our study indicates that antibodies against *S. epidermidis* at the concentrations used in this study may contribute to rather than prevent biomaterial-associated infection.

D04

The NikR protein mediates nickel-responsive induction of *Helicobacter pylori* urease via binding to the ureA promoter

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Introduction. To survive in its acidic gastric habitat, *Helicobacter pylori* requires high-level production of the nickel-containing metalloenzyme urease. The nickel-regulatory protein NikR was previously shown to be involved in acid- and nickel-responsive induction of urease expression and activity, but the molecular mechanism behind this regulation is so far unknown. The aim of this study was to further investigate the role of the NikR protein in the regulation of the urease virulence factor.

Methods. *H. pylori* reference strain 26695 and its isogenic NikR mutant were grown in Brucella media supplemented with 20 and 200 μM NiCl2, and/or 20 μg/ml chloramphenicol when appropriate. Urease expression was determined by urease activity measurement and SDS-PAGE. Transcriptional regulation of urease genes was monitored by Northern hybridization, while gel mobility shift assays and DNAse footprint assays were used to characterize the interaction of recombinant *H. pylori* NikR with the ureA promoter.

Results. The transcription of the urease genes and urease activity was nickel-induced in wild-type *H. pylori*, whereas this nickel-induction was absent in the NikR mutant. Supplementation of cultures with the translation inhibitor chloramphenicol also abolished most of the nickel-responsive induction of urease activity, demonstrating that not altered mRNA stability, but increased transcription is responsible for nickel-responsive induction of urease expression. Recombinant NikR protein was able to bind to the ureA promoter only in the presence of nickel. Removal of a palindromic sequence from the ureA promoter also abolished binding of NikR.

Conclusion. The NikR protein directly binds the ureA promoter of *H. pylori* in a nickel- and sequence-dependent manner, resulting in nickel-responsive activation of urease expression. This indicates that NikR functions as activator of urease gene transcription, which contrasts with the repressor only function thusfar attributed to this class of regulatory proteins.

D05

UreA2B2: a second urease system in the gastric pathogen *Helicobacter felis*

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Introduction. Urease activity is essential in host colonization by gastric *Helicobacter* species, and thus the enzyme urease is considered to be one of the major virulence factors of the animal pathogen *Helicobacter felis*. Murine infection with *H. felis* is a model for human *H. pylori* infection and has been used frequently to test the efficacy of urease-based vaccines against *Helicobacter* infection.

Aim. To investigate the urease system of *H. felis*.

Methods. Urease protein expression was monitored in western blots using polyclonal antisera against *H. pylori* urease. Urease activity was determined by measuring the production of ammonia in a colorimetric assay. Inactivation of the *H. felis* urease genes was achieved through insertion of a kanamycin cassette into the ureB gene and a chloramphenicol cassette into the ureB2 gene.

Results. Immunoblot analysis of *H. felis* strains with urease-specific antibodies showed that the majority of strains (4/7) displayed two immunoreactive bands of 67 and 70 kDa. The 67 kDa protein was identified as the urease large subunit UreB, whereas the 70 kDa protein displayed only 71% identity with this subunit. It was than tentatively named UreB2. The gene encoding the UreB2 protein was cloned and sequenced and shown to be organized in a gene cluster named ureA2B2. This gene cluster was present in all tested *H. felis* strains, even in those strains where UreB2 expression was absent. Urease activity of wild-type *H. felis* was 8.9 ± 7.0 U. Inactivation of the ureB gene led to complete absence of urease activity (0.1 ± 0.1 U), whereas inactivation of the ureB2 gene resulted in lowered urease activity (6.4 ± 5.8 U, p=0.043).

Discussion. The gastric pathogen *H. felis* expresses 2 sets of urease subunits, a unique feature amongst bacterial pathogens. The exact function of the UreA2B2 system is currently unknown; although the UreA2B2 proteins do not seem to constitute an active urease enzyme, this may well be by the absence of expression of the urease accessory proteins. The UreA2B2 urease may contribute to pathogenesis of *H. felis* infection, possibly by allowing antigenic variation or a switch in urease expression in unfavourable conditions.

D06

Helicobacter pylori modulates the Th1/Th2 balance through phase variable interaction between lipopolysaccharide and the dendritic cell lectin DC-SIGN

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Introduction. The human gastric pathogen *Helicobacter pylori* phase variably expresses Lewis (Le) blood group antigens in its lipopolysaccharide (LPS), but the biological significance of Lewis expression and phase variation is unclear.

Methods. We studied Le+ and Le- *H. pylori* variants, derived from a single clinical isolate, for their ability to bind to monocyte-derived dendritic cells (DC) and investigated the immunological consequences of this interaction with regard to internalization of bacteria and maturation and cytokine production of DC. Furthermore, the influence of DC, primed with Le+ or Le- *H. pylori*, on development of naïve (CD45RA+ CD4+) T-cells was investigated.

Results. Le+ *H. pylori* variants are able to bind to the C-type lectin dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN), and induce increased IL-10 production by DC. Binding to DC-SIGN by DC does not affect IL-12p70 production. However, DC incubated with Le+ *H. pylori* block T helper 1 (Th1) development as measured by IL-4 and IFN-gamma production. In contrast, Le- variants escape binding to DC and induce a strong Th1 response. In gastric biopsies challenged *ex vivo* with Le+ variants that bind DC-SIGN IL-6 production is decreased, which is indicative of increased immune suppression.

Conclusion. Our data indicate a role for LPS phase variation and Le antigen expression by *H. pylori* in suppressing immune responses through DC-SIGN, which may contribute to the chronic nature of *H. pylori* infection.

D07

Mannose cap-lacking mutants in mycobacterial lipoarabinomannan

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Introduction and goals. The surface glycolipid lipoarabinomannan (LAM) is implied to play a role in mycobacterial persistence. The mannose cap of LAM binds DC-SIGN and signals towards dendritic cell (DC) inactivation and/or blocks phagolysosome fusion. However, such studies compared non-isogenic, purified LAMs. We describe the isolation of capless mutants and their characterization.

Methods and results. Synthetic oligosaccharides representing the non-reducing terminus of LAM were synthesized $\{(man)_n ara$, n=1-3; ara; $(ara)_6\}$ and polymerised to polyacrylamide. A library (N > 200) of monoclonal antibodies (Mabs) was screened with the neoglyco-conjugates and 5 Mabs specific for the mannose cap were identified. In parallel, two strains of *M. marinum* were independently subjected to random mutagenesis with the *mariner* transposon (via phage mycomarT7 and a *mariner/sacB* containing plasmid, respectively). The transposon libraries were screened in colony blot with a mannose-specific Mab and several non-reactive colonies isolated. SDS-PAGE analysis of the mutants indeed proved the synthesis of LAM without a mannose cap. No pleiotropic effects were observed and in vitro growth was normal. Genetic complementation of these mutants restored cap synthesis and resulted also in cap expression in *M. smegmatis*. Interaction studies of the capless mutants with DC and macrophages demonstrate that wild-type *M. smegmatis* hardly binds to DC, while a complemented *M. smegmatis* binds well. Infection of macrophages showed that a capless mutant of *M. marinum* surivided as well as it wild type parent. Preliminary studies in infected adult zebrafish indicate that the capless mutant survived less well as compared to parent.

Conclusion. A procedure to isolate mannose cap-less mutants in mycobacterial LAM is presented. This will yield novel info on the biosynthesis of LAM and will allow elucidation of the role of the mannose cap in mycobacterial persistence.

D08

Chemotaxis Inhibitory Protein of *Staphylococcus aureus* defines a versatile structural fold

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Introduction. Chemotaxis Inhibitory Protein of *Staphylococcus aureus* (CHIPS) is a protein excreted by the majority of *S. aureus* strains. Via separate active sites, CHIPS binds the C5a- (C5aR) and formylated peptide receptor (FPR), blocking phagocyte responses. Thereby, CHIPS is an important virulence strategy of *S. aureus*. CHIPS blocks the two major steps in neutrophil recruitement, the respons to bacterial formylated peptides as well as the response to the major complement derived chemoattractant C5a. The capacity of CHIPS to inhibit neutrophil chemotaxis makes this protein a promising candidate anti-inflammatory drug.

Methods. Using Nuclear Magnetic Resonance we determined the solution structure of the CHIPS fragment that is responsible for C5aR antagonism (CHIPS31-121). Site directed mutagenesis was used to create mutant CHIPS proteins that were expressed using an *E. coli* expression system and tested in an in vitro assay for altered activity.

Results. The protein has a compact fold comprising of an ÿ-helix (residues 38-51) packed onto a four-stranded ÿ-sheet. Strands ÿ2 and ÿ3 are joined by a long loop with a relatively well-defined structure. Anti-CHIPS monoclonal antibodies, directed against the ÿ-helix, block the C5a-inhibition completely. Truncated CHIPS56-121 lacking the ÿ-helix is inactive indicating an important role for this structural element. Point mutations within the ÿ-helix confirm the major involvement of arginine 44 in C5aR antagonism. An arginine at position 46 is important for structural integrity.

Conclusion. The majority of the folding motif is also present in Staphylococcal and Streptococcal superantigen-like proteins with unknown function (SSL5, SSL7, SPE-C). Structure based sequence alignment identified arginine 46 as a conserved motif in these structural homologues. The high structural similarity between CHIPS and the non superantigen exotoxins plus the fact that they are all excreted proteins, are indicative of a closely related function. We hypothesize that a GPCR-modulating activity is present in these and many other proteins. The CHIPS folding motif can serve as a scaffold for the development of a novel class of anti-inflammatory therapeutics.

D09

Staphylococcal Complement Inhibitor (SCIN) prevents complement activation via all three pathways

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Introduction. The complement system plays an important role in host defense and can be activated via the classical, lectin and alternative pathway (CP, LP and AP). All pathways result in the activation of C3 via surface-associated C3 convertases: C4b2a (CP/LP) and C3bBb (AP). The subsequent deposition of C3b molecules at pathogenic surfaces is essential for their recognition by immune cells. Here we describe the discovery of Staphylococcal Complement Inhibitor (SCIN), an excreted 9.8 kDa protein that interferes with all three complement pathways.

Methods. Phagocytosis was performed using FITC-labeled *S. aureus*, normal human sera and freshly isolated neutrophils. Human IgM-, mannan- or LPS-coated plates were incubated with human sera to analyze C3b/C4b deposition via the CP, LP or AP respectively. Factor B (fB) and C2 were studied by Immunoblotting. Immunofluorescence techniques were used for analyzing interactions of SCIN with specific complement components on bacteria/zymosan.

Results. SCIN, produced by 90% of *S. aureus* strains, strongly reduced phagocytosis and killing of *S. aureus* by human neutrophils in a concentration of less than 1 μg/ml. This was shown to be a result of inhibition of C3b deposition on *S. aureus*. Three pathway-specific ELISAs showed that SCIN inhibits C3b deposition via all complement pathways: SCIN completely blocked AP-mediated C3b deposition at 0.3 μg/ml, the CP and LP were inhibited with 50% at 10 μg/ml. Within the CP and LP, SCIN did not interfere with C4b deposition but specifically interacts with C2. On bacteria and zymosan we observed that SCIN stabilized surface-bound C2a and Bb. Binding studies revealed that SCIN directly bound to C3 convertases at the surface and thereby inhibited C3b deposition.

Conclusion. 1. SCIN is an important innate immunity evasion molecule since it efficiently prevents phagocytosis and killing of *S. aureus*.

2. SCIN interferes with all three complement pathways.

3. Complement inhibition by SCIN is a result of the unique interaction of SCIN with surface-bound C3 convertases.

D10

The crucial role of *Campylobacter jejuni* genes in autoimmune antibody induction

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Introduction. Molecular mimicry of *Campylobacter jejuni* (*C. jejuni*) lipo-oligosaccharides (LOS) with gangliosides in nervous tissue is considered to induce cross-reactive antibodies leading to the Guillain-Barré syndrome (GBS), an acute polyneuropathy. The aim of this study was to determine whether specific bacterial genes are crucial for the biosynthesis of ganglioside-like structures and the induction of anti-ganglioside antibodies.

Methods. The type of LOS biosynthesis gene locus was determined by PCR analysis in 21 GBS-associated and 21 control *C. jejuni* strains isolated from patients with uncomplicated enteritis. *Campylobacter* knockout mutants of potential GBS marker genes were constructed. The LOS structures of wild type and mutant strains were determined by mass spectrometry analysis. Immunoblot analysis with GBS patient serum and mice immunization experiments were performed to analyze the effect of the gene inactivations on anti-ganglioside antibody reactivity and induction.

Results. We demonstrated that specific types of the LOS biosynthesis gene locus are associated with GBS and with the expression of ganglioside mimicking structures. *Campylobacter* knockout mutants of two potential GBS marker genes, both involved in LOS sialylation, expressed truncated LOS structures without sialic acid, showed reduced reactivity with GBS patient serum and failed to induce an anti-ganglioside antibody response in mice.

Conclusion. To our knowledge, we demonstrate for the first time that specific bacterial genes are crucial for the induction of anti-ganglioside antibodies.

D11

The *Mycobacterium marinum*-zebrafish infection model: host transcriptome profiling

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Mycobacterium marinum causes a systemic tuberculosis-like disease in a large number of ectothermic animals. We used the *M. marinum*-zebrafish infection model for analysis of a host transcriptome response to mycobacterium infection at the organismal level. RNA isolated from adult zebrafish that showed typical signs of fish tuberculosis due to a chronic progressive infection with *M. marinum* was compared with RNA from healthy fish in microarray analyses. These microarrays comprised 45,465 zebrafish transcript annotations. Detailed transcriptome analysis revealed 159 genes whose regulation is strongly affected. Upregulated genes include many known components of the inflammatory response (complement factors, immunoglobulins genes and different T-cell specific proteins). Furthermore, homologues of many signal transduction genes with relationship to the immune response were induced (such as a chemokine receptor and an interleukin receptor homolog, Janus kinase 1 and SOCS3). The most obvious immune-related genes present in the datasets of downregulated genes were the MHC class I genes. In addition various MHC class II genes were also ownregulated. Future functional analysis of these genes may contribute to a better understanding of mycobacterial pathogenesis.

F01

Ecology of halo-alkaliphilic sulphur oxidizing bacteria

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Recently, a new group of obligately chemolithoautotrophic, haloalkaliphilic sulphur-oxidizing bacteria has been discovered. About 100 *Thialkalivibrio* strains were isolated from sediment samples of soda lakes from various geographical locations, such as Kenya, Egypt, Mongolia, and Siberia. Soda lakes represent the major types of naturally occurring highly alkaline environments, in which indigenous microorganisms are adapted to a double extremophily (i.e., high salt and high pH). All the *Thialkalivibrio* strains grow at a pH of around 10 in a culture medium strongly buffered with sodium carbonate and are able to oxidize reduced sulphur compounds.

The use of molecular biological techniques in microbial ecology has demonstrated an enormous microbial diversity in nature. Goal of our research was to investigate the genetic diversity of *Thialkalivibrio* isolates and the possible correlation with the geographical location from where they were isolated. Among a variety of existing genotyping techniques, rep-PCR was chosen for its high taxonomic resolution (subspecies and strain level), and robustness. By amplification of regions located between repeated sequence elements, which are interspersed along the whole bacterial genome, strain-specific patterns were obtained. Gel analysis was made using GelComparII software (Bionumerics, Belgium) and a genetic relatedness dendrogram was created, showing a very high genetic diversity within the *Thialkalivibrio* genus, and a tendency to cluster according to geographical locations.

F02

Analysis of the genome and proteome of the anammox bacterium *Kuenenia stuttgartiensis*

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The anammox bacteria are important players in the global nitrogen cycle and can be applied to remove ammonia cost

effectively from wastewater under anoxic conditions. To understand the metabolic properties the anammox bacteria an environmental genomic project was started with the anammox bacterium *Kuenenia stuttgartiensis*. The genome sequence of *K. stuttgartiensis* was recently determined at Genoscope, Evry, France. Analysis of the *K. stuttgartiensis* genome assembly revealed many ORFs with the classical heme C binding motif, CXXCH. At least one of the c-type cytochromes deduced from the genome analysis corresponded to the high molecular-mass cytochrome HAO, a 150 kDa trimer containing 24 heme groups. This enzyme constitutes about 10% of the total protein and is located in an unique prokaryotic organelle, the so-called anammoxosome. Other annotated genes potentially involved in the anammox metabolisms could be nitrite oxidoreductase/nitrate reductase, various cytochrome c, the BC1 complex, quinols and the NADH:ubiquinone oxidoreductase (nuo). The major proteins from *K. stuttgartiensis* were separated by 2D gel electrophoresis. As many as 200 protein spots were detected on 2D gels within a pH range of 4-7. Using MALDI/TOF MS and peptide mass fingerprinting about 50% of the analyzed protein spots were positively identified. Among these proteins were: HAO, nitrate reductase, two subunits of the nuo complex, and soluble subunits of the ATP synthase. Future studies will use pre-fractionation of cell extract into soluble and membrane proteins, and removal of dominant proteins like HAO by gel filtration.In addition cell extracts will be analysed by nano-LC coupled to Fourier Transform Ion Cyclotron Resonance MS (Nijmegen Proteomics Center). A further goal is the isolation of intact anammoxosome organelles and identification of its proteins to elucidate this role of the organelle in the anammox metabolism.

F03
Importance of cytochromes in the metabolism of the anammox bacterium *Kuenenia stuttgartiensis*

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The anaerobic ammonium oxidation pathway (anammox) plays an important role in the biogechemical Nitrogen cycle and it is a very important process in low-cost N-removal from wastewater. The organisms responsible for the process are the autotrophic planctomycete-like bacteria, among others *Kuenenia stuttgartiensis. K. stuttgartiensis* oxidises ammonium with nitrite to dinitrogen gas under anoxic conditions.To understand the metabolic properties of the anammox bacteria, a genomic project was initiated. The genomic sequence of *K. stuttgartiensis* revealed 108 ORFs with the classical heme C binding motif CXXCH and the unusual heme C binding motif CXXX(X)CH. In total 62 of these ORFs were annotated as cytochromes. Also the cyctochrome maturation pathway was identified. Twelve of the deduced C-type cytochromes corresponded to hydroxylamine oxidoreductase-like proteins, containing 8 heme groups per subunit. At least 2 of the HAO-like proteins were expressed. Other genes potentially coding for cytochrome proteins involved in the anammox metabolism were similar to nitrite reductase, nitrate reductase and the BC_1 complex, but various cytochromes were of unkonown function. Many of the cytochromes of *K. stuttgartiensis* were separated by 2D gel electrophoresis and LC-MS/MS. Using MALDI/TOF MS and peptide mass fingerprinting these proteins spots were positively identified. Among these hemoproteins were: HAO-like proteins, nitrite reductase, and cytochromes of the nitrate reductase cluster. Further studies will investigate the functions of the cytochromes and their role in the anammox metabolism.

F04/05
'*Haloquadratum walsbyi*'; isolation and preliminary insight in its genome

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Salt lakes are biologically highly active environments that are estimated to cover a similar surface area as fresh water systems. These include the main land salt lakes, but also the extreme hypersaline and anoxic basins found at great depth on the ocean floor.[1] These extreme environments are dominated by specialized microorganisms called halophiles. One of the most intriguing microorganism found in hypersaline ecosystems is the famous square archaeon first described in 1980 by Anthony Walsby.[2] This archaeon is of specific interest because of its unique shape and its abundance in hypersaline ecosystems, which suggests an important ecophysiological role. Ever since its discovery, the isolation and cultivation of 'Walsby's square archaeon' has been a holy grail for many microbiologists. Despite their abundance and easy recognition by microscopy all cultivation attempts have failed up to now, marking the organism as one of the unculturables. Cultivation of the square archaeon is essential to understand their ecophysiological role and the nature of their unique morphologically features.
Here I will report on the isolation and cultivation of the enigmatic square archaeon that I proposed to name '*Haloquadratum walsbyi*.[3] Besides summarizing some of its unique features I will also present preliminary data on the genome sequence.

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F06
Genomics tools used in food production

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Introduction. In our contribution we present an approach that aims at obtaining insight at the molecular level in the physiology of food poisoning and spoilage microorganisms when present in food ingredients and during food production.

Here we focus on bacterial spore formers. The spores that they produce are high to extreme heat resistant. In order to ensure commercial sterility, i.e. to prevent outgrowth of process surviving spores during practical storage conditions, currently severe thermal processes are applied. Such processes often lead to overkill since both spore load and heat resistance of the spores that may be present in the product at the moment of sterilization are unknown. The data should facilitate the development of a detection system and the optimization of process settings.

Methods. It is known that the heat resistance of spores is enhanced by the presence of metal ions (minerals) which are often present in ingredients. We have now performed a genome-wide analysis of gene expression of sporulating *Bacillus subtilis* cells both in the presence of extra calcium and in the presence of a food ingredient (Cumin) shown to contain significant levels of among others calcium.

Results. Our analysis extends the notion that some genes are expressed more in cells sporulating in the presence of metal ions compared to cells sporulating in their absence. This holds in particular for genes encoding some of the small acid soluble spore proteins (see for full experimental details and a description of all results Oomes and Brul, 2004 *Innov. Food Sci. Emerg. Technol.* 5, 307-316). All differentially expressed genes from former and current experiments are now evaluated for their suitability as biomarkers for spore thermal resistance

Conclusions. We have shown the potential of using genome-wide expression analysis in food microbiology to screen for putative biomarkers of high spore thermal resistance. Upon validation of these putative biomarkers we will use them to recommend food production process settings that lead to cost reduction and/or improved product quality.

F07
Genetic analysis of spore germination and the effects of thermal spore injury

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For over a century, thermal treatment is a key process employed in food preservation. Heat-resistant bacterial spores can survive mild thermal treatments and cause food contamination and spoilage. The current demand for mild food preservation techniques that maintain the current food safety levels has prompted us to investigate the effects of sub-lethal heat treatments on germination and outgrowth of *Bacillus subtilis* spores.

The process by which a dormant spore resumes vegetative growth (germination and outgrowth) was studied in great detail by time-resolved transcriptional analysis. These microarray studies for the first time revealed the highly dynamic genetic processes occurring during spore germination. The complex developmental programme of spore germination combines signals from unlocking spore dormancy, re-initiation of metabolism, active spore repair, initiation of DNA replication, chromosomal segregation, cell growth and septum formation. A set of master regulators was identified that play a crucial role in the early stages of spore germination. To study the effects of thermal spore injury on spore germination, reporter gene constructs were used of genes crucial for specific stages of spore germination and outgrowth. Heat-damaged spores showed a delayed initiation of germination and outgrowth, perhaps indicating an active repair mechanism. The germination process was also found to be asynchronous, as observed by variations in the progression through the critical phases of germination. This heterogeneity in germination of heat-treated spores is likely to reflect differences in the level and type of spore damage. Curiously, outgrowing cells of heat-treated spores were often filamentous and occasionally double in width, suggesting defects in cell division and or cell wall synthesis. Heat-treated spores were shown to be more sensitive towards environmental stress conditions such as weak organic acid stress and saline conditions. Insight provided in this study allows the identification of novel targets or strategies for innovative food preservation techniques.

F08
Characterization of the germination receptors of *Bacillus cereus* ATCC 14579

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Specific amino acids, purine ribonucleosides or a combination of the two are required for efficient germination of endospores of *Bacillus cereus* ATCC 14579. A survey including 20 different amino acids showed that L-alanine, L-cysteine, L-threonine and L-glutamine are capable of initiating germination of endospores of *B. cereus* ATCC 14579. In addition, the purine ribonucleosides inosine and adenosine can trigger germination of the spores. Advanced annotation of the *B. cereus* ATCC 14579 genome revealed the presence of seven putative germination (*ger*) operons, termed *gerG, gerI, gerK, gerL, gerQ, gerR,* and *gerS*. To determine the role of the encoded putative receptors in nutrient-induced germination, disruption mutants were constructed by insertion of pMUTIN4 into each of these seven operons. Four out of the seven mutants were affected in their germination response to amino acids or purine ribonucleosides, whereas no phenotype could be linked to the mutants with a disrupted *gerK, gerL* and *gerS* locus. The largest effect was observed in spores of the *gerR* mutant, as germination of these spores was not initiated for all tested amino acids, except L-glutamine. The *gerG* mutant showed significantly reduced L-glutamine-induced germination, which points to a role of this receptor in the L-glutamine germination-signaling pathway. Inosine triggered germination less efficiently in the *gerR, gerI* and *gerQ* mutants, suggesting that these operons play a role in ribonucleoside signaling. Efficient germination by the combination of L-glutamine with inosine was shown to involve the *gerG* and *gerI* operon, since germination of mutants lacking either one of these receptors was significantly reduced. Germination triggered by the combination of L-phenyl-alanine and inosine was lost in the *gerI* mutant, indicating that both molecules are effective at this receptor.

F09

Assessment of heat resistance of bacterial spores from food product isolates by fluorescent monitoring of dipicolinic acid release

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This study is aimed at the development and application of a convenient and rapid optical assay to monitor the wet heat resistance of bacterial endospores occurring in food samples. We tested the feasibility of measuring the release of the abundant spore component dipicolinic acid (DPA) as a probe for heat inactivation. Spores were isolated from the laboratory type strain *Bacillus subtilis* 168 and from two food product-isolates *B. subtilis* A163 and *Bacillus sporothermodurans* IC4. Spores from the lab strain appeared much less heat resistant than those from the two food product isolates. The decimal reduction times (D-values) for spores from strains 168, A163 and IC4 recovered on TSA were 1.4, 0.7 and 0.3 min, at 105 °C, 120 °C and 131 °C, respectively. The estimated *Z*-values were 6.3 °C, 6.1 °C and 9.7 °C, respectively. The extent of DPA release from the three spore crops was monitored as a function of incubation time and temperature. DPA concentrations were determined by measuring the emission at 545 nm of the fluorescent terbium-DPA complex in a microtiter plate fluorometer. We defined spore heat resistance as the critical DPA release temperature T_c, the temperature at which half the DPA content has been released within a fixed incubation time. We found T_c-values for spores from *Bacillus* strains 168, A163 and IC4 of 108 °C, 121 °C and 131 °C, respectively. On the basis of these observations we developed a quantitative model that describes the time and temperature-dependence of the experimentally determined extent of DPA release and spore inactivation. The model predicts a DPA release rate profile for each inactivated spore. In addition, it uncovers remarkable differences in the values for the temperature-dependence parameters for the rate of spore inactivation, DPA-release duration and DPA-release delay.

F10

Weak Acid Stress in *Bacillus subtilis*; the responses of *Bacillus subtilis* towards Sorbic Acid

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Weak organic acids have been used by the food industry as food preservatives for a long time. However, their precise mode of action is still unknown. Weak acids are most effective in their undissociated form, presumably since they penetrate the cytoplasmic membrane more easily. Therefore, both the pH of the environment and the pK_a value of the weak acid are important for its antimicrobial activity. In general it is believed that the weak acids enter the cell in their neutral form and acidify the cytosol were the molecules dissociate because of the higher internal pH. The protons have to be pumped out of the cell by the ATPase to maintain the internal pH by the cost of ATP. A constant influx of the protons will eventually deplete cellular energy. This leads to the hypothesis that the weak acid may cause an energy stress and that the transcription factor SigB, the regulator of the general stress response pathway, might be involved in the cellular response towards this stress. Although the undissociated form is most active, also the anion form of the weak acid is believed to contribute, be it minor, to its antimicrobial activity. It is suggested that organic acids, especially when more lipophilic, can interfere with the membrane permeability. Therefore, the lipophilic sorbic acid may induce transcription factor SigM, which is involved in maintaining membrane and cell wall integrity. In this study we will discuss the responses of *Bacillus subtilis* towards a sorbic acid induced stress by monitoring the growth inhibition at different pH's. We also discuss the transcriptional responses on sorbic acid stress and the involvement of the transcription factors SigB and SigM. Finally, a number of sorbate sensitive mutants were discovered and their characterization will be presented.

F11

Differential effects of nitrogenous fertilizers on methane consuming microbes: 'Microbial diversity matters to global fluxes!?'

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Ecosystems collectively determine the biogeochemical processes that regulate the Earth system. Loss of biodiversity from these ecosystems and the potential consequences has been a central issue in ecological and environmental sciences the last decade. Although micro organisms may comprise much of Earth's biodiversity and have critical roles in biogeochemistry and ecosystem functioning microbial diversity is not taking into account in the global biodiversity loss debate. Methane oxidising bacteria (MOB) may be a group of microbes whose diversity matters to global fluxes since these bacteria play a vital role in the global budget of the greenhouse gas methane. The process of methane consumption has been shown to be affected by application of nitrogenous fertilizers or by deposition of atmospheric nitrogen. The general results of fertilizer studies are rather contradictory, even within similar ecosystems. In the present the hypothesis was investigated whether the contradicting results observed are caused by differential effects of fertilizers on the methanotrophic community in forest and rice field soil. Forest and rice field soil was incubated in microcosms under a continuous flow of labelled $^{13}CH_4$ and supplemented with nitrate or ammonium. Effects of fertilizers on *in situ* type specific growth rates and type specific methane incorporation rates were investigated by extracting PLFA (phospholipid derived fatty acids) specific for different genera of MOB (Type I vs. Type II). Next to this TRFLP (Terminal Restriction Length Polymorphism) analyses was used based on the gene

coding for the pmoA (particulate methane monooxygenase) enzyme. Both the PLFA as the TRFLP approach gave the same clear result. Type I MOB were generally stimulated in both forest as well as rice soils whereas type II MOB were inhibited. This differential effect provides a case example that microbial diversity indeed matters to global ecosystem fluxes, especially when assessing and predicting impact of environmental change on biodiversity

G04/05
Eradication of carriage of methicillin-resistant *Staphylococcus aureus* in medical personnel

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Introduction. In the Netherlands personnel carrying methicillin-resistant *Staphylococcus aureus* (MRSA) are not allowed to work in hospitals and they are referred for MRSA eradication therapy. The objective of this study was to assess the efficacy of the MRSA eradication therapy as used in our hospital.

Methods. During an outbreak of MRSA in our hospital and the regional nursing homes, all 45 colonized subjects were seen at the outpatient clinic for MRSA eradication therapy. First line treatment consisted of: mupirocin nasal ointment and hygienic measures. Cultures were performed weekly for 3 weeks. In case of treatment failure, all household contacts were screened for MRSA carriage and treated accordingly if positive. Persons with treatment failures were subsequently treated with systemic antibiotics (second line treatment (co-trimoxazole and rifampicin, 10 days) or with third line treatment (low dose clindamycin, 90 days)), also in combination with mupirocin and hygienic measures. Persistent throat carriers of MRSA were offered tonsillectomy. Thereafter, they received another course of second line treatment.

Results. 45 persons were referred for MRSA eradication therapy. One person first wants to deliver her baby before starting MRSA eradication therapy, because she refuses to use mupirocin nasal ointment during her pregnancy. 34/44 (77%) persons were successfully treated with first line treatment only. Of the 10 persons with persistent carriage of MRSA 2/10 were effectively treated with second line treatment and 3/10 with third line treatment. Three persons were persistent throat carriers of MRSA. One person refused further treatment and was lost to follow up. Two persons had successfully removed their tonsils. The remainig 2/10 persons who failed first line treatment had (multiple) household contacts with MRSA carriage in combination with difficult to treat underlying diseases (leukemia, chronic otitis with MRSA, HIV with skin ulcers). They are still under treatment.

Conclusion. MRSA eradication therapy of medical personnel is often (about 80%) successful after mupirocin nasal ointment and hygienic measures only. However, treatment failures need close attention and specific therapy.

G06/07
Methicillin-resistant *Staphylococcus aureus* in companion animals

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Introduction. Information on methicillin-resistant *Staphylococcus aureus* (MRSA) in animals is limited. This presentation will focus on the prevalence, diagnosis and therapy of MRSA infections in pet animals.

Prevalence of MRSA in the Netherlands. *Staphylococcus aureus* strains isolated from clinical infections of companion animals (n=100) from our strain collection were screened for methicillin resistance by oxacillin agar screening. In addition, samples from healthy dogs (n=83), cats (n=91) and horses (n=200) were also screened for MRSA. Oxacillin-resistant strains were tested for the presence of the *mecA* gene by PCR. *MecA* positive strains were further analyzed using Pulsed Field Gel Electrophoresis (PFGE). Only two MRSA were found, both cultured from dogs with wound infections. One of these MRSA showed homology to a human MRSA (PFGE cluster 257).

Transmission of MRSA between humans and animals. Two additional MRSA were cultured from the nose of two healthy dogs whose owners were colonized with MRSA. The PFGE patterns of the canine isolates were identical to those cultured from their owners (PFGE cluster 35 and 28 respectively).

Diagnosis. The best sampling sites for dogs and cats are the mucosal membranes of the nose and the auricle of the ear. The laboratory techniques for isolation and identification of MRSA are the same as in humans.

Therapy. Two dogs were treated successfully with a combination of rifampin and doxycycline per os. The dogs with the wound infections recovered without antimicrobial therapy. The choice of the antimicrobials should be based on susceptibility testing as most MRSA cultured from animals are multidrug-resistant. Local treatment with mupirocin ointment is impractical.

Conclusions. MRSA can be found in healthy and diseases pet animals in the Netherlands, but the prevalence is low. MRSA can be transmitted from humans to pet animals and probably vice versa. Therefore, the risk of pets being the source of unexplained carriage or relapse of infection in humans should be recognized. The best places for sampling companion animals are the nose and auricle of the ear. MRSA positive dogs can be treated with a combination of two antimicrobial drugs per os.

G08/09

Panton-Valentine leukocidin positive MRSA: the Dutch situation

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Introduction. Methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the Panton-Valentine leukocidin (PVL) loci are reported worldwide and are a serious threat to public health. PVL-MRSA is mainly associated with furunculosis, severe soft-tissue infections and fatal necrotizing pneumonia, even in previously healthy (young) individuals.We assessed the prevalence of PVL-MRSA in the Netherlands in the periods 1987-1995 and 2000-2004, and determined their characteristics by molecular subtyping.

Methods. Multi-locus sequence typing (MLST), staphylococcal chromosome cassette *mec* (SCC*mec*) typing and toxin gene PCR. The MRSA isolates studied were obtained from the Dutch national MRSA surveillance program conducted by the National Institute of Public Health and the Environment (RIVM). PVL-MRSA isolates were compared with well-known global epidemic MRSA clones.

Results. Approximately 10% of all MRSA isolates sent to the RIVM harbored the PVL locus. The first Dutch PVL-MRSA isolate was found in 1988. Most isolates belonged to well-known global epidemic MRSA clones, like sequence type (ST) 8 and ST80. MRSA with ST8 is also found in the USA, where it is linked to widespread infections among jail inmates and in the gay community. MRSA with ST80 seems to be an European clone and has become widespread in the Netherlands since 2000. Most PVL-MRSA isolates carried SCC*mec* type IV, a supposed marker for community-acquired MRSA. Also other SCC*mec* types (mainly type I and III) were found, which is suggestive for hospital-derived MRSA.

Conclusion. We demonstrated the simultaneous co-emergence of PVL-MRSA, belonging to different pandemic clones, in the Netherlands. Most PVL-MRSA seem to be community-acquired, but hospital-associated isolates also seem to occur. The precise incidence of infections with PVL-positive *S. aureus* in our country is unknown, because community-acquired infections – especially superficial skin infections – are rarely characterized. Our data support the need for further studies to monitor and prevent the spread of PVL-MRSA, in both the community and the hospital environment, before additional resistance and virulence markers are acquired.

H01

Rapid detection of *Clostridium difficile*-associated diarrhea in a prospective multicenter study, using a new immunoassay and real-time PCR

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Introduction. *Clostridium difficile*-associated diarrhea (CDAD) is usually diagnosed by the detection of TcdA) and/or TcdB in faecal samples, or by toxinogenic culture. A recently introduced rapid immunoassay (Immunocard toxins A and B, Meridian) and an in-house developed real-time PCR were compared in a prospective multicenter study with conventional diagnostics.

Methods. In a prospective study of 4 months, 3 university hospitals participated and tested all faecal samples from patients with diarrhea admitted to the hospital for 3 days or longer for CDAD, irrespective of the physicians request. An enzyme-linked fluorescent assay (ELFA, Vidas CDA2) was used for detection of TcdA and the cytotoxicity assay on Vero-cells was applied as the 'gold standard'. Additionally, the immunocard toxins A and B (ICTAB) and a home-made real-time PCR for the detection of TcdB were included in the study. The sensitivity of real-time PCR was 1 colony forming unit (CFU) in saline and 1x10^5 CFU/g faeces.

Results. Of 369 faecal samples included in this study, 56 (15.2%) showed a positive result in one or more assays. The cytotoxicity assay was positive in 23 (6.2%) of 369 patients. In 10 (43%) of these 23 patients, the diagnosis CDAD was not considered by the physician. Using the cytotoxicity assay as the 'gold standard', sensitivity of ELFA, ICTAB and real-time PCR were 69.6%, 91.3% and 87.0%, respectively. The specificity of ELFA, ICTAB and real-time PCR were 95.4%, 96.2% and 95.4%, respectively. The positive predictive value and negative predictive value for ELFA, ICTAB, real-time PCR were 50% and 97.9%, 61.8% and 99.4%, and 55.6% and 99.1%, respectively. Of 56 samples positive in one or more assays, 53 were available for toxinogenic culture. The concordance of PCR and ICTAB with culture was 84.9% (45/53) and 81.1% (43/53), respectively.

Conclusion. The new rapid immunoassay is a very rapid and easy-to-perform test for the diagnosis of CDAD. It may be useful for guiding appropriate treatment. The real-time PCR is an excellent instrument to control nosocomial spread of toxinogenic *C. difficile*.

H02

The contribution of PCR and analysis of intraocular antibody production to the diagnosis of infectious uveitis

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Introduction. Uveitis, an intraocular inflammation, is in 20-30% of the cases of infectious origin. The most common causes currently identified, are infections with cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV) and *Toxoplasma gondii*. As the eye represents a secluded and immune-privileged organ, diagnostic testing of peripheral blood in uveitis is hardly ever conclusive. Therefore, detection of antibodies or nucleic acids in the intraocular fluid is mandatory for diagnostic microbiology. However, this is hampered by the limited volume of ocular fluid. Since the introduction of the polymerase chain reaction (PCR) in routine diagnostic microbiology, a number of laboratories use PCR as the only diagnostic assay on ocular fluids. Therefore, we determined the relative contribution of the PCR and the analysis of the intraocular antibody production using the Goldmann-Witmer coefficient (GWC) to the diagnosis of infectious uveitis.

Methods. Real-time PCR and analysis of the GWC ({specific ocular IgG/specific serum IgG}:{total ocular IgG/total serum IgG}) by enzyme immunoassay (EIA) were performed for CMV, HSV, VZV and *T. gondii* on 50 ul of intraocular fluid samples from 271 patients with uveitis. A GWC > 3 was considered indicative of intraocular antibody production.

Results. An infectious etiology was established in 73 patients (27%). Of these, 9 were diagnosed with CMV, 14 with HSV, 19 with VZV and 31 with *T. gondii*. Thirty cases (41%) were positive in both assays, 34 (47%) by GWC-calculation only and 9 (12%) by PCR only. For *T. gondii*, 65% of cases would have been missed if only PCR was performed. Herpesviral DNA was more readily detected early after onset of symptoms, while *T. gondii* DNA was not detected until 3 weeks after onset of disease. A positive GWC was found throughout the course of disease.

Conclusion. Analysis of the intraocular antibody production significantly contributes to the diagnosis of infectious uveitis and for an optimal diagnostic approach, both PCR and GWC are needed. In contrast to PCR, intraocular antibody analysis appears to be useful throughout the whole course of the disease.

H03

Real-time quantitative detection of herpes simplex virus DNA in the lower respiratory tract

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Introduction. The clinical relevance of herpes simplex virus (HSV) in brochoalveolar lavage (BAL) specimens is difficult to determine, as oral shedding of HSV may contaminate the BAL fluid. HSV presence in BAL specimens is generally assumed to be without clinical consequences, although lower respiratory tract (LRT) infections due to HSV are described of which most cases remained unrecognized until post-mortem autopsy. Quantification of HSV in BAL specimens could potentially differentiate LRT infection from oral contamination.

Methods. An internally controlled, quantitative real-time PCR (qPCR) assay targeting the UL30 gene encoding HSV DNA polymerase was developed and evaluated for specific detection of both HSV1 and HSV2. A retrospective study was performed among available BAL specimens collected during a 1-year period at the Leiden University Medical Centre from adult patients (18 years). Available BALs were analyzed using the HSV qPCR assay and an existing CMV qPCR assay. Routine microbiological results were recorded. HSV presence and loads in BAL specimens were related to patient characteristics, hospitalization data and outcome.

Results. The HSV qPCR was validated using the 2004 Quality Control for Molecular Diagnostics (www.qcmd.org) proficiency panel. The sensitivity of the assay was determined to be 5-10 virus particles per reaction and the specificity was excellent using DNA or RNA from a varied panel of respiratory pathogens. HSV was detected in 11 (19%) of 59 BAL samples, of which 7 (12%) had a viral DNA load higher than log 5 (> 100.000 copies/ml). HSV DNA loads exceeding log 5 in BAL specimens (n=7) were significantly associated with a strongly increased risk of mortality within 30 days (OR 11.5, CI 2,2-72.7), while the detection of CMV and low loads of HSV (< log 5) were not. Extensive HSV pneumonia was histologically proven in one case with log 8 HSV DNA in BAL fluid at autopsy.

Conclusion. HSV was the second most frequently detected virus (19%) among clinical BAL specimens. This study demonstrates the potential value of quantitative detection of HSV DNA in BAL fluid, to differentiate HSV infections associated with fatal outcome from other cases in which this virus is present in BAL fluid.

H04

Comparison of real-time PCR and conventional methods to determine etiology of community-acquired pneumonia

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Introduction. Detection of the etiological agent in community-acquired pneumonia (CAP) is frequently not determined by conventional methods. Polymerase chain reaction (PCR) has been shown to increase the sensitivity for detection of respiratory pathogens. However, use of PCR to detect a whole range of pathogens in a system for routine diagnosis has not been widely applied. Here, the use of real-time PCR for microbiological diagnosis in patients with CAP was assessed. Real-time PCR for diagnosis of 12 respiratory viruses and *Mycoplasma pneumonia*, *Legionella* spp. and *Chlamydophila* spp. (atypical bacteria) in patients with community-acquired pneumonia was performed.

Methods. Conventional techniques and multiplex real-time PCR for atypical bacteria and respiratory viruses were performed and compared on samples collected from 105 adults enrolled in a prospective study in a defined geographical area. All patients had an infiltrate on chest radiograph and a pneumoniae severity index (PSI) score was obtained at admission.
Results. Microbiological diagnosis was obtained in 52/105 (50%) patients by conventional techniques and in 80/105 (76%) by real-time PCR. Results could be obtained in one working day using real-time PCR whereas 2-3 weeks was required for serological diagnosis. Respiratory viral infections were detected in 15/105 (14.2%) of the infections by conventional methods but in 59/105 (56.2%) by real-time PCR methodology. Mixed infections were seen in 28/105 (26.6%) when real-time PCR was performed compared to 3/105 (2.8%) with conventional methods. The presence of a mixed infection by real-time PCR was significantly associated with severe pneumoniae (p=0.002). Human rhinoviruses and coronaviruses, OC43 and 229E were frequently identified pathogens in mixed infections but were also identified in 4 cases of severe pneumonia as the only microbiological pathogen.
Conclusions. The real-time PCR assays enabled sensitive diagnosis for the main viral and atypical bacteria in comparison to conventional methods in a way that clinically relevant results can be obtained. The presence of mixed infections may be important in the severity of pneumonia.

H05
Shorter time to identification of pathogens in positive blood cultures by FISH in routine practice

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Introduction. The effect on turnaround time of routine implementation of molecular methods like fluorescent in situ hybridisation (FISH) for identification of pathogens from positive blood cultures is not known.
Methods. FISH was performed on growth-positive blood culture fluids simultaneously with conventional identification. The panel of probes used allow identification of > 95% of pathogens most commonly found in positive blood cultures. Results and time points were collected for Gram-stain, conventional identification and FISH.
Results. Two hundred blood cultures were included. FISH allowed identification at species level of 162 pathogens (81%). With the available species-specific pobes, 97% were identified correctly; in 3 samples with *Staphylococcus aureus* and 2 with *Escherichia coli* identification was suboptimal. Eubacterial or panfungal probes were positive on all blood cultures with growth, including viridans *streptococci* (6), *Enterobacter cloacae* (4) and *Proteus mirabilis* (4).
Average time to identification by FISH was 3,5 hours for Gram-negative and 4 hours for Gram-positive organisms. Compared to preliminary identification (aurex, optochine, oxidase) time gain by FISH was 70 minutes ($p < 0.001$); for definite identification this increased to approximately 16 hours ($p < 0.001$).
Conclusion. Molecular identification by FISH correlates well with conventional identification. In five cases identification was suboptimal because of difficult interpretation of fluorescence due to large amounts of protein and cells. The panel of probes should be enlarged to permit identification of > 95% of pathogens; probes for viridans *streptococci*, *E. cloacae*, and *P. mirabilis* would be of interest.
FISH allows faster identification than conventional methods. This can be especially useful for cultures that are growth-positive in the afternoon as FISH results will be available the same day whereas conventional identification will require overnight culture at our laboratory. If the turn-around time of the FISH procedure could be further decreased and the panel of probes extended, FISH would provide a valuable diagnostic improvement to the microbiological laboratory.

H06
Luminex *x*MAP technology: a new reliable method to detect *Cryptococcus neoformans* and *Cryptococcus gattii*

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Luminex is a novel flow cytometry technique, which can be used to detect (fungal) pathogens. The method is based on a nucleotide hybridization assay and consists of a combination of species-specific capture probes and different sets of fluorescent beads.
Luminex probes for *Cryptococcus neoformans* and *C. gattii* have been developed based on the IGS (intergenic spacer) region. Two species specific probes were made (a general *C. neoformans* and a general *C. gattii* probe) and 6 group specific probes were developed for each of the haploid Amplified Fragment Length Polymorphism (AFLP) groups.
A set of 34 clinical strains, which were isolated from Dutch patients between 1977 and 2004 and genotyped by AFLP, were used to test the Luminex probes developed for the *C. neoformans* species complex. Because these clinical strains were mainly *C. neoformans* strains, an additional 20 *C. gattii* strains were included in the test set. The strains were tested in duplo.
The results were highly reproducible and all strains were identified correctly. In some cases even hybrid strains could be identified.
Although most hybrid cryptococcal strains do not have both IGS alleles the hybrids which have both alleles (according to cloning experiments) could sometimes be identified as hybrids. In these cases the strenght of the signal of one of the parents was comparable to a normal haploid strain, the strenght of the signal for the other parent was positive, but much lower than a normal positive signal.
Luminex is a reliable detection method of *C. neoformans*, *C. gattii* and in some cases of cryptococcal hybrids. In the future this method should be tested on clinical material, such as cerebrospinal fluid (CSF), to investigate the clinical relevancy of this method.

L01

Antimicrobial resistance in *Escherichia coli* in the Netherlands and Europe: results from the European Antimicrobial Resistance Surveillance System (EARSSS)

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Introduction. *Escherichia coli* is the most frequently isolated Enterobacteriacea from blood cultures in clinical settings in Europe. In Europe, fluoroquinolones (FQ) and third generation cephalosporins (g3cep) are increasingly used. In the Netherlands, this use apparently levelled of in recent years. We evaluated data on FQ and g3cep resistance among *E. coli* from the Netherlands as collected through the European Antimicrobial Resistance Surveillance System (EARSS) and compared the results with other European countries.

Methods. EARSS collects routine antimicrobial susceptibility test data, as tested according to standard protocols, from invasive *E. coli* isolates since 2001. From 2001-2003, 21 countries reported FQ and g3cep susceptibility test results, for 41,624 and 42,889 *E. coli* isolates, respectively. The Cochran-Armitage test was used for trend analysis.

Results. In 2001, *E. coli*-FQ resistance was 8.8% overall, in 2002 11.0% and in 2003 it reached 12.0%. This increase was found for seventeen countries, being statistically significant for seven (Austria, Bulgaria, Czech Republic, Germany, Spain, Hungary and Sweden). For the Netherlands these percentages were, respectively, 6%, 5% and 7%. In 2001 resistance was >= 10% in seven countries, in 2003 13 of the 21 countries passed this level. Compared to other European countries, FQ resistance was still low in the Netherlands, it ranked 15th of 21 countries. In general, resistance for g3cep was low, but it rose from 1.6% in 2001 to 2.5% in 2003. This increase was found for 11 countries, being statistically significant for seven (Austria, Bulgaria, Spain, Finland, Croatia, the Netherlands and Portugal). For the Netherlands it increased from 0.5% in 2001 to 1.4% in 2003, ranking it 11th of 21 countries.

Conclusion. 1. There has been a widespread increase in FQ and g3cep resistance in *E. coli* in Europe, coinciding with increased use of FQ and g3cep. Infections with *E. coli* may become increasingly difficult to treat in many European countries. 2. Resistance rates were still low in the Netherlands.

L02

Giardia and *Cryptosporidium* in the Netherlands

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Objectives. The studies were designed to get an insight into the incidence of protozoal-, bacterial-, and viral infections in patients with diarrheal complaints in different groups: patients consulting their general practioner and the dutch population. Here we focus on *Giardia* and *Cryptosporidium*.

Methods. Three studies were designed and conducted: 'Haarlem'study: 1994-1996, general practitioners, Haarlem region NIVEL: Case-control study in sentinel General practitioners practices (1996-1999), SENSOR: prospective population based cohort study with a nested case-control study in the Dutch population (1999). The studies differ in inclusion criteria and the diagnostic laboratory techniques used.

Results. Incidence of gastroenteritis in the NIVEL (GP) study (after correction) was 79.5 per 10.000 personyears. This means that 80.000-130.000 persons will consult a GP annually for gastroenteritis. Incidence of gastroenteritis in the population- based study was 283 per 1000 personyears. *Giardia* was detected in 14.8% of the cases in Haarlem, in 5.4% of the cases in the NIVEL study and 3.3% of the controls. For *Cryptosporidium* this was resp 3.3%, 2.1% and 0.2%. Detection of pathogens was influenced by age, season and duration of symptoms.

We were able to construct an algoritm for diagnostic workup in GI patients.

The main riskfactors for *Giardia* are: swimming OR: 6.8 (95%CI: 2.4-19.3); multivariate OR: 15.6 (3.2-77.1); contacts with person with GE 7.1 (1.8-26.6); multivar. OR 28.6 (3.2-255.6) family member attending primary school: 2.8 (1.4-5.8); multivariate OR 2.5 (1.0-6.3). The population attributable risk fraction (PARF) for all these factors was 49% in the GP patients and 76% for patients in the general population.

Conclusion. *Giardia lamblia* and *Cryptosporidium* are important pathogens in gastro-enteritis patients in the Netherlands. If transmission through water, infected persons and through school contacts could be eliminated, about 50%-76% of *Giardia* gastro-enteritis could be prevented. *Giardia lamblia* is the most frequently found potentially pathogenic intestinal protozoal species in patients with diarrheal complaints in the Netherlands

L03

Association between ciprofloxacin resistance *Escherichia coli* and integron class 1

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Introduction. A rapid increase in ciprofloxacin and cotrimoxazole resistant *Escherichia coli* strains was observed at haematology wards in two Dutch hospitals in 2000. Different aspects were studied to determine the cause of this increase.

Methods. One hunderd-sixty-one *E. coli* strains were isolated between 2000 and 2002. DNA fingerprinting was used to determine the genetic relationship between all strains. PCR analysis was used to detect the presence of integron class 1, followed by PCR-Restriction Fragment Length Polymorphism (RFLP) analysis for typing of the gene cassettes of strains harboring integron class 1. In addition, dot blot analysis was carried out to detect the presence of a quinolone resistance gene (qnr gene). Furthermore, conjugation experiments were performed to identify which

antibiotic resistance were co-transfered. Finally, part of the DNA gyrase A was sequenced to reveal the presence of mutations in this gene.

Results. AFLP revealed that all strains were genetically heterogeneous, which excluded a clonal outbreak of one particular strain. Detection for the presence of integron class 1, showed that 81% of the ciprofloxacin resistant strains contained a intI1 gene; in contrast, this gene was only present in 11% of the ciprofloxacin susceptible strains ($p < 0.0001$). However, only four different gene cassettes were found with PCR-RFLP analysis, suggesting a nosocomial mobile DNA element outbreak. The recently identified quinolone resistance gene was not present in the integron. In addition, conjugation experiments showed that ciprofloxacin resistance was not co-transferred together with integron class 1. Moreover, ciprofloxacin resistant strains harbored mutations in the DNA gyrase A gene, which are known to be responsible for ciprofloxacin resistance.

Conclusion. The evidence regarding the presence of integron class 1 in ciprofloxacin resistant *E. coli* strains indicates that ciprofloxacin resistance is associated with integron class 1. It was however clearly shown that ciprofloxacin resistance was not co-transferred and the qnr gene was not present. It can be concluded that the association of ciprofloxacin resistance and integron class 1 has no genetic basis.

L04

Sequential emergence of multiple adenovirus serotypes after pediatric stem cell transplantation

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Introduction. Adenovirus infections have the potential to cause fatal disease after stem cell transplantation (SCT), in particular in children. Adenoviruses occur in a variety of 51 serotypes from six different species. Recently, genotyping has also been applied to differentiate the adenovirus serotypes. Sequential occurrence of different serotypes could be demonstrated and occasionally discrepant serotypes were detected in different clinical specimens of the same patient.

Methods. A total of 96 viral isolates from specimens (feces, throat swabs, and urine) from 28 consecutive pediatric SCT recipients were serotyped. Episodes were differentiated by the occurrence of at least 2 culture-negative fecal specimens and at least one month apart. Serotyping was carried out by classical neutralization with initially pooled antisera on A549 cells, followed by type-specific neutralization. Genotyping by sequence analysis of part of the hexon gene was added for 19 isolates.

Results. 42 different adenovirus isolates were detected in 28 patients, including 4 isolates without an assigned serotype. In 18 patients (64%) only one serotype was detected, 10 patients (36%) showed multiple serotypes (2 in 7 patients, 3 in 2 patients, 5 in 1 patient). In 6 patients with multiple infections, serotype 31 was the initial serotype, only 1 single serotype infection was caused by this virus. Feces and throat swabs of the same sample date (n=10) always showed identical serotypes, feces and urine (n=8) isolates were different in 2 cases. Genotyping by sequencing demonstrated identity on the adenovirus subgroup level in 100%, on the serotype level in 80%, when conclusive results could be obtained. Adenovirus related mortality was highest among single-serotype infections (10 in 18, vs. 1 in 10).

Conclusion. More than one single serotype of adenovirus can be detected after SCT in a substantial proportion of cases. A sequential emergence of dominant serotypes is observed during immunological reconstitution. This finding will be relevant for diagnostic purposes, for immunotherapeutic interventions and for insight in the pathogenesis of this problem.

L05

Outbreak of *Bordetella pertussis* on a neonatal intensive care unit (NICU) and the role of macrolide prophylaxis

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Objective. To investigate the spread of *Bordetella pertussis* infection on a neonatal intensive care.

Methods. Retrospective descriptive evaluation of *B. pertussis* infection by serology and nasopharyngeal swab PCR and the role of macrolide prophylaxis.

Results. In June 2004, a staff member of the NICU developed a common cold that gradually evolved into the classical symptoms of pertussis (paroxysmal cough and vomiting). The *B. pertussis* serology was positive (PTX-IgG > 100 U/ml). In July, blood and nasopharyngeal swabs were collected from all staff members with upper respiratory complaints (n=27). 3/27 staff members had positive *B. pertussis* serology and 2/27 'suspect' ('non-conclusive' on retesting). In 1/27 staff members the PCR was positive (negative on retesting). As spread of *B. pertussis* was proven, nasopharyngeal swabs and blood were collected from 42 neonates. 5/42 neonates had a positive PCR. All patients, their parents and staff members then received prophylaxis (azithromycin or erythromycin) and the ward was closed for one week. None of the neonates developed neonatal pertussis. Nasopharyngeal swabs were obtained again one week after erythromycin therapy; 0/21 samples were PCR positive (including the 5 previously positive neonates). A second blood sample was obtained 8 weeks after start of erythromycin therapy; none of the neonates seroconverted. Blood samples from staff members (n=88) were also tested 3-4 weeks after start of prophylaxis. 1/88 staff members, seroconverted, but without any signs or symptoms of pertussis.

Conclusions. An index pertussis case infected 3 staff members and further spread of *B. pertussis* to patients was detected. Macrolide treatment prevented the development of overt disease in patients with *B. pertussis*. Seroconversion without respiratory complaints may occur under prophylactic therapy.

L06

Molecular epidemiology of Ampicillin resistant *Enterococcus faecium* in the UMC-U hospital

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Genetic population analysis of *Enterococcus faecium* identified a genetic lineage, complex-17, that has spread globally, characterized by ampicillin resistance, the presence of a pathogenicity island and associated with hospital outbreaks. Since ampicillin resistance serves as a marker for epidemic isolates we analyzed the presence of ampicillin resistance among clinically relevant enterococci collected between 1994-2003 at the UMC-U. In total 130 ampicillin resistant enterococci were found all *E. faecium* (ARE). There was an increase in the number of ARE infections between 1994 and 2003 and E. faecalis to *E. faecium* ratio's in the first 20 enterococcal blood isolates of each year (1994 to 2004) revealed a relative increase of blood stream infections caused by *E. faecium* (p=0.001). Screening of 240 patients on three at risk wards between March and October 2004 revealed that 67 (27.5%) patients were colonized with ARE. In the same period, ARE were infrequently found (n=19, 2.9%) in 650 fecal samples from non-hospitalized patients (p < 0,001). All ARE were genotyped using Multiple Locus Variable number of tandem repeat Analysis (MLVA). Among all ARE that underwent MLVA (n=216), 47 different MLVA types were found of which 36 belonged to complex-17, represented by 184 isolates (85%). MLVA typing revealed unnoticed spread of at least two ARE clones, which in both cases resulted in infection of severely ill patients. It also showed the ongoing spread of a vancomycin-susceptible ARE clone, which caused, as a vancomycin resistant strain, an epidemic in 2000. Our findings demonstrates an increase in infections caused by ARE and unnoticed spread of genetically related ARE-isolates, belonging to complex-17, during the last ten years in the UMC-U. The increase of ARE with epidemic potential within hospital settings represents a health risk, as these bacteria may acquire the vancomycin-resistance transposon.

M01

Recurrent brain abscess caused by *Cladophialophora bantiana* in a drug abuser: case report

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Introduction. *Cladophialophora bantiana* is a neurotropic, darkly pigmented mould that causes cerebral phaeohyphomycosis either in normal or in immunocompromised patients. CNS infection has a high degree of mortality (over 70%), and requires both surgical and antifungal chemotherapy. The natural habitat of *C. bantiana* is cuurently unknown. Trauma and exposure to dust have been supposed to be the main cause of infection with a particular predilection for males. Only a limited number of cases have been reported from Europe and, to our knowledge, the case we describe is the first in the Mediterranean.

Methods. The strain of *C. bantiana* was isolated from a cerebral abscess removed surgically from a 29 year-old male drug abuser. The mould developed initially on Sabouraud's dextrose agar with cloramphenicol after fifteen days of incubation at 30 °C as a black powdery colony. A slide culture on potato dextrose agar and a subsequent incubation at 40 °C showed the characteristic morphology and the thermotolerance typical of this species.

Results. The patient underwent three surgical interventions before diagnosis was established. However, once the infectious agent was identified after the third surgical intervention, the patient was successfully treated for 21 days with fluconazole. In fact two subsequent computed tomography exams, performed at, respectively, two and ten months after the last intervention did not show any abscess.

Conclusions. The mortality with *C. bantiana* is particularly high and in the majority of cases surgical and/or pharmacological treatment is unsuccessful. Death usually occurs within one year of infection onset. Moreover it was to be expected that a delay in the diagnosis could, in this case, have worsened the prognosis. It is then surprising that this patient could survive in spite of delayed antifungal chemotherapy. Our current hypothesis is that the strain of *C. bantiana* isolated from this patient is different, with regard to its virulence, from previously described strains. This hypothesis is currently under investigation by molecular analysis of the strain.

M02

Identification of Dermatophytes isolated from tinea capitis in western China using ITS sequencing

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Objective. Tinea capitis is a common dermatophyte infection of the scalp in children in western China. Over the past 20 years, the most common etiologic agent initially was *Trichophyton schoenleinii*, followed later by *T. violaceum*. Until recently, identification of dermatophytes was mainly based on the cultivation of fungal isolates on special media and on microscopic morphology. Molecular methods such as sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA have proven to be useful for identification of dermatophytes. Genotypic differences are considered more stable and more precise than phenotypic characteristics. The aim of this study is to identify the spectrum of species causing tinea capitis by molecular methods and to establish epidemiological trends in the western China.

Methods. A total of 78 isolates were collected from clinically suspected tinea capitis patients in 2003. DNA extraction of all strains was performed using the Fast Prep kit. PCR amplifications were done with primer ITS1 and ITS4. The entire ribosomal DNA internal transcribed spacer (ITS1-5.8S-ITS2) region was sequenced. *T. violacum* was identified by PCR using a microsatellite primer set (T1).
Results. Of the 78 strains examined, five species of dermatophytes (*T. violaceum, T. schoenleinii, T. tonsurans, T. ferrugineum* and *T. verrucosum*) were identified by ITS sequencing down to the species level. The remaining species were identified by specific PCR using the T1 primer set. Phylogenetic trees were generated based on an analysis of alignment data in comparison with reference strains. The results showed that *T. violaceum* (34 strains), *T. schoenleinii* (26 strains) are the preponderant species in the area investigated, while *T. tonsurans* (8 strains), T. ferrugineum (8 strains) and *T. verrucosum* (2 strains) are less prevalent. With microsatellite markers, one polymorphism was found among the strains of *T. violacum* [GT10 repeats, and a substitution and indel of four nucleotides (GGCC)]. This was type C compared to literature data.
Conclusions. Most strains show a good correspondence of sequencing data and morphology identification, especially *T. violaceum*. The spectrum of species causing tinea capitis in western China is quite different from that of other areas in the world. Differences in specific prevalences may be related to factors such as local climate, habits and availability of medical facilities.

M03
A case of a child with cystic fibrosis and infection with *Aspergillus fumigatus* and a *Pseudoallescheria boydii*: clinical parameters and serology

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Background. A 5-year-old girl diagnosed with cystic fibrosis at that age developed the clinical picture of Allergic Bronchopulmonary Aspergillosis (ABPA) at the age of 7 years. The diagnosis was based on clinical manifestations (shortness of breath, increased productive cough, decreased exercise tolerance, wheeze on auscultation) and laboratory examinations (decreased pulmonary function tests [FEV_1 59%], a rise of total IgE from 71 to 1254 kU/l, elevated total eosinophils [4.54 10^9/l], a sputum culture positive for *A. fumigatus* [first 4 months before ABPA-diagnosis] and positive IgE and IgG to *A. fumigatus*). The patient is (with a temporary interruption) still being treated with itraconazole. Further, initial therapy consisted of prednisolon for a period of 3 months daily and 4 months later a period of alternate day prednisolon. 5 months after the ABPA was diagnosed *Ps. boydii (Pb)* was cultured in sputum. The aim of this paper is to discuss the clinical characteristics in time together with IgG ELISA for both *A. fumigatus (Af)* and *Pb*.
Material and methods. Clinical parameters and outcome of sputum cultures were compared with serology on multiple occasions. An ELISA assay to *Pb* was developed using the culture medium extract from the *Pb* sputum strain. The culture medium was prepared by filtration and dry-freezing according to a routine *Aspergillus* protocol.
Results. After treatment the patient's symptoms improved and FEV_1 returned to normal. The IgE level drastically lowered, but with considerable variability. Initially, titres for both *Af* and *Pb* were negative. At first, only *Af* was cultured from the sputum and serum demonstrated specific IgG and IgE to *Af* at the time of diagnosis for ABPA. The IgG titre for *Pb* was already positive before the sputum cultures became positive for *Pb* and remained positive for both *Af* and *Pb* during the period that both fungi were present in the sputum cultures. Later the *Af* disappeared from the sputum cultures, while sputum *Pb* remained positive. During the latter period titres for both *Af* and *Pb* remained strongly positive. ELISA IgG cross- inhibition experiments and double-immuno-diffusion tests, suggest that there was no cross reactivity between *Af* and *Pb*.
Conclusion and discussion. This is the first report that describes infection with *Af* and *Pb* together with IgG ELISA titres to both fungi and corresponding clinical parameters. The possibility that a specific antibody assay has been developed to *Pb* will be discussed.

M04
Fungal manganese superoxide dismutase (MnSOD) genes: from phylogeny to the molecular diagnosis of invasive mycoses

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Background. Fungal pathogens represent an ever-increasing threat to human health. The increasing population of immunocompromised individuals, together with the growing diversity of involved fungal species, and the emergence of resistance to antimycotic drugs, have compounded the problem. Currently, the definitive diagnosis is still based on the histological evidence of tissue invasion and/or the isolation and identification of the fungal agent, which is important to drive the antifungal therapy. In fact, culture-based diagnosis lacks of sensitivity, especially in early infection stages, and in vitro fungal growth can take many days or weeks. Considering these fungal infections are associated with high mortality, molecular tools are needed to detect earlier the infection in order to administer early an adequate therapy, and to adjust it in terms of an accurate quantitative-PCR follow up of the infection course. The aim of this project is to develop a molecular tool for detecting deep fungal infections
Methods. The MnSOD gene was selected as a target, regarding recent published data (including *Pneumocystis* papers of our own group) that have demonstrated its accuracy to fungal species identification.
Results. First step included sequencing and phylogenetic analysis of the MnSOD gene in the main medically important fungi. In a second step, alignment of MnSOD sequences led to the identification of a nucleotidic specific motif that can be used to detect the main opportunistic pathogenic fungi using PCR.
Conclusion. An interesting observation was the topology of the MnSOD-based tree, exhibiting MnSOD paralog genes

in Ascomycota. Also, MnSOD gene polymorphism revealed to be a more accurate indicator of molecular clock than 18S rRNA genes.

M05
Identification and pathogenicity of clinical isolates of genus *Exophiala* from the U.S.A.

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Objective. Numerous members of the genus *Exophiala* are potential agents of human and animal mycoses. The majority of these infections are superficial, but also fatal systemic infections are known. The aim of this presentation is to establish which *Exophiala* species are recurrent in the clinical lab.
Methods. We re-identified 210 clinical isolates from the U.S.A., which had a preliminary morphological ID, by sequencing internal transcribed spacer (ITS) region of the ribosomal DNA. The results were listed and evaluated in relation to the infections caused.
Results. Molecular IDs were, in order of frequency: 56 *E. dermatitidis* (26.7%), 53 *E. oligosperma* (25.2%), 37 *E. 'xenobiotica'* sp. nov. (17.6%), 12 *E. phaeomuriformis* (5.7%), 12 *E. lecanii-corni* (5.7%), 8 *E. jeanselmei* (3.8%), 7 *E. bergeri* (3.3%), 6 *E. spinifera* (2.9%), 6 *E. mesophila* (2.9%), 3 *Phialophora europaea* (1.4%), 3 *E. 'equina'* sp. nov. (1.4%), 3 *Rhinocladiella similis* (1.4%), 2 *E. attenuata* (1.0%), 1 *E. heteromorpha* (0.5%), and 1 *E. salmonis* (0.5%). Strains were repeatedly isolated (28.1%) from systemic infections involving lung, heart, brain, bone marrow, blood, spleen, bile, stomach and intestines. Localized or superficial lesions (49.0%) included cutaneous and subcutaneous lesions, eyes, sinus maxillaris, throat, ear, lymph node, joint, breast, mucous membranes, nail, scalp and hair infections. The systemic infections were preponderantly caused by *E. dermatitidis, E. oligosperma, E. lecanii-corni, E. phaeomuriformis, E. 'xenobiotica', E. heteromorpha* and *E. 'equina'*. Strains of *E. bergeri, E. spinifera, E. mesophila, E. jeanselmei, Rhinocladiella similis, E. attenuata* and *P. europaea* mainly induced superficial and local infections.
Conclusion. Black yeasts of genus *Exophiala* are mainly known for their superficial and local infections, but systemic infections are relatively frequent. Main agents of such infections are *E. dermatitidis, E. oligosperma, E. phaeomuriformis* and *E. 'xenobiotica'*, and these species can be regarded as the most important clinically relevant agents of *Exophiala*. Since relatively few unknown ITS signatures were encountered, we suppose that the list of opportunistic *Exophiala* species in temperate climates is nearing completion, when a number of new species (*E. 'xenotiotica', E.' equina'*, in prep.) have been described.

M06
Relation of halotolerance to human-pathogenicity in the fungal Tree of Life: an overview of ecology and evolution under stress

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Objective. Despite the known phylogenetic diversity of the xerotolerant fungi, we have made a remarkable observation when comparing the distribution of xerotolerance in the fungal Kingdom with that of fungi able to invade warm-blooded animals. The aim of this presentation is to find a possible explanation for this phenomenon.
Methods. Ordinal relationships were established on the basis of SSU rDNA sequence data. Known xerotolerant resp. -philic and halotolerant resp. -philic species were attributed to their fungal orders. Known agents of vertebrate pathogenicity and opportunism were listed, and scattered cases were evaluated.
Results. At present, a total of 106 orders of fungi are known. Tolerance of low water-activity is apparent in only ten of these. Pathogenicity and consistent opportunism (BioSafety Levels 2 or 3) are also found in ten orders. Both properties are uncommon in the fungal Kingdom. Nonetheless, the two lists show total overlap: eight orders with xerotolerance also contain opportunistic fungi of BSL 2-3, while the remaining three contain occasional opportunists (BSL-1). However, with only a few exceptions, species exhibiting xerotolerance have no BSL attribution at all or belong to BSL-1.
Conclusion. The distribution of xerotolerance and clinical significance strongly suggests that the backbones of each of these eight orders encode properties that are useful for both life strategies. Focusing on individual species, we notice, however, that xerotolerance and pathogenicity seem to be mutually exclusive. If xerotolerance is regarded as a condition to cope with general stress, presence of properties underlying this ability provides the fungus with an armament to survive types of stressful conditions other than decreased water activity. Low degrees of xerotolerance therefore may mark different starting points of subsequent evolution, either into a direction of an even higher degree of stress tolerance, or in another direction, leading to disruptive selection or to adaptive sympatric speciation. Osmotolerance and may be regarded as a new virulence factor.

M07
Rapid diagnosis of PCP and resistance to co-trimoxazole using real-time PCR

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Introduction. *Pneumocystis jiroveci* pneumonia (PCP) is an opportunistic infection of immunocompromised patients. Polymerase chain reaction (PCR) methods have been described which offer improved sensitivity and specificity.

However, assessment of the use of real-time PCR in the diagnostic laboratory is less well described. Additionally rapid detection of resistance mutations to co-trimoxazole (Co-T) might improve clinical management of patients. Here, conventional microbiological staining methods were compared to conventional PCR and real-time PCR for diagnosis of *P. jiroveci* and positive samples were assessed for the presence of resistant mutations.

Methods. Eighty-four sequential bronchoalveolar lavage samples from patients analyzed by methanamine silver and Giemsa staining methods were stored at -70 °C and subsequently analyzed by conventional and real-time PCR. The BALs were collected over a 20-month period. The real-time PCR was designed to the dihydropteroate synthase (DHPS) to be specific for *P. jiroveci* and enable sequencing of the PCR product to determine resistance mutations at nucleotide positions 165 and 171.

Results. The staining methods showed that 16/84 (19%) patients had PCP. The real-time PCR and the conventional PCR both detected *P. jiroveci* in 19/84 (22.6%). All the samples positive by microscopy were positive. Using the staining methods as the 'Gold Standard', the sensitivity, specificity, positive predictive value and negative predictive value for the PCR methods were 100%, 96%, 84% and 100% respectively. The mean cycle threshold (Ct) value for the 16 stain positives was 31.5 and for the 3 stain negative/PCR positives was 41.5. Analysis of the clinical records and microbiological results of the 3 discrepant samples showed that PCP was the most likely clinical diagnosis. The 19 positives were all sequenced and no resistance mutations were found and results were available in one working day.

Conclusions. Real-time PCR for *P. jiroveci* can provide rapid, sensitive and specific diagnosis for PCP in BAL samples. Additionally the Ct value may be useful in determining the amount of infection. Using this method targeting the DHPS gene rapid results can also be obtained for resistance to Co-T.

Mo8

Multi-locus sequencing raises new questions in the *Cryptococcus neoformans* species complex

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Cryptococcus neoformans and *C. gattii* are basidiomycetous yeasts that are important human pathogens of mainly immunocompromised people. Previous studies have resulted in the recognition of two species: *C. neoformans*, with varieties *grubii* and *neoformans*, and *C. gattii*. The observation of mating resulted in the description of the teleomorph *Filobasidiella*. Mating can occur between strains of opposite matingtypes (MATalpha and MATa).

Different genotyping methods such as Amplified Fragment Length Polymorphish (AFLP) and PCR fingerprinting show a division in 7 groups. These groups correspond to the current classification, but 4 extra groups can be found in *C. gattii*.

We performed multi-locus sequencing on 160 strains from different origin (clinical, environmental, geographical) to obtain a better understanding of the species complex.

Multi-locus sequencing showed that natural hybrids (environmental and clinical) between *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* do not have both rDNA alleles of ITS (internal transcribed spacer) and IGS (intergenic spacer). Even after cloning, rDNA from only one of the parents could be found. However, in hybrid laboratory strains both alleles were present. Therefore it seems that cryptococcal hybrid strains lose the additional genetic material (such as rDNA).

By sequencing the mitochondrial gene ATP6 we found that a hybrid strain always inherits the ATP6 allele from the MATa parent. This is concurrent with the results from Yan and Xu who found that mitochondria are always inherited from the MATa parent.

However we did not get the same result with the other mitochondrial region included in our study as all MtLrRNA (mitochondrial large ribosomal subunit RNA) sequences obtained from hybrids were *C. neoformans* var. *neoformans* sequences, irrespective of the mating-serotype combination of the hybrid.

Multi-locus sequencing resulted in a better resolved structure of the species complex, but especially in the case of hybrids, new questions, e.g. on mitochondrial inheritance, were raised.

Mo9

Primary hepatic invasive aspergillosis (IA) in a hematopoietic stem cell transplant (HSCT) recipient

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A 53-year-old female patient underwent a HSCT from an unrelated donor to treat a chronic lymphatic leukemia. During the neutropenic period circulating aspergillus antigen galactomannan (GM) was detected which showed an increasing trend. A high resolution CT (HRCT) scan of the thorax and brain showed no evidence for a fungal infection. However, treatment with itraconazole was started and the GM ratio became negative. However, over the following 2 months the GM level remained between 0.5 and 1.4 which was interpreted as false-reactivity, since repeat HRCT of the lungs showed no evidence of IA and the patient was clinically in good condition. The patient developed graft-versus-host-disease and a post-transplantation lymphoproliferative disorder (PTLD) with several lesions in liver and spleen on the HRCT. Treatment with retuximab was initiated. During this therapy (day 115 post-HSCT) she was admitted with a septic shock. Diagnostic work-up showed that one hepatic lesion in the liver had progressed while the other lesions had responded to retuximab treatment. The plasma GM ratio had increased to 15.5 and a biopsy of the lesion in the liver was performed. Microscopy of the liver biospy showed hyphae consistent with aspergillus although a yeast infection could not be ruled out. Culture of the liver biopsy grew *Aspergillus fumigatus*. Voriconazole treatment was started and the GM ratio declined to 2.0. A follow-up HRCT scan showed decreasing lesions in the liver and spleen. The patient is still being treated with voriconazole.

Conclusion. Circulating GM was the first indication of primary hepatic IA in this patient. Persistent and rising GM is an strong indication of IA and should prompt to additional diagnostic procedures if imaging of lung and brain are negative.

M10
Failure of caspofungin (CAS) as primary treatment of proven invasive aspergillosis (IA) in a hematopoietic stem cell (HSCT) transplant recipient

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CAS is accepted as salvage therapy in patients with IA and is undergoing evaluation as first-line therapy. We describe a 29-year-old male allogeneic HSCT recipient who received CAS as treatment of probable IA, based on characteristic pulmonary lesions on the HRCT and the presence of circulating aspergillus antigen, galactomannan (GM, serum ratio 2.6). Voriconazole was contraindicated in this patient because of significant elevation of liver-enzymes during a previous treatment episode. Shortly after commencing CAS therapy the GM ratio in serum increased to 33.3 after which it started to decrease. However, after an initial clinical response, the patient detriorated despite the return of granulocytes. Also a HRCT of the lungs showed progression of the pulmonary infiltrates. The patient became respiratory insufficient and developed multi-organ failure. He was transferred to the intensive care unit and treatment was changed to ambisome. The clinical condition of the patient deteriorated further and circulating GM levels increased indicating progressive infection. Ultimately the patient died on day 30 after HSCT. At autopsy, tissue of the upper lobe of the left lung was obtained and cultures grew *Aspergillus fumigatus*. The strain had a low minimal inhibitory concentration (MIC) for CAS (0.125 mg/l).
Conclusion. This patient with proven IA failed to primary therapy with CAS despite in vitro activity of the drug against the infecting strain. Furthermore, significant increase of circulating GM during therapy with CAS was noted, which has been observed in experimental models previously.

Q01/02
Mechanisms of genetic variability in foodborne bacterial pathogens

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The increasing availability of genome sequence information and the advent of microarray technology are providing new insights into the genetic basis of bacterial diversity and the mechanisms involved in gene transfer that lead to the acquisition of new genetic traits in different species and genus. The introduction of DNA into microbial genomes, referred to as horizontal DNA transfer, can occur in several ways i.e. by transformation, bacteriophages, or via conjugative transposons and plasmids and this has the potential to radically alter the life-style of a bacterium. Surveys of microbial genomes have revealed that up to 20% of the genome of some bacteria constitutes horizontally transferred DNA and the retention of this DNA over evolutionary time contributes to species diversification. Clusters of genes or islands that are important in virulence and have an average base composition different from the bulk of the genome are commonly referred to as pathogenicity islands (PI) and they have been found in a variety of Gram-positive and Gram-negative bacteria. In many cases PI are flanked by sequences associated with DNA transfer indicating that they have been spread among members of the bacterial kingdom by horizontal transfer, especially via plasmids. There are also examples where particular genes have been lost from bacterial lineages either because they fail to provide any further benefit or because they interfere with adaptation to a new ecological niche. In this lecture the contribution of lateral gene transfer to the evolution of food-borne bacterial pathogens will be discussed including recent data on the use of DNA microarray hybridisations to investigate uncharacterised of Campylobacter jejuni, Salmonella and Listeria. The variety of possible DNA transfer and transposition mechanisms operating in these pathogens will also be highlighted.

Q03
Phenotypical characterization, antimicrobial resistance and molecular typing of 23 clinical isolates of *Nocardia farcinica* in the Netherlands

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Background. A recent cluster of 4 disseminated *Nocardia farcinica* infections among immunocompromised patients in our hospital, prompted us to investigate the relatedness of *N. farcinica* strains in more detail.
Methods. In total, 25 *N. farcinica* strains were included: 23 isolated from clinical specimens identified by 16S rRNA gene analysis, and 2 ATCC strains. Of 23 strains, 4 were isolated in hospital A in the period from july-sept. 2004, 14 were collected in hospital B and 5 were cultured in hospital C. All strains were investigated phenotypically by API 32C Yeast identification system and conventional tests. Primers selected for random amplification polymorphic DNA (RAPD) analysis, were DAF4 and RTG6, as they provided sufficient bands for typing studies. Amplified fragment length polymorphism (AFLP) was optimized and applied on all isolates Susceptibility tests were performed by E-test and conventional disk diffusion assay.
Results. All 25 strains were negative for esculin hydrolysis, acetate and citrate utilization, resistant to lysozym, and had a good identification code based on the last four digits of the API 32C profile for *N. farcinica*. All isolates were susceptible to amikacin and ciprofloxacin, but resistant to gentamicin, tobramycin and erythromcycin. Median MIC values for imipenem (0.5 ug/ml) were 1.5 times lower than meropenem (1.5 ug/ml). An inducible resistance was observed in all strains to ciprofloxacin with erythromycin as inducer. All 4

epidemic strains were related with AFLP, 2 ATCC strains were identical as were two isolates from hospital C. All remaining isolates revealed unique patterns.
Conclusion. *N. farcinica* can be identified correctly with conventional phenotypical test. An unknown macrolide inducible resistance to ciprofloxacin was observed among all isolates. AFLP is currently the typing method of first choice.

Q04
Functional microbiomics: elucidation of the functionality of the human GI-tract microbiota

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Introduction. The human gastrointestinal tract (GI-tract) harbours a complex microbial community consisting of mainly anaerobic bacteria, collectively known as microbiota. While our insight into the diversity of the microbiota has been increased greatly by analysis of the 16S ribosomal RNA gene sequence variability, we have only limited knowledge of its *in vivo* functionality. Therefore, the aim of this research is to investigate prokaryotic gene expression of the human microbiota by the molecular method cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP).
Methods. The RNA fingerprinting method cDNA-AFLP has previously been successfully used to investigate gene expression in plant pathogens and yeast without knowledge about their genomic diversity. In short, cDNA-AFLP visualizes transcript diversity on a polyacrylamide gel after selective amplification of specific restriction fragments obtained from cDNA fragments synthesized from total RNA.
Results. cDNA-AFLP analysis of total RNA extracted from human faecal samples resulted in clear fingerprints that contained about 1,200 TDFs (Transcription Derived Fragments). Sequence analysis of 65 randomly selected TDFs and subsequent homology searches in protein and nucleotide databases were performed to unravel their identity. Two sequences of approximately 0.4 kb could clearly be identified as transcripts from prokaryotic origin with a known function. In addition, 9 sequences showed no or very low homology to any of the searched databases as may have been expected since the vast majority of GI-tract bacteria have not been sequenced yet. Moreover, 41 sequences turned out to be of prokaryotic ribosomal origin and another 13 sequences showed highest homology to ribosomal RNA of human origin.
Conclusion. We demonstrated that cDNA-AFLP analysis of RNA extracted from GI-tract samples is a useful approach to study *in vivo* microbial gene expression in the human GI-tract. Currently, the addition of several mRNA-enriching steps to the RNA isolation and cDNA-AFLP approach are validated, in order to reduce the fraction of TDFs of ribosomal RNA origin in the cDNA-AFLP profiles.

Q05
A cost-effectiveness study comparing real-time PCR with traditional culture for detection of *Salmonella* spp. and *Campylobacter jejuni* in feces

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Introduction. We have developed a real-time PCR for the detection of *Salmonella spp*. and *Campylobacter jejuni* in feces. Positive and negative results can be obtained within 24 hours, whereas with traditional culture the outcome is only available after 2-4 days. Based on this remarkable difference in turn-around time our objective is to change the standard workflow in our laboratory: Culture will be performed only on the small fraction of PCR positive or blood containing samples (~10%). To estimate the economic consequences of this method we performed a cost-effectiveness study comparing both methods.
Methods. Each year more than 10.000 fecal samples are cultured at our laboratory. Our own historical data indicate that 3.0% of the samples are positive for *Salmonella spp*. and 4.7% are positive for *Campylobacter spp*., whereas other bacterial pathogens like *Shigella spp*., *Yersinia enterocolitica*, and *Escherichia coli O157* are only seen in less than 0.3% of the cases. Based on these data the total costs of culturing were calculated and compared with costs for PCR detection and subsequent culturing of positive samples. The total workload and all material costs were included in the calculations.
Results. Culture based detection of *Salmonella spp*. and *Campylobacter spp*., resulted in an average cost per sample of € 8.70. PCR processing and subsequent culturing of positive samples of *Salmonella spp* and *Campylobacter jejuni* showed an average cost per sample of € 8.80.
Conclusions. PCR processing of fecal samples is cost-effective for the detection of *Salmonella spp*. and *Campylobacter jejuni* and general implementation in a clinical laboratory results in a considerable reduction in turn-around time.
However, in such an approach less frequently detected pathogens, including *Shigella spp*., *Yersinia enterocolitica*, and *Escherichia coli O157* are not consistently detected.

Q06
Clonal *Campylobacter jejuni* strains are deficient in DNA competence

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Campylobacter jejuni is considered to be the major cause of bacterial gastro-enteritis in humans. For a long time, epidemiology was based on phenotypic traits using classical methods such as serotyping. With the advent of genomics, typing methods based on genome composition (genotyping) are more common. Genotyping can be used to analyse the population structure of *C. jejuni*, which is identified as genetic diverse with a few clonal lineages. As *C. jejuni* is natural competent, we investigated whether *C. jejuni* strains from clonal lineages are impaired in DNA uptake via natural transformation.

The genetic relationship of 30 *C. jejuni* strains with Penner serotypes O:1, O:2, O:19, O:41 and O:55 was determined with amplified fragment length polymorphism (AFLP). Their transformability was investigated with natural and electro-transformation experiments.
AFLP analysis yielded unique patterns for most of the strains belonging to serotypes O:1 and O:2, indicating genetic diversity within non-clonal lineages. Within serotypes O:19, O:41 and O:55 AFLP profiles were very similar, suggesting that genetic diversity was minimal (clonal lineages). Natural transformation experiments revealed that clonal strains could not be transformed, neither with homologous nor with heterologous plasmid DNA, while for most non-clonal strains, kanamycin-resistant transformants were obtained. Electro-transformation yielded transformants for both clonal and non-clonal strains.
Taken together these results suggest that a deficiency in natural transformation contributes to the existence of the clonal lineages O:19, O:41 and O:55. DNA binding and uptake assays will be performed to further dissect the nature of the defect.

Q07
Marine anaerobic ammonium oxidizing bacteria

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The availability of fixed inorganic nitrogen limits primary productivity in many oceanic regions. So far only the conversion of nitrate to N_2 by heterotrophic bacteria was believed to be an important sink for fixed inorganic nitrogen. However, recent independent studies showed that marine anammox bacteria converted ammonium anaerobically with nitrite to N_2 in the Black Sea, the Skaggerak, and the Gulfo Dulce.[1-3] Nutrient profiles, fluorescently labelled RNA probes, ^{15}N tracer experiments, and the distribution of specific anammox ladderane membrane lipids indicated that the anammox bacteria were responsible for the loss of fixed nitrogen in the suboxic zone of these ecosystems. Phylogenetic analysis of 16S ribosomal RNA gene sequences showed that the marine anammox bacteria of the Black Sea, named *Candidatus* 'Scalindua sorokinii', were only distantly related to the waste water species *Candidatus* 'Kuenenia stuttgartiensis' and *Candidatus* 'Brocadia anammoxidans'.[3] The estimated contribution of marine anammox bacteria to the loss of fixed inorganic nitrogen ranged from 30-70%. The widespread occurrence of ammonium consumption in many suboxic marine settings indicates that anammox bacteria may play a very important role in the oceanic nitrogen cycle.[3,4]

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Q08
Improvements in the secundary laboratory diagnosis of tuberculosis

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The secundary laboratory diagnosis of tuberculosis involves identification, drug susceptibility testing, and epidemiological typing of cultured mycobacteria. Not only the quality, but also the turn-around-time of the laboratory diagnosis is of importance. The RIVM receives about 1000 *Mycobacterium tuberculosis* complex isolates and about 400-500 non-*M. tuberculosis* complex (Atypical) mycobacterial cultures per year.
In 2004, reversed line blot assays were introduced for identification of *M. tuberculosis* complex- as well as all relevant Atypical mycobacteria. Therefore, identification of mycobacteria is nowadays performed in a few hours. Because these identification methods are PCR-based, they can be applied directly to weakly positive liquid cultures.
Drug susceptibility testing (DST) of all *M. tuberculosis* complex isolates in the Netherlands is performed for therapy guidance and to facilitate (inter) national surveillance of resistance. Unfortunately, for most tuberculostatics only a phenotypic method is available. At the RIVM a proportional minimal inhibition concentration (MIC) method on 7H10 agar is used for all first- and second line drugs. The quality of this method is controlled by proficiency studies of the WHO. Only for the detection of rifampicin resistance, a sensitive molecular method is available, but in 2005 the performance of a new molecular method for INH resistance will be tested.
IS6110 restriction fragment length polymorphism (RFLP) typing of *M. tuberculosis* complex isolates has been used successfully in the past decade to investigate transmission of tuberculosis in the Netherlands at various scales. This has provided insight into the natural history of tuberculosis infection in this country and facilitated contact investigation and source case finding. It also helped in detecting laboratory cross-contaminations (2-3% of the positive mycobacterial cultures in the Netherlands). Because RFLP typing is technically demanding and time consuming, new typing methods are being investigated. Variable Number of Tandem Repeat typing, which is based on PCR seems a promising candidate to eventually replace RFLP typing.

Q09
Nalidixic acid resistance in *Campylobacter lari*

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Campylobacter lari is a phenotypically and genotypically very diverse species that widely occurs in the environment and occasionally has been reported to be involved in intestinal and extra-intestinal infections in humans. Classical *C. lari* strains are resistant to the quinolone nalidixic acid and do not produce urease, but variants susceptible to nalidixic acid and/or capable of urease production exist. The aim of

this study is to investigate how these properties correlate with the various lineages and what the molecular basis of quinolone resistance in *C. lari* is.
A set of 250 *C. lari* strains from different sources were phylogenetically analyzed by amplified fragment length polymorphism (AFLP), and were tested for nalidixic acid resistance and urease production. Eleven genogroups were identified of which three were nalidixic acid resistant. Urease activity was restricted to four nalidixic acid susceptible genogroups. The observed phylogenetic clustering of nalidixic acid resistant strains and urease producing strains suggests that the acquirement of these properties is an early event, after which other events caused the genetic diversity in the descendants that make up the respective genogroups. For a subset of 48 strains that represent all genogroups, the molecular basis of nalidixic acid resistance was studied by antibiotic resistance typing and sequence analysis of the quinolone resistance determining region (QRDR) of the *gyrA* gene. None of the strains was multidrug resistant, showing that a multidrug efflux pump does not contribute to nalidixic acid resistance in *C. lari*.
Sequence analysis of the QRDR of *gyrA* showed that quinolone resistance of *C. lari* correlates with an amino acid substitution at position 86. The phylogenetic clustering of exclusively nalidixic acid resistant strains in certain genogroups suggests that, once acquired, mutations in *gyrA* leading to quinolone resistance do not revert.

Q10
Identification of proteins binding to the pseudomurein cell wall of *Methanothermobacter thermautotrophicus*

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The domain archaea are typified, amongst other characteristics, by the presence of a cell wall composed of pseudomurein. Although the structure and exact characteristics of pseudomurein are known for some time, the enzymes involved in pseudomurein processing have not been studied. So far only two phage enzymes, PeiW and PeiP, have been shown to degrade pseudomurein. These proteins share a molecular architecture and both contain N-terminal repeats of unknown function and a catalytic domain in the C-terminal halve. Initial experiments showed that the PeiW protein was able to bind rapidly (~1 min) and in large amounts (~4?107 PeiW/cell) to *Methanothermobacter thermautotrophicus* cells. To further study PeiW binding, truncated PeiW derivatives where characterised that missed the N-terminal repeats or the catalytic domain, respectively. These experiments showed that the N-terminal repeat region only was involved in pseudomurein-binding. The PeiW and PeiP sequences were next used for the identification of pseudomurein-binding domains encoded in the *M. thermautotrophicus* genome. An analysis of the modular architecture of the *M. thermautotrophicus* proteins that contained the PeiP and PeiW catalytic domain showed the presence of two ORFs with N-terminal repeats that shared no sequence homology with PeiW or PeiP. Further sequence analysis indicated the presence of this 150 amino acid repeat unit in 10 ORFs of which 9 contained a predicted signal peptide. Microscopical analysis of *M. thermautotrophicus* cells incubated with a fusion protein consisting of the 150 aa repeat unit fused to Green Fluorescent Protein unambiguously showed that this domain is involved in pseudomurein-binding. In conclusion, the identification of the pseudomurein-binding domain present in 10 ORFs of the *M. thermautotrophicus* genome has revealed previously unknown protein candidates involved in the biosynthesis and modification of pseudomurein during its life cycle.

Q11
A proteomics approach to study the copper homeostasis - development relation in *Streptomyces lividans*

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Introduction. In the absence of Cu-ions (copper bound by the Cu-chelator: bathocuproine disulfonic acid (BCDA)), development is frozen in the vegetative growth phase but vegetative growth itself is not affected. Upon the addition of Cu^{2+}, development proceeds with aerial growth. Genome sequence of the filamentous bacterium *Streptomyces coelicolor* revealed that at least 13 putative Cu-proteins are encoded. Most of them are secreted or membrane bound.[1] Analysis of the extracellular proteome is used to trace secreted (Cu-) proteins involved in or controlled by Cu availability and/or development.
Methods. *Streptomyces lividans* solid cultures were cultivated in the presence of 10mM Cu^{+2} or 20mM BCDA. Extracellular protein expression was studied by 2D gel electrophoresis followed by MALDI-TOF-MS analysis of in-gel trypsin digested proteins.
Results. The 2D profile of the extracellular proteome showed that expression levels of several proteins were induced by Cu deprivation.[2] 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. A direct link between copper dependent development and respiration is strongly supported by this observation.
Individual knockout mutants were generated of each of the copper proteins, among which a unique multi copper oxidase[3], and the morphology of these mutants was analyzed. Only one of the mutants was affected in development.
Conclusion. A large number of extracellular proteins undergoes post-translational modifications. During growth *Streptomyces lividans* suffers from phosphate starvation.
The *sco* mutant has a bald phenotype demonstrating the relation between Cu-homeostasis and respiration.

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R

Introduction Enteroviruses in clinical practice

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Enteroviruses are among the more frequently isolated viruses in daily practice but their clinical impact is not well known. They cause usually mild infections and on some occasions infection is merely coincident with unrelated pathology. In contrast, enteroviruses can cause severe infections (meningo-encephalitis, pancreatitis, myocarditis). They are also associated with chronic conditions like heart failure and type 1 diabetes mellitus. The session will focus on new aspects, including newer virus types, diagnostic methods and a putative relationship with diabetes. Finally, the need for better surveillance is discussed.

R01

A novel human enterovirus in faecal samples from HIV-1 infected persons and patients with acute flaccid paralysis

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Following two independent lines of study, we have discovered a new serotype of human enterovirus. We screened a Dutch cohort of 201 HIV-1 positive subjects from 1994-1995 with nested degenerate primers targeting conserved domains within the picornavirus 5'-untranslated region (5'-UTR). Independently, the same virus was isolated from faecal samples of four individuals with acute flaccid paralysis in Cambodia. We provisionally have designated the virus as enterovirus 90 (EV90).

EV90 can be cultured on RD cells, but not on HEP-2, HEL, Vero or diploid cells of human fetal lung. Upon infection, RD cells exhibited increased rounding and refractility, nuclear pyknosis and eventually detachment from the culture surface. In hematoxilin/eosin stained preparations, large numbers of nuclei displaced to the periphery were observed in infected RD cells, but not in the non-infected control samples. EV90 is neither neutralized by the conventional pooled 'anti-entero' sera nor by specific antisera against representative enterovirus strains. Phylogenetic analysis based on the capsid VP1 sequence revealed that this virus belongs to the species human enterovirus A (HEV-A), but is distinct from any of the previously described HEV-A strains. Instead, EV90 proved relatively closely related to simian enteroviruses that were recently assigned to HEV-A. The complete 7.4 kb genome sequences of two EV90 isolates from the Dutch and Cambodia cohorts shared 86% nucleotide sequence identity and exhibited 68% identity to the closest HEV-A strains, coxsackie A16 and enterovirus 71. Similarity plot analysis showed that EV90 remained distinct from all other HEV strains throughout all polyprotein domains, suggesting absence of inter- and intra-species genomic recombination. By contrast, its 5'-UTR formed a monophyletic cluster with members of HEV-C and HEV-D (group I), rather than with other HEV-A and HEV-B strains (group II). A large screen of faecal samples to assess EV90's prevalence and associated clinical symptoms is currently underway.

R02

A newly identified parechovirus causing sepsis-like syndrome in neonates

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From June through October 2004 fourteen infants were admitted to the hospital with symptoms varying from aseptic meningitis to sepsis-like syndrome. All of the infants were 2-8 weeks old and had acute onset of fever, and were irritable. The majority of the admitted patients had accompanying symptoms such as lethargy, anorexia and tachypnoea. They all received antibiotics (amoxicillin and ceftriaxone), some of them also received aciclovir. All improved clinically after 3 to 5 days. Polymerase chain reaction on cerebrospinal fluid was negative for enterovirus and herpes simplex virus. Nasopharyngeal swabs and feces were taken for viral culture on four different cell lines (HEL, Vero, Hep-2 and LLCMK2). After 5 to 10 days of incubation cytopathologic effect was found on the Vero cells, characterized by rounding of cells, condensation of nuclei, eventually resulting in cell-lysis. Immunofluorescence for enterovirus was negative. The isolates could not be serotyped using different pools of neutralizing antibodies to different enteroviruses. Furthermore the enterovirus-specific PCR was negative. Electron microscopy showed a picornavirus. Because of the growth characteristics and resistance to chloroform, a parechovirus was suspected. A new PCR for parechovirus was developed and all isolates were tested positive in this parechovirus-PCR. Subsequent analysis of the amplified product revealed parechovirus type 3.

To our knowledge, this is the first report of clinical disease caused by parechovirus type 3 in the Netherlands. Genotyping of the different isolates showed that in these 14 infants there were at least 3 sublineages of parechovirus 3, indicating that parechovirus is endemic in the population. Serologic studies are underway to investigate the seroprevalence of antibodies to this newly identified parechovirus type 3.

R03
Molecular diagnosis of Enteroviruses

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Introduction. Human enteroviruses (HEV) infections are very common. Fortunately, the vast majority of these infections is self-limiting with no serious sequelae. However, the diseases range from the very serious (poliomyelitis, meningitis) to the benign (common cold) and together, they cause enormous hardship in the human population. Moreover, the vast incidence of infections by these highly adaptable viruses leads to the continuous emergence of new virulent strains. These may be either highly virulent leading to fatalities particularly in children (e.g. enterovirus 71) or they may have changed their pathogenic phenotype (e.g. the evolution of coxsackievirus A24, causing the common cold, into coxackievirus A24v causing acute hemorrhagic conjunctivitis to millions of humans in the nineteen seventies). Enterovirus diagnosis is therefore clinically very important. Molecular detection is a routine tool to diagnose enterovirus infections in a variety of clinical specimens and, in fact, the method of choice to diagnose enterovirus meningitis. In general broad-spectrum PCR systems are used directed against the conserved 5'NTR of this virus group. However, no molecular detection technique is yet approved as a standard for routine laboratory use.
Aim. To evaluate clinical utility of the conventional in-house two-step PCR, previously developed by us (Zoll et all, JCM 1992), the commercially available real-time PCR-based Artus RealArt™ Enterovirus LightCycler® test and the NASBA-based bioMérieux real-time NucliSens EasyQ Enterovirus Assay, for the detection of enterovirus in cerebrospinal fluid (CSF) samples.
Results. The detection limit of the molecular assays was determined with 10-fold dilutions of an enterovirus strain and with the Third European Union Concerted Action Enterovirus Proficiency Panel. The new test systems had a broad dynamic range and displayed high sensitivity, reproducibility, and specificity in these test. Cerebrospinal fluid specimens were investigated in an ungoing comparative study. The data obtained so far showed a comparable clinical sensitivity of the in-house RT-PCR and the NucliSens EasyQ Enterovirus Assay. The Artus RealArt™ Enterovirus LightCycler® test appeared to be less sensitive for the detection of enterovirus in clinical specimens.
Conclusion. Detection of enteroviruses is essential in the diagnosis of enteroviral meningitis versus bacterial meningitis, thereby resulting in timely and appropriate clinical management that can amount to significant cost savings to the patient and health care system. Molecular diagnosis facilitate early diagnosis of infection. The Artus RealArt™ Enterovirus LightCycler® test was found to be significantly less sensitive that the other systems analyzed. The NucliSens EasyQ Enterovirus Assay appears to be sensitive and specific. In contrast to the in-house RT-PCR, the NucliSens EasyQ Enterovirus Assay includes an enterovirus-specific internal control, is easy to perform and is standardized for routine practice.

R04
External quality assessment of nucleic acid amplification techniques for the detection of Enteroviruses

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Since the first description of the polymerase chain reaction (PCR), various nucleic acid amplification techniques (NAT's) have been applied extensively to detect infectious agents, including enteroviruses (EV's), for diagnostic as well as research purposes. EV's are important causes of morbidity and mortality, particularly in neonates and infants. In addition, they have been correlated with various chronic conditions such as diabetes, chronic fatigue syndrome, ALS, post-polio syndrome etc. While the added value of NAT's over conventional virus culture for the diagnosis of enteroviral infections, particularly of the CNS, are now well recognised, the significance of NAT results in elucidating the role of EV's in various chronic conditions is less clear. One of the reason for this is the lack of reference materials and external quality assessment programmes.
Thus, with initial financial support from the EU, an annual proficiency testing programme for quality control of NAT's in diagnostic virology was started in 1998 by QCCA, and later continued by QCMD. Proficiency panels consisted of 12 freeze-dried samples with various concentrations of heat-inactivated EV's from different genogroups, including a dilution series of Coxsackie A9 virus. A questionnaire on technical aspects accompanied the panel. The number of participants increased from 63 (1998) to 112 (2004) laboratories in 22 countries worldwide. Although vastly improved compared to the early days of NAT, the false-positivity rate ranged between 1.2% and 5.2%; no improvement was seen through the years. Sensitivity varied more than 1000-fold between laboratories, and was not significantly related to the type of assay used (nested/semi-nested/single PCR; in-house/commercial), extraction- or detection system used (gel electrphoresis/hybridisation/real-time). Careful selection of appropriate primers and probes and meticulous optimisation and execution of the assay appear to be the most important determinants for maximum performance. Most laboratories use in-house methods. The results of the proficiency testing programme emphasise the continued need for an external quality assessment programme, and for the development of International Standards for enteroviruses.

R05
Enteroviruses and type 1 diabetes mellitus (T1D)

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Background. Type 1 Diabetes (T1D) is a chronic metabolic disease with loss of insulin-producing β cells, which is associated with an autoimmune process that starts long before

the disease becomes clinically manifest. Both, genetic and environmental factors are involved, among which enterovirus infections. Their action may be twofold: (1) enteroviruses may trigger onset of autoimmunity against β cells, supposedly by disturbing immune regulation, (2) enteroviruses are a frequent cause of pancreatitis during which they may harm β cells.

Aim of the study. To explore whether enteroviruses can infect dendritic cells (DC) which are the conductors of the immune response, including immune regulation.

To explore modulating effects of enterovirus on immune function.

To investigate whether evidence exists for enteroviral infection at onset of T1D.

Results. We did previously show that enteroviruses cannot infect peripheral blood mononuclear cells (PBMC) *in vitro*. Now we show that they neither infect monocytes. However, monocyte-derived DC are susceptible to infection by ECHO viruses (EV) but not Coxsackie B viruses (CVB). EV kill DC within 24 hours, which occurs mainly by necrosis although there is simultaneous activation of the apoptotic pathway. Poor expression of the Coxsackie-Adenovirus Receptor on DC is held responsible for the resistance to CVB infection, since transfection with infectious CVB-RNA resulted in unrestricted replication. Unexpectedly, surface expression of activation markers, cytokine production, as well as gene-expression (micro-array) revealed no activation of DC, supposedly because of immune-evasive activity of the virus. Preliminary data indicate that functional lymphocyte assays become impaired when DC are first infected *in vitro*.

Meanwhile, we searched blood from 11 new T1D patients for viral RNA (RT-PCR) and found enteroviral RNA in 4/11 patients but in none of age-matched 20 controls. Throat swabs were invariably negative.

Conclusions. The data suggest that EV can modulate immune responses by infecting DC. Enteroviral infection of immune cells seems to be involved at onset of T1D in a proportion of the patients.

R06
Enterovirus surveillance: 1996-2003

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Enterovirus (EV) surveillance was set up in 1996 to document the absence of wild poliovirus circulation in the Netherlands. All 20 clinical virology laboratories report to RIVM the number of stool samples tested for the presence of EVs by cell culture on poliovirus-sensitive cell lines, the numbers of EVs detected and typing results of these isolates. Separate data are collected for children < 15 years of age, and older persons. Presence of polioviruses in stools is excluded for untyped or untypable EVs at RIVM by culture on L20B cells and by molecular methods. Quality of data is controlled by participation of all laboratories in a yearly proficiency test.

Up to 2003 a total of 5148 EV isolations were reported from 72361 stool samples (EV isolation rate: 7.1%). More than 95% of the EV isolations and about 80% of the stool samples are from children under 15 years of age. Laboratories linked to academic hospitals report lower EV isolation rates as public health laboratories: 4.6% vs. 9.8%.

No wild poliovirus was detected. The real strength of the present system of EV surveillance is the regular detection of vaccine-polioviruses, although in the Netherlands IPV is used for polio vaccination. 25 polioviruses have been reported from 24 stool samples; 16 of these samples were obtained from asymptomatic children, recently vaccinated in countries using OPV. In 8 cases, vaccine poliovirus isolation was an artefact, related to concurrent analysis of poliovirus-positive samples from proficiency panels. In the aftermath of local OPV vaccination campaign in Rotterdam, in response to the polio outbreak in the Cape Verdic Islands, mixtures of vaccine polioviruses were detected in 5 asymptomatic patients. The present EV surveillance system functions well to document the absence of wild poliovirus in the Netherlands and can easily be extended to monitor important parameters for other EV-caused diseases such as aseptic meningitis and diabetes mellitus.

R07
Introduction tumor virology in clinical practice

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The role of viral infections in the etiology of malignant tumors is firmly established in several instances. These include: the two human *gammaherpesvirinae* Epstein-Barr virus (EBV) and human herpes virus 8 (HHV8), the causative agents of chronic viral hepatitis, hepatitis B virus (HBV) and hepatitis C virus (HCV), several types of human papillomaviruses (HPV) and the human T cell lymphotropic virus (HTLV), type I.

These well-described associations represent not just a mere curiosity but already at present are of great practical relevance for diagnostic purposes, either in cases with clinical suspicion or for screening purposes. Potentially, the role of these viral infections in the origin or maintenance of tumor growth also offers opportunities for preventive and therapeutic options.

For these reasons, a session has been organized in which recent Dutch contributions to this field will be summarized, with an emphasis on the clinical relevance of the associations between viral infections and malignant disease.

R08
Human papillomavirus (HPV) in cervical and cutaneous tumors

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Human papillomaviruses (HPV) are small, double-stranded DNA viruses that infect mucosal and cutaneous epithelium. The HPV types infecting the genital mucosa fall into two categories: high-risk and low-risk. The low-risk HPV types, e.g. HPV6, cause genital warts and laryngeal papillomas. The high-risk HPV types, like HPV16, infect the multilayered

squamous cell epithelium of the cervix and are causative agents of cervical cancer. This is mainly based on the presence of transcriptionally active HPV DNA in nearly all cervical cancers and the activity of the E6 and E7 viral genes. The E7 protein of the high-risk HPV types acts as a viral oncoprotein deregulating the cell cycle thereby driving suprabasal cells of the squamous cell epithelium into S-phase while the E6 protein prevents that these cells go into apoptosis by binding and degrading p53. This process but also p53-independent activities of E6 lead to cell immortalization and accumulation of mutations in these cells. Ultimately this may lead to cervical cancer.

The HPV types infecting the cutaneous epithelium can be subdivided in classic cutaneous wart types and the epidermodysplasia verruciformis (EV) types. EV is a rare syndrome characterised by numerous warts and a high-risk of squamous-cell carcinoma (SCC) on sun-exposed skin. Although ultraviolet radiation is the most important causative factor of cutaneous squamous-cell cancer, (sero)epidemiological studies implicated also EV-HPV in their aetiology. Based on recent molecular-biological studies the role that EV-HPV types possibly play in cutaneous carcinogenesis is different from the role that genital HPV types play in cervical cancer. Cutaneous SCC is probably caused by an interaction between the EV-HPV types and ultraviolet radiation. The E6 protein of some EV-HPV types has been shown to impair the process of DNA repair and to prevent apoptosis of cells exposed to UV radiation. This may lead to survival of UV DNA-damaged cells. The resulting genetic instability of these cells in combination with HPV-induced extended cell proliferation due to the action of the E7 viral protein may lead to actinic keratoses and SCC. At the later stages of carcinogenesis HPV does not seem to play an important role.

R09
Human herpes virus 8: virology and disease

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Kaposi's sarcoma (KS) manifests itself clinically by reddish skin lesions and is most aggressive in HIV infected individuals co-infected with human herpes virus 8 (HHV8). Before the discovery of HHV8 the frequency of association of cytomegalovirus (CMV) infection with KS led to consider CMV as a possible etiologic agent of KS. However, Chang and Moore discovered a novel herpes virus in KS samples in 1994. They named it Kaposi's sarcoma-associated herpes virus (KSHV) or HHV8, which is now considered to be the cause of all variants of KS, including AIDS KS, classic KS, endemic KS and iatrogenic KS. HHV8 is also the causative agent of other diseases such as primary effusion lymphoma and multicentric Castleman's disease (MCD).

To study the relationship between HHV8 DNA load and clinical symptoms we have developed and evaluated a quantitative real-time (Taqman) PCR assay. This assay is very sensitive, specific and easily reproducible and can be used for different clinical samples such as serum, plasma and PBMC's. Recently, we described an HIV-1 negative, non-transplant patient, who developed HHV8-associated MCD and KS after 17 years of immunosuppressive treatment with cyclosporin A. The patient was found to be HHV8 IgG seropositive at presentation, making an acute HHV8 infection unlikely. Chemotherapy with liposomal doxorubicin resolved both symptoms of MCD and KS. A concomitant decline in the HHV8 viral load in serum /plasma, as determined by our real-time PCR assay, was observed.

HHV-8 DNA is more often detected in patients progressing towards KS than in infected patients who do not develop disease, suggesting that the level of viremia may be a prognostic marker for future KS development. We analysed longitudinal serum samples from 19 AIDS-KS patients, ranging from 2 years before KS till 2 years after KS diagnosis. We found no correlation between the HHV8 DNA viral load and progression to KS or disease stage. However, we measured a significant impact of the start of antiretroviral therapy. In particular, a decline of HHV-8 DNA in serum was observed after the start of anti-HIV therapy with protease inhibitors.

R10
Epstein-Barr virus and nasopharyngeal carcinoma: practical role of viral DNA detection

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Objective. To evaluate the role of quantitative measurement of Epstein-Barr virus (EBV) DNA in the clinical management of nasopharyngeal carcinoma (NPC) patients in a non-endemic area.

Methods. EBV DNA load in plasma samples from 20 Dutch NPC patients was determined. For each patient pre-treatment and post-treatment samples were included. Three patients were regularly sampled during treatment. RNA in situ hybridization for the detection of EBV-encoded RNAs (EBERs) was performed on nasopharyngeal biopsies in all cases.

Results. Eighteen NPCs were EBER positive and showed a positive EBV DNA load in plasma at time of diagnosis (median 4.2 $\log_{10}$ copies/ml; range: 2.6 $\log_{10}$-5.1 $\log_{10}$). Patients with EBER negative NPC's (2/20) had undetectable EBV DNA load. Sixteen out of eighteen EBER positive NPC patients completed therapy resulting in complete remission and concurrently EBV DNA became undetectable. In three longitudinally evaluated cases EBV DNA gradually became undetectable within four weeks after start of therapy. Two patients developed distant metastasis with concomitant increase in EBV DNA load. One EBER positive NPC patient developed an EBER negative metastasis during follow-up and EBV DNA load remained undetectable at time of recurrence.

Conclusions. Detection of EBV DNA in plasma correlated with the EBER-based diagnosis of EBV-association of the tumor. The disappearance of EBV-DNA after treatment confirmed the complete regression of the tumors on treatment. Concurrent increase of EBV DNA load was seen with the occurrence of metastasis in all but one case. EBV DNA load appears to be a useful tumor marker in NPC patients from a non-endemic area.

R11

Hepatocellular carcinoma and hepatitis B and C virus

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Patients with chronic liver disease, especially those with established liver cirrhosis, are at increased risk for developing hepatocellular carcinoma (HCC). HCC is the cause of more than 500 000 deaths annually throughout the world. In HCC patients, one or multiple tumors may be present in the liver, with an estimated growth of 0.5 to 4 cm in diameter per year. Tumor development may be screened for by measuring alfa-foetoprotein in serum, or by ultrasound. Small tumors can be resected. Other treatment options are alcohol injections and liver transplantation. Five-year survival is 20-50%. A mutation at codon 249 of the tumor-suppressor gene p53 is very often found in HCC.

Both hepatitis B virus (HBV) and hepatitis C virus (HCV) may cause chronic liver disease and these patients are therefore at increased risk for developing HCC. Factors predisposing for the development of HCC are a chronic inflammatory process combined with active viral replication. Furthermore, alcohol and other toxic substances like aflatoxins increase the incidence of HCC due to their hepatotoxic effect, especially in subjects with decreased liver function.

There is a strong epidemiological link between the prevalence of HBV and the occurrence of HCC. Specific viral oncogenes, however, have not been found in the HBV genome. The HBV genome can be integrated in the host genome in HBV infection. The association of HBV with HCC is probably due to incidental activation of cellular oncogenes during the integration process and by the chromosomal rearrangements during the integration. This may explain why even HBV carriers without active liver disease, like perinatally infected subjects, are also at increased risk for developing HCC.

HCV is a non-integrating virus. The association of HCV and HCC is probably due to the persistent inflammatory processes in the liver. The yearly conversion rate from HCV-associated cirrhosis to HCC is 3% in Europe and the U.S., to 6% in Japan. The average time from infection to development of HCC is 30 years, ranging from 15 to 45. In HCV-associated HCC, the proposed oncogenic event is the regeneration and clonal expansion of committed liver cells after damage by genotoxic agents in the cirrhotic liver.

S01

Isolation of clinically relevant nontuberculous mycobacteria in Zambia; eight case reports

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Introduction. The isolation of nontuberculous mycobacteria (NTM) always raises the question on their clinical significance and the decisive factor in such cases is usually based on the diagnostic criteria defined by the American Thoracic Society (ATS). In an ongoing tuberculosis surveillance in several hospitals in Zambia we found a high percentage of NTM isolated from various specimens including that are normally sterile. Eight patients from whom NTM were cultured from sterile body sites are presented.

Methods. The study population consisted of 213 patients; 99 female and 114 male adults over 15 years of age. All patients had signs and symptoms for longer than two weeks. During three consecutive days sputum was collected from patients with a productive cough and two separate specimens were cultured for mycobacteria. Depending on the clinical picture pleural fluid, ascites, abscess material or enlarged lymph nodes were obtained in a sterile manner and cultured for mycobacteria.

Results. Out of 213 patients, 69% were HIV positive. *Mycobacterium tuberculosis* was isolated from 44 patients (77% HIV positive). A specimen from a sterile body site was collected from 25 patients (60% HIV positive). From eight of these specimens NTM were isolated and these cases are presented. From four patients *M. lentiflavum* and from one patient *M. goodii* was isolated of a sterile body site specimen. In order to exclude the possibility of a laboratory contamination AFLP DNA typing was performed which showed that different strains were isolated and, therefore, a laboratory contamination could be considered unlikely.

Conclusion. 1.The medical relevance of the NTM isolations reported here appears certain for at least half of the cases on basis of the diagnostic criteria of the ATS.

2. This is the first report of *M. lentiflavum* and *M. goodii* infections in Africa.

3. Our AFLP mediated strain typing method supports the clinical relevance of the isolates on basis of genetic diversity observed among the clinical isolates.

4. Clinically relevant infection due to NTM seems to occur in HIV positive as well as in negative patients in sub-Saharan Africa.

S02

Recurrent chickenpox in renal transplant recipients

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Chickenpox caused by infection with *varicella-zoster virus* (VZV) is a common childhood disease. It is generally accepted that the original infection confers lifelong protection against symptomatic reinfection with the virus. Despite the presence of specific antibodies, latent VZV may reactivate and cause zoster, a disease characterised by a dermatomal distribution of a vesicular rash and neuralgia. It is well known that immunocompromised patients are more prone to reactivation of endogenous VZV and can have atypical zoster with dissemination or visceral involvement. Within the last six months we observed three cases of a less recognized clinical entity, i.e. exogenous reinfection with VZV resulting in clinical chickenpox. The diagnosis was based on the typical itching rash of chickenpox and detection of VZV in skin lesions two weeks after exposure to VZV, which is consistent with the incubation period for varicella, combined with well documented evidence of previous exposure, and absence of the hallmarks of reactivation, i.e. a dermatomal distribution

and neuralgia. To our knowledge a total of 20 cases of apparent exogenous reinfection with clinical chickenpox have been reported in the medical literature, including patients with impaired immunity due to haematological malignancies and SLE. Giving the growing population of immunocompromised patients, clinical reinfection with VZV is likely to be encountered more frequently. Our observations suggest that VZV exogenous reinfection already is a common, although often missed, clinical problem in transplant recipients. Therefore, caregivers of immunocompromised patients should instruct there patients about the risk of varicella on exposure, even though they have a history of previous chickenpox, detectable antibodies to the virus, or both. In addition, VZV reinfection should not be confused with primary chickenpox or atypical zoster. Because of the benign natural course of VZV reinfection an accurate diagnosis can prevent unnecessary aggressive treatment and patient distress.

S03
Pulmonary *Enterobius vermicularis* infection, a case report

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Introduction. *Enterobius vermicularis* infection is a common parasitic infection almost always confined to the gut, but extra-intestinal infections have been reported. We describe the unexpected presence of *E. vermicularis* in the lung of a patient with mild pulmonary symptoms.
Examination and findings. A 38-year old woman presented with epigastric pain, diarrhea and nausea, later followed by dyspnoea and the production of sputum. The patient had a history of bilateral infiltrating ductal carcinoma five years earlier, for which she underwent mastectomy, adjuvant chemotherapy and radiotherapy. One year before, she had rectal bleeding, associated with massive intestinal *E. vermicularis* infection as observed during endoscopy. At the time, mebendazole (100 mg, single dose) treatment was instituted and repeated after 2 weeks.
A CT scan of the thorax showed an abnormal density in the basal lobe of the right lung. A pulmonary metastatic lesion of breast carcinoma was suspected and diagnostic excision was performed. Pathological examination showed necrotizing granulomatous inflammation, but no malignant cells. The Ziehl-Neelsen and auramine staining were negative for mycobacteria nor were other micro-organisms found in PAS stained material. The granulomatous area contained several round to oval structures with a double layered wall, measuring around 55 by 25 mm, strongly suggestive of *E. vermicularis* eggs. Also a cross section suggestive of a whole degenerated worm was found. A high number of eosinophils was found in the cell rich periphery surrounding the worm. Blood examination revealed eosinophilia (12.2%) and raised IgE levels. The patient was treated with albendazole 400 mg two times daily for 4 weeks.
Conclusion. In this patient pulmonary enterobiasis was diagnosed, based on the typical microscopic appearance of *E. vermicularis* eggs in lung tissue, combined with a recent history of massive intestinal enterobiasis. Pulmonary enterobiasis should be considered in the differential diagnosis of unexplained pulmonary lesions in patients with a history of intestinal *E. vermicularis* infection.

S04
Endemic Echinoccocosis in the Netherlands?

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Patient is a female, born in 1961, operated in 1975 for lung disease, after supposed contact with sheep (diagnosis unknown at moment of presentation). She presented herself in the summer of 2004 with pain in the back and right upper quadrant of the abdomen. CRP 163; Eosinophiles:1.28 (N: 0.4); liver function normal. Imaging: CT scan abdomen: suspect for *Echinococcus*. Serology: *Echinococcus* ELISA positive 1:80.
Therapy: PAIR (PunctureAspiration Injection Re-aspiration). On microscopy *Echinococcus* was confirmed in the laboratory. Letters from operation in 1975 were still available: the lung disease was diagnosed at that time as *Echinococcus*. Patient had two sessions of PAIR (29-09 and 4-10-2004) because of residue in the cyst. She was treated with albendazol 3 courses of three weeks. The procedure was complicated due to an infection with *S. aureus* for which she was treated with clindamycin orally.
In november 2004 she still complained to be tired and with lack of energy. The CRP had dropped to 12. In December 2004 the CRP had slightly risen and patient was followed closely.
Relevant in the history: She was given a dog for her 11 th birthday. The dog died when she was 17. There were no other contact with dogs nor any travelling in known *Echinococcus* endemic areas.
After PAIR in 2004, cyst fluid was sent to RIVM for further identification. DNA of cyst fluid was isolated and used for molecular identification by PCR and sequencing. The isolate was identified as *E. granulosus* and to our surprise subtyped as genotype 5, that means the *E. granulosus* genotype originating from cattle.Comparing the DNA sequence of this *E. granulosus* G5 with the DNA sequence of the same marker from a previously described patient from the Netherlands (Bowles ea 1992), revealed two identical isolates.
Conclusion: This is the second time that *E. granulosus* genotype 5 has been isolated in a Dutch patient not travelling abroad. This case might indicate that there is still an endemic cattle strain in the Netherlands with a potential for human infections. It is important that clinicians are aware of this possibility.

S05

Genetic diversity of encapsulated and non-encapsulated *Trichinella* by studying the 5S rDNA tandemly repeated intergenic region and isolation of the first *T. pseudospiralis* in the Netherlands

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Trichinellosis is a cosmopolitian zoonotic parasitic disease caused by different *Trichinella* species present in meat of infected animals (pig, wild boar or horse meat). Molecular identification increases the knowledge on the geographic distribution and the epidemiology of the different *Trichinella* species. We analyzed the 5S rDNA of encapsulated and the non-encapsulated *Trichinella* species to be used as an molecular marker to study genetic diversity and identification tools. The amplification of the tandem repeated of the 5S rDNA intergenic region of the encapsulated species of *Trichinella* resulted in a 751 bp fragment, whereas the 3 non-encapsulated species, *T. pseudospiralis*, *T. papua* and *T. zimbabwensis* show a fragment of 800 bp. Although the size of the 800 bp PCR fragments of *T. zimbabwensis* and *T. papua*e is similar to that of *T. pseudospiralis*, there are differences in the 5S rDNA intergenic regions among the 3 non-encapsulated species. The phylogenetic analysis of the 5S rDNA intergenic region shows the three non-encapsulated *Trichinella* species clustering together and well separated from the encapsulated species. In addition, a single PCR based method allows to distinguish non-encapsulated and encapsulated species between them. We used this assay to identify muscle larvae of wild boar. In an ongoing study in wild boar between 2003-2004, only one animal was positive in the digestion method with 1,32 LPG. Molecular identification of the larvae using the 5S tandemly repeated intergenic spacer based PCR showed an 800 bp fragment. DNA sequencing analysis of the PCR product showed that the 3-prime end was homologous with the DNA sequence of the non-encapsulated *T. pseudospiralis*. Hence, this was the first isolation of *T. pseudospiralis* in a wild boar in the Netherlands. The infection load of the animal revealed a potential human infection risk.

V01/02

Introductie HL7-standaard

B.L. Kabbes RI CMC

Managing partner/senior consultant KABBES&PARTNERS Management & ICT Zorg, bestuurslid Stichting HL7 Nederland, lid Technische Commissie, coordinatieteam Projectbureau, lid NEN standaardisatiecommissies, lid Standaardisatie Organen Overleg (SOO), the Netherlands

De Health Level Seven(HL7)-standaard is de afgelopen 15 jaar uitgegroeid tot de wereldwijde standaard voor gegevensuitwisseling in de zorg. De introductie van de HL7-standaard in Nederland in 1993 voorzag in een grote behoefte en HL7 werd binnen 10 jaar de nationale standaard. In 2002 werd door NEN formele erkenning verleend als NEN-norm (NEN 7503). Vrijwel alle ziekenhuizen en ICT-leveranciers gebruiken de HL7-standaard voor de gegevensuitwisseling tussen het ZIS en de diverse afdelingssystemen. De HL7-standaard bevat kant-en-klare, gedetailleerde berichtenspecificaties voor vrijwel alle deelgebieden en domeinen in de zorg: registratief, financieel, medisch, verpleegkundig, onderzoek en behandeling, aanvragen/uitslagen, medisch dossier, DBC's, etc. In de presentatie zal een toelichting worden gegegeven op de algemene kenmerken van HL7-berichtenstructuren, voorbeelden, toepassingswijze, invoering en actuele stand van zaken. Voor Nederland wordt de standaard beheerd door de Stichting HL7 Nederland (met 150 lid-organisaties). Ingegaan zal worden op de rol en structuur van de Stichting HL7 Nederland, de internationale HL7-organisatie en de wijze waarop de HL7-standaard wordt onwikkeld en onderhouden. Ten slotte zal het belang van de HL7-standaard bij de uitrol van nationale ICT-projecten vanuit Nictiz worden toegelicht.

V03/04

Informatie en berichtenstromen toegespitst op de medische microbiologie m.b.v. Hl7-berichten

W. Kalis

Voorzitter van Health&Clinical groep Hl7, the Netherlands

De presentatie zal ingaan op welke informatie- en berichtenstromen een rol spelen bij de communicatie tussen een medisch microbiologisch laboratorium en verschillende andere systemen zoals Ziekenhuis Informatie Systemen (ZIS), Elektronisch Patiënten Dossier (EPD) en Huisarts Informatie Systemen (HIS). Specifiek staan we stil bij het rapporteren van de bacteriologische uitslagen (kweken) m.b.v. van het HL7-protocol, toegespitst op de functionele kant.

Vragen die daarbij een rol spelen:

- Welke Hl7-berichten spelen daarbij een rol?
- Wat zijn de statussen van het resultaat, wanneer mag een resultaat worden ingezien door een ontvanger?
- Welke gegevens versturen we naar de andere partij, b.v. EPD?
- Hoe is het geregeld met de verschillende tabellen en codes die deel uitmaken van het uitslagbericht? Welke standaarden kunnen hiervoor worden gebruikt: de Semantische Standaard en Snomed?
- Hoe moet de ontvanger het bericht interpreteren?

V05/06

IHE: Integrating the Healthcare Enterprise

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IHE (Integrating the Healthcare Enterprise) is een internationaal breed ondersteund initiatief van gebruikers, vertegenwoordigende instituten en leveranciers van ICT-systemen op het gebied van de gezondheidszorg.

IHE is ontstaan uit een initiatief van de RSNA (Radiology Society of North America) en de HIMSS (Healthcare Information and Management Systems Society). IHE is begonnen binnen het domein van de Radiologie en heeft zich inmiddels uitgebreid naar andere domeinen zoals Cardiologie, Apotheek en Laboratorium. Inmiddels zijn er overkoepelende IHE-organisaties in Noord Amerika, Europa, Japan, Italie, Duitsland en Nederland.

Doelstelling IHE. IHE stelt zich ten doel het bevorderen en coördineren van initiatieven om de interoperabiliteit (uitwisselen en delen van medische informatie) te bewerkstelligen tussen medische ICT-systemen van verschillende leveranciers in gezondheidszorginstellingen. IHE bereikt dit doel door middel van het opstellen van zogenaamde Integratie Profielen. Een Integratie Profiel is een gemakkelijk te begrijpen specificatie van bij elkaar behorende functies die het gebruikers en leveranciers makkelijk maakt om te communiceren over wat er benodigd is voor de ondersteuning van de werkprocessen in een domein. Deze specificatie identificeert 'Actoren', zoals opdrachtgever (PLACER) en opdrachtuitvoerder (FILLER). Ook worden transacties tussen deze actoren om specifieke data uit te wisselen ten behoeve van een specifieke klinische taak gedefinieerd en beschreven in een zogenaamd IHE Technical Framework. IHE is geen organisatie die standaarden ontwikkelt, maar IHE maakt in het Technical Framework gebruik van algemeen geaccepteerde standaarden zoals HL7 en DICOM.

IHE organiseert zogenaamde Connect-a-thon's en demonstraties. Een Connect-a-ton is een connectivity marathon waarbij het mogelijk gemaakt wordt voor leveranciers om Integratie Profielen te valideren ten behoeve van de demonstratie van de Integratie Profielen op bijvoorbeeld congressen georganiseerd door de HIMSS of RSNA-organisaties. De resultaten van de Connect-a-thon worden op de website van IHE gepubliceerd. Naar aanleiding van de resultaten van de Connect-a-thon hanteren leveranciers zogenaamde IHE Integration Statements om de conformance te beschijven met het IHE Technical Framework.

Het IHE Laboratorium Technical Framework. Midden 2003 is het initiatief genomen om een IHE international Laboratory Technical Committee op te richten met deelnemers uit onder meer Nederland, Italie, Frankrijk en Japan. Besloten werd om in het eerste Technical Framework het plaatsen van laboratoriumaanvragen (Orders) en het uitvoeren van klinische laboratoriumtesten (Oberservations) op te nemen. Daarnaast is opgenomen het beschikbaar maken van resultaten inclusief validatie status aan zorgverleners.

Het Integratie Profiel: 'Laboratory Scheduled Work Flow' is in 2004 gepubliceerd en de Technical Committee werkt nu aan profielen als Lab Point of Care Testing, Lab Code Set Distribution, Lab Data Automation en Lab Patient Information Reconcillation.

W01/02
Cost-effectiveness of routine real-time PCR for the aetiological diagnosis in adults hospitalised with lower respiratory tract infections

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Introduction. Few hospitals have introduced nucleic amplification techniques for the routine diagnosis and treatment of lower respiratory tract infections (LRTI) in adults. The costs appear to be an important impediment to routine implementation.

Methods. To determine the diagnostic value of real-time PCR techniques in a routine setting, we collected nose-throat samples of immunocompetent patients at different days after admission to our hospital for antibiotic treatment of LRTI. Samples were evaluated by virus culture and by real-time PCRs for adenoviruses, coronavirus OC43, 229E and NL63, influenzavirus A/B, parainfluenzavirus 1-4, rhinoviruses, RS virus A/B, human metapneumovirus and *Chlamydia pneumoniae, Legionella pneumophila* and *Mycoplasma pneumoniae*. In addition, blood and sputum samples were taken for culture and acute and reconvalescent serology samples were evaluated for the respiratory viruses and the atypical pathogens.

Results. 107 consecutive patients (57 men) were included of whom 55 (51%) had pneumonia (chest X-ray showing infiltrate). Respiratory viruses were detected by virus culture in 8 patients (7%) and by real-time PCR in 37 patients (35%). Most frequently influenzavirus A (n=21), rhinovirus (n=6) and coronavirus (n=6) were detected. In 3 cases virus was not detected in the first sample (taken on the day of admission to the hospital) but only in follow-up samples. In 19 of 37 patients (51%) with a virus infection as determined by real-time PCR, respiratory complaints were present for 3 days or less. Added to sputum and blood cultures, real-time PCR increased the number of patients in which a causative microorganism was identified from 32 (30%) to 61 (57%). However, reporting of real-time PCR results resulted in partial or total cessation of antibiotic treatment in only 6 patients (11%). Overall, antibiotic use was comparable in the intervention and the control group.

Conclusion. Viral agents play a substantial role in the aetiology of hospitalised patients with LRTI. real-time PCR significantly increases the number of aetiological diagnoses in immunocompetent adults admitted with LRTIs. So far, however, it did not result in a decrease of antibiotic use or cost.

W03
Similar reduction of cytomegalovirus DNA load by oral valganciclovir and intravenous ganciclovir on pre-emptive therapy after renal pancreas transplantation

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Background. Pre-emptive treatment of CMV infection in transplant recipients aims at prevention of clinical disease by early detection. However, current treatment requires the intravenous (iv) administration of ganciclovir for 2 weeks, which is a considerable burden for the patient. In this observational study, the efficacy of the new oral prodrug valganciclovir was compared with iv ganciclovir.

Methods. To facilitate the introduction of valganciclovir, a therapeutic guideline was developed to use this drug under controlled conditions with regard to safety in renal pancreas transplant recipients requiring CMV therapy. Subsequently, a group of 57 consecutive transplant recipients was evaluated. Onset and treatment of CMV infections were followed by frequent monitoring of CMV DNA in plasma by quantitative real-time PCR. Details of antiviral therapy were documented.

Results. In 15 out of 57 transplant recipients, a total of 27 anti-CMV treatment episodes were recorded: 18 with valganciclovir (900 mg twice daily) and nine with iv ganciclovir (5 mg/kg twice daily) as initial treatment. Median CMV DNA load reduction during treatment was 0.12 log10/day in the valganciclovir group and 0.09 log10/day in the ganciclovir group. There were no haematological side effects in any group and no patient developed signs of clinical CMV disease.
Conclusion. Similar reduction of CMV DNA load was observed during pre-emptive treatment with oral valganciclovir and iv ganciclovir in transplant recipients. Oral valganciclovir would provide an attractive and safe alternative for pre-emptive CMV treatment in renal pancreas transplant patients, however, confirmation in larger randomized studies would be desirable.

W04
Quantitation of 16S ribosomal DNA and RNA as a new approach to monitor the presence and state of viability of bacteria

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Introduction. In the field of molecular diagnostics much effort is put in development of sensitive and reproducible quantitative DNA amplification. These methods however do not indicate whether the micro-organism is stil viable. Therefore, a universal method, based on quantitation of DNA and RNA, was developed to study the presence and state of viability of bacteria.
Methods. For reproducible quantitation a reliable and stable RNA standard was set up based on the MS2 bacteriophage containing 16S RNA. This standard was assessed in real-time PCR. In addition the same target was assessed for DNA quantification in real-time PCR. A quantitative molecular growth curve of DNA and RNA was created. In addition the effects of bacteriostatic and bacteriocidic antibiotics were determined. The standard was further employed to quantify 16S ribosomal DNA and RNA during the growth curve of *E. coli* in broth as well as in platelet concentrates (PCs).
Results. With the RNA standard a sensitivity of 14 copies/PCR was achieved. Detection of DNA persisted during the whole life cycle of bacteria: the amount of DNA increased in the log-phase, remained similar during the stationary phase and declined during the death phase.
A correlation between detection of 16S rRNA and viability of bacteria was also observed: RNA increased with increasing DNA, remained stable during the stationary phase and then diminished rapidly to undetectable levels, which coincided with loss of viability.
When bacteria were exposed to chloramphenicol at the log-phase of growth, the amount of RNA stayed constant for two hours and then disappeared completely. Similar effect was also observed when bacteria were treated with amikacin. However with this bactericidal agent RNA disappeared faster than with chloramphenicol.
Conclusion. It is possible to quantify both RNA as well as DNA in clinical samples with the use of the RNA standards developed. This approach combining quantitative results of DNA en RNA may prove useful in monitoring the effect of antibiotics during treatments of infections with bacterial pathogens.

W05
Representational difference analysis and real-time PCR for strain-specific quantification of porcine commensals closely related to *Lactobacillus amylovorus*

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Introduction. Previous studies have shown that strains closely related to *Lactobacillus amylovorus* are widely distributed as common porcine intestinal commensals. Phenotypic characterization, however, has revealed significant differences with the type strain of *L. amylovorus* (DSMZ 20531^T). Current molecular ecological techniques, including ribosomal RNA-targeted approaches, are only of limited value for the assessment of such microdiversity that can have significant functional impact.
Methods. Here we report on the development of a novel strain detection system based on isolation of specific genomic fragments by representational difference analysis (RDA) and their further detection by real-time PCR.
Results. RDA was firstly adapted to study the microdiversity between highly similar genomes of three porcine isolates related to *L. amylovorus*. Unique nucleic acid fragments for one of the isolates, strain 001^T, were revealed after subtractive hybridization. Furthermore, real-time PCR amplification of the strain-specific genomic fragments using Biorad iCycler equipment was evaluated. Real-time PCR detection of serially diluted genomic DNA was linear for cell counts ranging from 10^6 to 10 cells per PCR assay. The detection specificity was also validated using genomic DNA of the *L. amylovorus*-like isolates and type strains of related *Lactobacillus* spp. Moreover, the *L. amylovorus*-like strain 001^T was successfully quantified in ilea lumen samples when the last strain was administered to weaning piglets challenged with enterotoxigenic *Escherichia coli* K88.
Conclusions. We developed a sensitive and specific approach based on RDA and real-time PCR aiming to detect and quantify a specific bacterial strain in porcine intestinal samples.

W06
Inter-laboratory agreement of three real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples

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Introduction. *Pneumocystis jiroveci* pneumonia (PCP) is an opportunistic infection with a poor prognosis. Diagnosis of PCP by microscopy requires trained personnel and is cumber-

some. Polymerase chain reaction (PCR) to diagnose PCP shows great promise, but lacks inter-laboratory validation and standardisation. The goal of this study was to evaluate the inter-laboratory agreement of three real-time PCR assays for the detection of *P. jiroveci* in bronchoalveolar lavage (BAL) fluid samples.

Methods. Three tertiary care centers (LUMC Leiden, UMCN Nijmegen and azM Maastricht) collected 124 BAL fluid samples tested by microscopy (May-Grünwald Giemsa, methenamine silver stain and/or immunofluorescence). All 124 BAL samples were tested blindly. Each center used commercial DNA extraction methods and an in-house real-time PCR The targets used for the PCR were the major surface glycoprotein (UMCN and azM) or the dihydropteroate synthase gene (LUMC). PCR was performed on Lightcycler (UMCN), iCycler IQ (LUMC) or ABI Taqman (azM). Kappa values for inter-laboratory agreement were calculated.

Results. Of 41 samples positive by microscopy for PCP, 40 were positive in all three PCR methods. The remaining gave a high Ct value (Ct=36.6) in azM and was negative in LUMC and UMCN, indicating a low parasite burden in a patient after seven days of cotrimoxazole treatment. Sixty-nine out of 83 PCP negative samples were negative in all three PCR methods. High Ct-values (greater than 36) were found in a single assay (n=8), in two assays (n=1), and in all three assays (n=5). The latter five samples included one patient in whom PCP was diagnosed one week earlier. Inter-laboratory agreements were as follows: LUMC- UMCN: 0.88, LUMC-azM: 0.93, and UMCN-azM: 0.88.

Conclusions. 1. The present study shows excellent inter-laboratory agreements for three different in-house real-time PCR assays. 2. It confirms the usefulness of real-time PCR assay for the detection of PCP in BAL fluid samples. As a spin-off of this study the implementation of a proficiency testing panel is considered.

X01

The PhoP/PhoQ system controls expression of the intramacrophage type three secretion system of *Salmonella enterica*

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Introduction. *Salmonellae* contain a unique type three secretion system, termed Spi/SsA, which is pivotal for the ability to cause systemic disease and functions exclusively when the bacteria are inside eukaryotic cells. The two component system SsrB/SpiR directly controls the specific intracellular expression of this type three secretion system but the signals that govern it in the intracellular compartment remain largely unknown.

Methods/Results. Using promoter fusions to the Green Fluorescent Protein, we demonstrated that the intracellular transcription of the Spi/Ssa system requires the PhoP/PhoQ two-component system, which detects low Mg^{2+} conditions and is a major virulence determinant. We established that the PhoP protein regulates expression of the response regulator *SsrB* at a transcriptional level and of the sensor SpiR at a post-transcriptional level. We demonstrated binding of the PhoP protein to the *ssrB* promoter both *in vivo*, using chromatin immunoprecipitation in *Salmonella*-infected macrophages, and *in vitro* using purified PhoP protein. In addition, we identified a region in the 5th untranslated region of the *spiR* message that is required for PhoP-mediated regulation.

Conclusion. The PhoP/PhoQ two component system controls the intramacrophage expression of *spi/ssa* genes by regulating the SsrB/SpiR system, suggesting that Mg^{2+} limitation is a crucial signal to denote the intra-phagosomal compartment to the bacteria. Furthermore, these findings indicate that the major role of the PhoP/PhoQ system in the virulence of Salmonella could be partly due to its control of the Spi/Ssa intracellular type three secretion system.

X02

PPE protein Rv2430c is secreted by pathogenic mycobacteria

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One of the major surprises of the analysis of the *Mycobacterium tuberculosis* genome was the observation that almost 10% of its coding capacity (167 genes) was devoted to the production of two protein families, the so-called PE and PPE proteins. These proteins have not been identified yet in any non-mycobacterial species and are only present in low numbers in non-pathogenic Mycobacterium species, such as *Mycobacterium smegmatis*. In this research we focussed on the Rv2430c/Rv2431c operon of *M. tuberculosis*, which encodes a PPE gene and a PE gene, respectively. The Rv2430c gene-product, also known as PPE41, is known to be an immunodominant antigen of *M. tuberculosis*[1]. This operon was expressed in three different species, *i.e. M. smegmatis*, *M. marinum* and *M. bovis*. Both the pathogenic bacteria efficiently secreted PPE41, whereas the protein was associated with cell pellets of *M. smegmatis*. PPE41 secretion was dependent on the presence of the small PE protein encoded by Rv2431c. A specific mycobacterial secretion system is encoded by the extended RD1 region, which transports small proteins devoid of classic signal sequences. Although PPE41 also lacks a signal sequence, secretion of this protein was not affected by an RD1 mutation in *M. marinum*. Pathogenic mycobacteria apparently have a second dedicated secretion system. Secretion of PPE41 in human macrophages was analyzed by differential cell disruption and immunofluorescence microscopy.

References

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X03

Development of genomic array footprinting to identify conditionally essential genes in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a major cause of infections such as pneumonia and meningitis in both children and adults worldwide. Given the limitations of the current capsule polysaccharide vaccine as well as the increasing antibiotic resistance among circulating strains, identification of novel antimicrobial target genes remains of utmost importance. The availability of genomic sequences of several *S. pneumoniae* strains has triggered the use of genome-based techniques such as transcriptional profiling and proteomics to identify novel potential candidates for vaccine and/or drug development. However, as yet no high-throughput genome-wide technique to efficiently screen for conditionally essential genes in *S. pneumoniae* is available. Therefore, we are currently developing genomic array footprinting (GAF), a method combining genome-scale mutagenesis and microarray technology to identify genes important for survival of the bacterium during infection. We have established both *in vitro* and *in vivo* transposon mutagenesis protocols to create mutant banks. To provide suitable probes for microarray analysis, chromosomal DNA from the mutant pool was extracted and used as template for the generation of mutant-specific probes. To this end, several procedures were tested for sensitivity and specificity of amplification of transposon-insertion sites. Finally, amplified products were labelled and hybridized to an amplicon-based microarray. Genes essential for *S. pneumoniae* under certain conditions will be characterized by differential hybridizations patterns obtained before and after challenge of the mutant pool. In addition to being a fast and feasible way to identify conditionally essential genes of *S. pneumoniae*, we expect GAF to be easily adaptable for use with other bacterial species as well.

X04

Different roles of the *Neisseria meningitidis* outer membrane export proteins in susceptibility to antimicrobial agents

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Introduction. Multicomponent multidrug efflux systems in Gram negative bacteria consist of an inner membrane located energy dependent translocase, a periplasmic membrane export protein and an outer membrane export protein. The outer membrane export protein, can associate with different translocases and periplasmic membrane export proteins. Previous work and complete genome analysis showed that in *Neisseria gonorrhoeae* only one outer membrane export protein, the well-characterized MtrE, is present. In the genome sequences of *Neisseria meningitidis* MC58, we found two genes, NMB1714 and NMB1737, encoding outer membrane export proteins based on conserved domains. Homologs of these genes were also encountered in the other available genome sequence of *N. meningitidis* Z2491.
Aim. To assess the activity and specificity of both outer membrane export proteins of *N. meningitidis*.
Methods. Knockout mutants of *N. meningitidis* H4476 were constructed by insertional mutagenesis, in which either NMB1714 or NMB1737 was inactivated. In addition, a double knockout mutant was made, in which both genes were inactivated. Susceptibility to various antimicrobial agents was evaluated by E-test and disc diffusion.
Results. The susceptibility to a wide range of antimicrobial agents is increased in the NMB1714 knockout strain, and even more increased in the NMB1714/NMB1737 double knockout. Surprisingly, the NMB1737 knockout strain has a decreased susceptibility to part of these antimicrobial agents.
Conclusion. It is concluded, that the meningococcal MtrE homolog NMB1714 is part of the main multidrug efflux systems, whereas the role of NMB1737 in multidrug efflux is very limited. In addition, the results suggest that there is competition between the two outer membrane efflux proteins for the periplasmic and inner membrane proteins.

X05

Comparative analysis of the BvgAS transcriptional regulon in *B. pertussis* and *B. bronchiseptica*

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The *Bordetella* Bvg regulon provides an excellent tool for comparative expression analysis to identify determinants of pathogenicity. To this end, we constructed a *Bordetella* microarray representing all *B. pertussis* putative ORFs as well as *B. bronchiseptica* sequences that do not have *B. pertussis* homologues. To assess both ends of the regulatory spectrum (Bvg^+ and Bvg^-), we compared expression profiles of *B. pertussis* and *B. bronchiseptica* strains genetically 'locked' in the respective phenotypic phase. In addition, we examined environmentally modulated cultures. Bvg-activated products of both species fall into a wide but similar range of functional categories, relatively enriched for cell surface and transport protein transcripts. About a third of these genes were expressed by both species, such as adhesins, toxins and type III secretion genes. The major differences were that only *B. pertussis* expressed pertussis toxin, whereas *B. bronchiseptica* expressed multiple fimbrial genes, transcriptional regulators, and uncharacterized ORFs. We propose that these loci provide, at least in part, a genetic basis for particular host adaptations. In contrast, most Bvg-repressed genes, many of which encode motility, chemotaxis, and transporter functions, were differentially expressed only in *B. bronchiseptica*. *B. pertussis* expressed capsule biosynthesis genes in the Bvg^- phase, and shared Bvg^- genes included the *wlb* locus. Thirty-eight genes identified by microarray analysis were confirmed to have altered transcript levels by real-time PCR (R^2=0.92). Further, our microarray data clearly showed that, rather than

functioning as an on-off switch, Bvg controls a spectrum of gene expression states that exist along a regulatory continuum. Multiple loci were maximally transcribed between the Bvg^+ and Bvg^- poles, under semi-modulating conditions. In addition to *bipA*, the first identified Bvg-intermediate phase gene, examples include loci involved in energy metabolism and phenylacetic acid degradation. We postulate that the complexity of gene expression states governed by BvgAS reflect the need for temporally and/or spatially defined programs of gene expression during the infectious cycle.

X06

The role of the bacterial CpG sensing Toll-like receptor 9 in *Chlamydia trachomatis* female genital tract infection: the knockout mouse and human candidate gene approaches

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Background. Toll-like receptors (TLRs) is required for the recognition of bacterial CpG motifs and could potentially play an important role in the susceptibility to and severity of Chlamydia trachomatis (CT) infection.

Aim. To assess the role of the TLR9 in CT female genital tract infection using: 1) a knockout murine model (primary and secondary infection), 2) Candidate gene approaches in a cohort of subfertile women.

Methods. C57BL/6 TLR9-/- and C57Bl/6 mice were infected and reinfected with CT (serovar D) and a number of different infection parameters were evaluated. The frequencies of TLR9 -1237T > C and TLR9 +2848 G > A single nucleotide polymorphisms (SNP) were determined in Dutch Caucasian subfertile women with (n=48) and without (n=236) tubal pathology and healthy controls (n=147). In addition, SNP analyses were performed in subfertile women with serological responses to C. trachomatis, with and without tubal pathology. Finally, haplotype analyses based on these two TLR9 SNPs were performed.

Results. Murine Model: All initial infection parameters were identical between controls and TLR9 deficient mice. However, we observed that in TLR9 deficient mice the median duration of infection was significantly shorter compared to reinfected control mice (4.5 vs 12.5 days, p=0.02).

Human SNP Analysis: Differences in TLR9 genotypes were borderline significant (p=0.05) between women with or without tubal pathology as well as for seropositive women and the risk of having developed tubal pathology.

Human Haplotype Analysis: A clear difference existed when comparing the risk of having developed tubal pathology for women with serological responses to CT: haplotype I was found more frequent in women with tubal pathology (51%) as compared to those without (29%) and for haplotype III the reverse was found: 7% vs 24% (haplotype distribution comparison: p=0.011).

Conclusion. Both approaches showed a potential relevant role for TLR9 in mediating CT infection especially in relation to tubal pathology. Whether this role is due to linkage should be assessed by further research.

Y01

PLAY, an abundant ascospore cell wall protein in *Talaromyces macrosporus*

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Ascospores formed by the fungus *Talaromyces macrosporus* belong to the most resilient eukaryotic structures observed to date. In addition, they show constitutive dormancy, that means that they do not germinate as a result of the presence of proper nutrients alone, but need another trigger to alleviate the inhibition of germination. The ascospores can survive prolonged heat treatments at 85 °C and also survive high pressure treatments (800 MPa) which are used as a novel non-thermal preservation method for food products. Short treatments of heat or high pressure even do activate these spores to germinate. Upon activation these cell germinate, which is characterised by trehalose breakdown, glucose release and a sudden ejection of the inner cell through the outer, very thick, ascospore cell wall. The latter process is dubbed prosilition. Activation of ascospores by heat was accompanied by changes in the ascospore cell wall structure as judged by an increase of the permeability for fluorescent probes, X-ray diffraction patterns and electron paramagnetic resonance studies on cell wall fractions. Activation in buffer was very low at 65 °C, but strongly increased at 70 °C or higher. These temperatures were also associated with the release of large amounts of a small protein from the cell wall. This protein is the most abundant protein of ascospores, responsible for at least 5% of the total protein. The encoding gene was cloned after determining the N-terminal sequence of the protein. The genome of *T. macrosporus* contains one copy of this gene and its expression is strictly related to the formation of fruiting bodies (that contain ascospores). Further research is done to evaluate its role in dormancy and heat-resistance.

Y02

Stress-response regulation in *Cryptococcus neoformans*

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Cryptococcus neoformans (*Cn*) is the causative agent of cryptococcal meningoencephalitis. There is accumulating evidence that *Cn* is a facultative intracellular pathogen, residing in macrophages and endothelium. The molecular mechanism conferring resistance to phagolysosomal killing in these cells is a key unresolved issue. To gain insight into the fungal

adaptive strategies, serial analysis of gene expression was used to map genes differentially expressed in an intraphagocytic environment. Comparing gene-profiles of *Cn* serotype D B3501 cells recovered from human umbilical vein endothelial cells (HUVEC) to those from free-grown *Cn* revealed the up-regulation of the cryptococcal homologue of the *SKN7* two-component stress response regulator gene from *Saccharomyces cerevisiae*. Studies with *Cn* cells disrupted for *SKN7* revealed an increased susceptibility to t-butyl hydroperoxide (100% lethality at 0.7 mM, versus 1.2 mM for wildtype) and significantly lower survival rates in HUVEC infection experiments. Mice experiments reveal that *SKN7* gene disruption strongly attenuates cryptococcal virulence in vivo.

Y03
Fungi deploy specialized hyphae for waste processing

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Mycelial fungi play a central role in element cycling by degrading dead organic material such as wood. Fungal colonization of a substrate starts with the invasion of exploring hyphae. These hyphae secrete enzymes that convert the organic material into small molecules that can be taken up by the fungus to serve as nutrients. Here, we show that exploring hyphae of *Aspergillus niger* differentiate with respect to enzyme secretion; some strongly express the glucoamylase gene, while others hardly express it at all. Waste processing by fungal hyphae, therefore, seems to be a job for specialists.

Y04
Isolation of septal pore caps from basidiomycetous fungi

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The septal pore cap (SPC) structure covers the dolipore, a septal pore surrounded by a donut-like swelling, in many basidiomycetous fungi. The SPC is a membranous structure associated with endoplasmic reticulum. The morphology of the SPC is diverse within the different phylogenetic groups of basidiomycetes and can be divided in several main categories: vesiculate or tubulate, imperforate, and perforate. Though electron microscopical (EM) studies revealed the SPC in great detail, the function of the SPC is only poorly understood. Our aim was to isolate and enrich SPCs to characterize its proteins and genes that are involved in the formation of the SPC. This will lead to a better understanding of the role of SPCs in basidiomycetous cells.
We successfully enriched SPCs from *Rhizoctonia solani* cell fractions. After EM studies we observed that the plug material at the orifice of the septal pore channel stayed attached via fibrillar material to SPCs. Protein electrophoresis showed that a 18 kDa glycoprotein was enriched in the SPC fraction. This protein was N-terminally sequenced. We raised antibodies against this protein to perform immunolabeling studies. From our observations we think that the SPC may be involved in the production of plugging material. Alternatively, the SPC may function as a repository of the plugging material that can be released upon plugging the septal pore during i.e. stress situations. Based on our results, genetic studies will be performed in other basidiomycetous fungi, like *Schizophyllum commune* and *Coprinus cinereus* to strengthen our hypothesis.

Y05/06
Analysis of shared proteins: a promising method to resolve the eukaryotic Tree of Life

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Our understanding of the Tree of Life (TOL) is still fragmentary. Until recently, molecular phylogeneticists built trees based on ribosomal RNAs and selected protein sequences which, however, usually suffered from lack of support for the deeper branches. Now, phylogenetic hypotheses can be based on the analysis of full genomes. Here we present results from a phylogenetic analysis of concatenated sequences of orthologous genes present in the genomes of 21 fungal, 3 animal and one plant species. A total of 531 proteins occurring in all the genomes studied, were analyzed using four different phylogenetic methods. Our results agree well with current hypotheses on the phylogeny of higher fungi. However, the single tree that we inferred from our dataset shows for the first time excellent nodal support for each branch, which suggests that it reflects the true phylogenetic relationships of the species involved. Our results demonstrate that eukaryotic phylogeny may strongly benefit from the use of full genome comparisons, as we demonstrate here for the fungal kingdom.

P01
Community-acquired pneumonia caused by *Legionella longbeachae* in an immunocompetent patient

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Introduction. The genus Legionella includes over 40 different species of fastidious Gram-negative bacilli, with over 60 described serogroups. These organisms are ubiquitous in environmental and man-made watersystems, and many

have been shown to cause human disease, most commonly pneumonia. The vast majority of such cases are due to *L. pneumophila*. A minority is due to other species, most commonly *L. micdadei, L. bozemanii, L. dumoffii,* and *L. longbeachae.* Described here is a case of pneumonia due to *L. longbeachae* in the Netherlands in an immunocompetent patient.

Case report. In July 2003, a 67-year-old previously healthy man was admitted to the emergency department of our hospital (Diaconessenhuis, Meppel) with a history of progressive malaise, cough and fever. He was transferred to the intensive care unit where he underwent intubation for hypoxia and respiratory distress. A complete blood count revealed a white blood cell count of 14.900 cells/µl and a C-reactive protein value of 387 mg/l. Serological tests for *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Chlamydia psittaci, L. pneumophila* and PCR on *M. pneumoniae* and *C. pneumoniae* were negative. Bacterial cultures (blood, sputum) remained negative. An immunochromatographic membrane test (Binax now; Binax, USA) to detect *L. pneumophila* serogroup 1 soluble antigens in urine had been performed, but the result was negative. Because Gram stain showed numerous leukocytes without bacteria, the possibility of Legionellosis was considered. *L. longbeachae* was identified by 16 S rRNA based PCR assay and sequence based typing. An acute serum sample showed a single elevated IgM titer of 1: 512 and IgG titer of 1:64 against *Legionella non-pneumophila* spp., indicating the presence of acute disease. The patient fully recovered after initiating appropiate antibiotic treatment.

Conclusion. since most laboratory tests for *Legionella* cannot detect infections caused by *non-pneumophila Legionella* spp, culture on legionella-selective media or PCR should be considered when diagnosing pneumonia with an unknown etiology.

P02

Recurrent urinary tract infection caused by *Actinobaculum schaalii*: a case report

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We report here a case of recurrent urinary tract infection caused by *Actinobaculum schaalii*, a rarely found *Actinomyces*-like microorganism. *Actinobaculum* is a catalase-negative, Gram-positive rod-shaped bacterium without branching. The genus includes three species; *A. suis, A. schaalii* and *A. urinale*. A fourth species, *A. 'massilia'*, is not validly published. Until now only three cases of human urinary tract infections with *Actinobaculum spp.* are reported.

Our patient was a 82-year-old male who had been treated for a prostatic carcinoma with internal radiation treatment. In march 2004 he presented with symptoms of cystitis. Microscopic analysis of the urine revealed 11-20 leukocytes/high power field and no epithelium. On Gram-stain the urine contained Gram-positive rods. After 24 hours of incubation in air plus CO_2 over 10^5 tiny colonies were detected on Columbia Colistin Nalidixic Acid agar (CNA). After 48 hours the colonies became slightly alpha-haemolytic. Identification of the strain by the API-Coryne system (bioMerieux) resulted in an unacceptable profile. The strain was sensitive to all tested antibiotics and the patient was successfully treated with amoxycillin-clavulanate without further determination of the isolated bacterium. However, one month later symptoms of cystitis reappeared and a second urine culture was performed. Now the urine revealed 20-40 leukocytes/high power field and the same Gram-positive rods appeared. Identification of the strain with API-Coryne resulted in the same unacceptable profile as formerly. Biochemical patterns showed that our isolate was related to *A. schaalii* and *A. 'massilia'*. This time Polymerase Chain Reaction (PCR) with universal 16S rRNA primers was performed on lysate of the cultured colonies. The PCR product was sequenced bi-directional. Database comparison revealed that our strain, *A. 'massiliae'* and *A. schaalii* are different genomospecies within the nomen-species *A. schaalii*. The patient was treated with trimethoprim-sulfamethoxazole and responded well to this therapy.

In conclusion, we described a case of recurrent urinary tract infection in a 82-year-old male caused by *Actinobaculum schaalii*.

P03

A case of Varicella Zoster Virus (VZV) related progressive outer retinal necrosis (PORN) after allogenic Stem-Cell transplantation

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Commonly reported complications of disseminated VZV infections in severely immunocompromised patients (e.g. stem cell transplant recipients) include VZV pneumonia, encephalitis and hepatitis. Recently, VZV has been associated with another unusual presentation referred to as progressive outer retinal necrosis (PORN) syndrome. The PORN syndrome is described as a distinct form of VZV necrotizing chorioretinitis found almost exclusively in patients with the acquired immunodeficiency syndrome (AIDS). Only, a few cases in non-AIDS patients have been reported. Here we present the first laboratory confirmed case of VZV associated PORN in a SCT recipient despite adequate treatment for occult disseminated zoster like lesions. Five weeks after a haplo-identical allogeneic stem cell transplantation the patient presented with typical zoster skin lesion with dermatomal involvement. Despite appropriate treatment with acyclovir, zoster-like lesions re-occurred twice and eventually the patient reported visual loss of the left eye and subsequently the right eye. Upon ophthalmic examination multifocal, coalescing retinal lesions were observed. VZV DNA was detectable in plasma at presentation of zoster skin lesions and declined to undetectable levels after each treatment episode. VZV DNA was also detected in anterior chamber and vitreous fluids of both eyes during ophthalmic involvement (HSV, CMV and EBV DNA were undetectable). The nature of the retinal lesions, their rapid spread, the lack of response to treatment, the lack of more prominent inflammatory reaction in vitreous and anterior chambers as well as the involvement of VZV but not other herpes viruses are typical of PORN. Despite antiviral treatment combined with vitrectomy

and profylaxis treatment for retinal detachment, visual acuity of both eyes dropped progressively. Aggressive antiviral treatment, with vitrectomy and profylaxis for retinal detachment can result in useful vision for the duration of the patients? life. However, no single treatment regimen has been reported to successfully treat all cases of PORN. Additional investigation will be required to determine the best treatment for patients with this disorder.

P04
Microbial diversity of artificial phototrophic biofilms grown under different light conditions

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Phototrophic biofilms may be defined as interfacial microbial communities mainly driven by light as energy source. The development of the microbial community of freshwater phototrophic biofilms was investigated during experiments in a specially developed flow-lane-incubator with precise control of light, temperature, velocity conditions and with nutrient-adapted artificial medium. Biofilm growth was monitored by measuring the decrease of subsurface light below the polycarbonate substratum.
DNA was extracted from biofilm 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 different stages of biofilm development. Extraction and sequencing of the specific DGGE bands revealed the identity of the dominant species at different stages of biofilm development. Although the biofilm was always dominated by oxogenic phototrophs, it was obtained that the species composition changes drastically as the biofilm matures under different light intensities, temperatures and velocity conditions. These ecological features and the subsequent functional relationships may be key parameters for exploitation, control and modelling of aquatic phototrophic biofilms.

P05
Two patients with *clostridium cadaveris*-bacteremia

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Introduction. *Clostridium cadaveris* is a strict anaerobic Gram-positive rod and is the most prominent bacterium during the decay of dead bodies. Human infections with *C. cadaveris* are very rare.
Methods. We describe two immunocompetent patients with *C. cadaveris*-bacteraemia that were encountered during the period 2003-2004. Identification of blood-isolates was performed using standard laboratory methods in combination with 16S-PCR. An extensive review of the literature was performed to identify risk-factors for *C. cadaveris*-bacteremia.
Results. The first case was a 75-year old male who was admitted with fever and abdominal pain. Initially, empiric antibiotic treatment was started, but a laparotomy performed 6 days after admission showed a perforated diverticulitis. Subsequently, a sigmoidectomy was performed. Blood-cultures obtained during the period before surgery showed *C. cadaveris*. The patient recovered without problems.
The second case was a 68-year-old male who was admitted with high fever, diarrhea and abdominal pain. Blood cultures grew *C. cadaveris*. A gastro-intestinal origin was suspected but extensive examinations showed no apparent abnormalities. Patient's condition improved and antibiotics were stopped after 4 weeks of therapy. Two weeks later, the patient became septic and blood cultures again grew *C. cadaveris*. The patient improved with antibiotics but after 8 days suddenly collapsed and died. Post-mortem examination demonstrated signs of chronic cardiac disease, with an old, large thrombus in the left atrium. No abnormalities were seen at any other site. Review of all published case-histories, including ours, showed a total of 7 cases of *C. cadaveris*. Infection is generally associated with poor general condition and underlying malignancy or severe immune suppression. In these situations a gastro-intestinal source of *C. cadaveris* is common.
Conclusions. *C. cadaveris* mostly leads to infections in immunocompromised patients and/or patients with bad overall condition. The source of the infection is usually the abdomen. In immunocompetent patients, gastro-intestinal events may cause bacteremia with *C. cadaveris*.

P06
Novel approach for *Acinetobacter* outbreak management and total Intensive Care disinfection: hydrogen peroxide vapour

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Introduction. During a 1-month period, several multi-resistant *Acinetobacter* strains were isolated from 4 intensive care unit (ICU) patients. Infection was documented in 1, others were colonised. Using Pulsed Field Gel Electrophoresis all strains were identical. Despite rigorous ICU cleaning and cohorting the strain continued to spread. Suspecting an environmental source we closed the ICU for new admissions and transferred patients to other wards. After discharge Sterinis® (Gloster Sante Europe Labège Cedex - France) hydrogen peroxide vapour technique was used to disinfect the area. We investigated the effect of this disinfection strategy.
Methods. Four evaporators releasing H2O2 gas were positioned in the ICU. Two to three gas release cycles were used to reduce microbial load. The ICU was sealed for 24 hours. Microbial validation was performed using Rodac plates; before and after the process 60 samples were taken from environmental surfaces and checked for microbial growth. 51 McConkey agar plates inoculated with the outbreak strain were placed, as well as 6 spore strips.
Results. One *Acinetobacter* strain was found on the telephone in the nurses post during the pre-check. Rodac plates taken after the procedure showed no *Acinetobacter* growth.

Furthermore, the growth of non-pathogenic bacteria as seen on the pre-check plates was almost reduced to zero on the post-check plates. The evaporation process was able to prevent growth in most places on the Mc Conkey agar plates inoculated with *Acinetobacter* except for some niches. After this new disinfection procedure no new *Acinetobacter* colonisation or infection was seen in our ICU. All (disposable) materials present in the ICU were used as usual after the procedure.

Conclusion. After H2O2 gas disinfection all disposable materials could be used as usual causing economic benefits over other strategies. Taking into account the shortcomings of this new procedure, such as difficult to reach areas for gas evaporation, this disinfection strategy might become a powerful tool in the environmental elimination of outbreak strains in the ICU setting.

P07
Trichomonas vaginalis detection by real-time PCR

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Introduction. A new real-time PCR assay for the detection of *Trichomonas vaginalis* (TV) in genital swabs was compared with traditional TV culture. Using PCR the prevalence of TV in women and men was determined. Finally, the possible use of urine samples was briefly investigated.

Methods. Swabs from 1977 women and 92 men suspected of having a TV infection were tested by PCR and culture. The swabs for PCR were collected in 2-SP transport medium after which the nucleic acids were released using the (Ct/Ng) Roche Specimen Preparation kit. Primers and probe for TaqMan PCR were selected from the TVK3/TVK7 repeat gene.

Results. 27 samples were PCR and culture positive, 11 samples were positive by TV PCR only, and no samples were positive by culture only. The 11 discordant PCR positive/culture negative samples were retested using an alternative real-time PCR assay directed at the TV (tubuline gene. The confirmatory PCR was positive in 9/11 samples. The sensitivity and specificity of the new TV PCR was 100% and 99.9% respectively, as compared to 71% and 100% for culture. From 7 of the TV PCR positive patients a urine sample was tested before initiation of antibiotic treatment. All 7 urine samples were TV PCR positive. From 9 female patients new swabs were taken 2 weeks after initiation of therapy. 7/9 of these swabs were TV PCR negative. The other 2 were from patients from which there was doubt about the compliance of the medication.

The prevalence of TV (by PCR) in the study group was 1.9% in women and 1.1% in men. In swabs from 1321 additional patients, which were not cultured for TV (but sent to the laboratory for Ct and/or Ng PCR), the prevalence of TV (by PCR) was 1.0% in women and 0.2% in men.

Conclusions. Real-time PCR is the method of choice for the diagnosis of *Trichomonas vaginalis* infections in women with vaginal discharge and men with non-gonococcal urethritis. In addition, TV PCR should also be considered in women with a primary suspicion of Ct or Ng. After antibiotic treatment TV DNA becomes undetectable within a few weeks. Preliminary results show that testing urine samples may be equally effective as testing swabs.

P08
Comparison of N-actetyl-L-cysteine-NaOH and Sulphuric Acid decontamination methods for recovery of mycobacteria from clinical specimens

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Introduction. Culture is considered the gold standard for the detection of *Mycobacterium tuberculosis*, but most clinical sputum samples contain a variety of micro-organisms that may overgrow *M. tuberculosis*. Decontamination of these samples is therefore crucial in preventing contamination of the mycobacterial culture. However, also the recovery of mycobacteria is negatively influenced by decontamination. We compared the NaOH-N-actetyl cysteine (NaOH-NALC) and the sulphuric acid decontamination procedure in the detection of mycobacteria using the Mycobacteria Growth Indicator Tube (MGIT).

Methods. From 142 Zambian patients 219 sputum specimens were collected and subjected to mycobacterial culture. These specimens were divided in two samples (in total 438 samples). One half of the specimen was decontaminated with NaOH-NALC and the other half with sulphuric acid.

Results. From the 438 samples a total of 261 (60%) cultures yielded growth of mycobacteria, representative of 22 different species. *M. tuberculosis* was most commonly recovered (n=62), followed by *M. lentiflavum* (n=33) and *M. intracellulare* (n=31). The sulphuric acid method was more successful than the NaOH-NALC method in recovering mycobacteria in MGITs (146 versus 115 respectively, $p < 0.001$). Of the 146 positive mycobacterial cultures recovered after sulphuric acid decontamination 28 were *M. tuberculosis*, 84 nontuberculous mycobacteria (NTM) and 34 acid fast bacterial isolates could not be identified to the species level. The 115 mycobacteria recovered by NaOH-NALC method consisted of 34 *M. tuberculosis* strains, 55 NTM and 26 acid fast bacteria that could not be identified. Comparing the two decontamination methods the recovery of NTM in the sulphuric acid group was significant higher than in the NaOH-NALC group ($p < 0.001$). In contrast, no significant difference was found for the recovery of *M. tuberculosis*.

Conclusion. 1. Significantly more NTM were recovered by the sulphuric acid decontamination method than by the NaOH-NALC method (84 versus 55 respectively).

2. There was no significant difference between the sulphuric acid decontamination method and the NaOH-NALC method for the isolation of *M. tuberculosis*.

P09

Identification and susceptibility testing of staphylococci by direct inoculation from positive BACTECblood culture bottles

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Introduction. Shortening the turnaround time of microbiological analyses for rapid identification and susceptibility testing of bacteria can lead to a significant reduction of patient morbidity, mortality and cost, in particular for patients with septicemia. This study explores the possibility of combining direct inoculation of tube coagulase, DNase and VITEK 2 from BACTEC blood culture bottles to achieve rapid determination and susceptibility testing of staphylococci.
Methods. Direct inoculation of bacterial suspension to the VITEK 2 P523 susceptibility card, tube coagulase test and DNase was made after differential centrifugation of blood cultures of organisms with staphylococcal morphology on Gram stain. *Staphylococcus* spp. isolated from solid-medium blood cultures were identified by standard laboratory methods which were the standard against which the sensitivity and specificity of the rapid tests were compared.
Results. Between April and June 2004, a total of 70 strains were investigated; 37 *Staphylococcus aureus* and 33 coagulase-negative staphylococci (CNS). All strains were correctly identified as *Staphyloccus aureus* or CNS, by comparing the results with those using a pure overnight culture. According to the NCCLS breakpoints, antimicrobial susceptibility testing with the VITEK 2 system gave an overall 99,6% correct category agreement (range 94,6% - 100%), 0,1% very major errors and 0,3% minor errors among *Staphylococcus aureus* isolates. Antimicrobial susceptibility testing gave an overall 97,4% correct category agreement (range 90,3%-100%), 0,9% very major errors and 1,7% minor errors among CNS isolates.
Conclusion. results obtained from direct inoculation of blood culture bottles containing stafylococci for determination and susceptibility testing seem safe enough for immediate reporting.

P10

An improved phenotypic test for detection of Enterobacteriaceae producing extendend-spectrum and AmpC beta-lactamases

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Introduction. For clinical laboratories the detection of extended spectrum beta-lactamase (ESBL) production is important. Phenotypic test are often difficult to interpret. Furthermore, ESBL production can be masked by high-level expression of AmpC beta-lactamases. In this study we evaluated a modified double-disk test (MDDT) for detection of ESBLs in Enterobacteriaceae including AmpC-producing species. The MDDT was compared with the combined double-disk test (CDT) and E-test ESBL strips. Genotypic characterization of ESBL genes was taken as golden-standard for ESBL detection.
Methods. PCR and sequence analysis of the ESBL genes, TEM, SHV and CTX-M was performed on 67 strains (8 species) that were resistant for one or more extended-spectrum cephalosporines, obtained at the department of medical microbiology (AMC). MDDT was performed by placing disks of ceftazidime, cefotaxime, cefpodoxime and cefepime at distances of 30 and 20 mm (center to center) from a disk containing amoxicillin plus clavulanic acid (amox+clav). For detection and induction of AmpC, a cefoxitin disk was used. The CDT consisted of a disk containing ceftazidime plus clavulanate was included in the MDDT. Three E-test strips containing ceftazidime or cefotaxime or cefepime, with and without clavulanic acid were used.
Results. PCR and sequence analysis detected 56 isolates as ESBL positive, 35 SHV-12, 3 SHV-2, 2 SHV-5, 9 CTX-M-15, 4 CTX-M-16, 1 CTX-M-3 and 2 TEM-52. The CDT identified 40 isolates as ESBL positive. The three E-tests together, detected 38 isolates as ESBL positive.
ESBL detection by MDDT of ceftazidime, cefotaxime, cefpodoxime and cefepime versus (amox+clav) were 44, 42, 42 and 44 isolates, respectively. In total, the MDDT detected 52 isolates as ESBL positive.
Conclusion. The MDDT for ESBLs detection is more sensitive than the double-disc synergy test and the E-tests which are widely applied in the routine microbiological diagnostics.

P11

Serological diagnosis of rickettsial diseases, evaluation of two approaches

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International travel has an increasing impact on health care, as travelers can be exposed to a wide range of exotic pathogens including rickettsial diseases: scrub typhus *Orientia tsutsugamushi*, spotted fever group for instance *Rickettsia conorri* and the typhus group *Rickettsia prowazekii* and *Rickettia typhi*. Rickettsiae are transmitted via arthropods including ticks, mites, fleas and lice. Specific optimal conditions determine the geographic distribution of the vector and the risk areas for rickettsioses. Although rickettsiae can be isolated from or detected in clinical specimens, serological tests still remain an indispensable tool in the diagnosis of rickettsioses despite the fact that specificity is suboptimal due to extensive antigenic cross-reactions within the family *Rickettsiaceae*. The indirect immunofluorescence assay is considered the test of choice. In this study we compared the results of the commercially available serological assays used at the RIVM with those of the in-house IFA used in the reference laboratory in Marseille. For this, we selected 148 serum samples from our biobank of patients with positive IFA results and for which background information was available. All sera were re-tested in the reference lab in Marseille. Sera were scored positive or negative using the

currently used criteria in each laboratory. Test comparison showed poor correlation, with similar results for 36% of the sera. Preliminary results of this comparative evaluation suggested that the commercial assay yielded significantly more positive results (87 versus 22 respectively), for which especially the *O. tsutsugamushi* titers from the commercial assay could not be confirmed by the laboratory in Marseille (2 out of 23). The discrepant results could in part be explained by the more stringent application of cut-off criteria, where initial Ig screening is done in a starting dilution of 1: 100 by the reference laboratory in Marseille. Both the IFAs showed cross-reaction within the *Rickettsiaceae* family 65 and 73% respectively. The complete analysis of results will be presented.

P12
Rapid detection of *Campylobacter jejuni* DNA in feces by real-time PCR

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Introduction. *Campylobacter jejuni* is the major cause of bacterial gastro-enteritis in the Netherlands. Conventional diagnosis is based on detection of *C. jejuni* in feces and consists of selective culture and identification, which can take several days. The culture approach is biased by a viable but non-culturable state (VNC) *C. jejuni* can acquire under physiological stress and starvation conditions, as well as postal delay, which compromises sensitivity more for detecting *C. jejuni* when compared to other bacterial enteric pathogens. Cost-effective PCR based screening of feces is feasible (ICAAC 2004 poster O-1626) and will decrease the turn around time significantly. However in this approach a sensitive PCR is mandatory. This study describes the development of a real-time PCR method for the direct detection of *C. jejuni* in feces.
Methods. The real-time PCR assay for *C. jejuni* targets the *mapA* gene (considered specific for *C. jejuni*) and is combined with a very efficient 'in-house' DNA extraction from feces. The sensitivity was assessed with spiked fecal specimens and the assay was validated with *C. jejuni* culture positive (n = 191) and culture negative (n=224) fecal specimens.
Results. The analytical sensitivity was determined at 100 CFU/g feces. In clinical fecal specimens the assay performed with a sensitivity and specificity of 96%. No cross-reaction with other enteric pathogens was observed, although 3 fecal samples designated as C. coli positive by culture were also detected by *C. jejuni* PCR. This may be attributed to hippurate-negative *C. jejuni* or double-infections with C. coli and VNC *C. jejuni*. PCR also detected *C. jejuni* in 2.5% of the samples negative for enteric pathogens by conventional methods, which may result in improved detection of *C. jejuni* associated gastro-enteritis by PCR. PCR inhibition was not observed in any of the 415 samples tested.
Conclusion. Sensitive and cost effective molecular screening of fecal specimens for *C. jejuni* is feasible and has great potential as a means of rapid detection of bacterial gastro-enteritis. Extended validation is required to establish if the detection rate of *C. jejuni* associated gastro-enteritis will be improved by PCR.

P13
Sequence-based typing of microbial pathogens

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Introduction. Typing of microbes is essential to detect outbreaks and to set up surveillance programs. To date, most typing methods are based on banding patterns. However, such typing methods are only partially successful since reproducibility, speed, nomenclature, and interchange of results between different laboratories and countries can be difficult. Sequence-based typing methods circumvent these problems.
Methods. Two sequence-based typing methods were studied: the *Staphylococcus* Protein A (*spa*) typing method and the *Streptococcus* M-protein (*emm*) typing method. For *spa* typing 100 isolates were tested, most of which were methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, and for the *emm* typing 50 group A *Streptococcus* isolates, including so far non-typable isolates, were used. In both methods lysates were prepared, followed by PCR and sequencing. The *spa* sequences were analysed with Ridom Staphtype software and the *emm* sequences by using the emm BLAST function at the website of the National Centers for Disease Control (www.cdc.gov/ncidod/biotech/strep/strepblast.htm).
Results. After optimization of PCR and sequence reactions the sequence-based typing of bacteria is relatively easy. All isolates could be typed and a few new *spa* types and *emm* (sub)types were obtained. The intralaboratory reproducibility for both sequence-based typing methods was 100%. Spa typing seems already suited for the detection of MRSA outbreaks, since all outbreak isolates resulted in the same *spa* type. More MRSA isolates need to be tested in order to see whether the *spa* typing method is suited for surveillance programs. The *Streptococcus emm* typing showed better results in comparison to the results obtained by routinely performed reversed line blot, with the addition that so far non-typable isolates could also be typed and a higher reproducibility was obtained.
Conclusion. Sequence-based typing of *S. aureus* and S. *pyogenes* is highly reproducible. An unambiguous typing nomenclature was obtained for each isolate tested so far, and it facilitates the exchange of typing data which is of great importance in establishing (international) surveillance programs.

P14
Amplified fragment length polymorphism analysis of Propionibacterium isolates implicated in contamination of blood products

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Introduction. Despite major efforts to reduce the rate of transfusion-transmitted bacterial infections, contamination of blood products with bacteria still occurs. Platelet concentrates (PCs) are frequently associated with transmission of bacterial infections. Yet, few is known about the source and mechanisms of the contamination. Contamination can originate

from skin (skin plug) during venipuncture, or can be caused by inadequate disinfection of the donor arm at the phlebotomy site or by transient bacteraemia. Propionibacterium is the most prevalent organism implicated in contamination of PCs. By means of amplified-fragment length polymorphism (AFLP) analysis, it was intended in this study to investigate the cause of contamination of PCs with Propionibacteria.

Methods. AFLP was employed to study 66 isolates derived from 33 PCs and 33 related red blood cells concentrates (RBCs). In addition 12 clincal skin isolates and 4 culture collection reference stains were included. Representative strains of each cluster were further analysed by DNA sequencing of the 16S ribosomal RNA gene.

Results. The AFLP results together with sequencing analysis of the 1200 bp of the 16S ribosomal RNA gene revealed the existence of three main groups: two groups (55%) consisting of isolates that did not originate from skin surface and another group (45%) comprising bacteria belonging to the skin surface flora. This latter group showed complete homology with reference strains of P. acnes.

Conclusion. The AFLP is reproducible and gave invaluable information about the nature of Propionibacteria contaminating PCs. The findings provide evidence for the involvement of the sebaceous (dermis) part of the skin as a potential source of contamination of blood products with Propionibacterium spp.

P15

Introduction of TcdA-negative, TcdB-positive *Clostridium difficile* in a general hospital in Argentina

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Introduction. *Clostridium difficile* causes nosocomial antibiotic associated diarrhea and pseudomembranous colitis. The main virulence factors are toxins TcdA and TcdB. Clinically important TcdA-negative, TcdB-positive (A^{-B+}) *C. difficile* strains from different countries have occasionally been reported, but epidemics are rare.

Methods. In a prospective study to determine the incidence of CDAD in a 200-bed general hospital in Argentina, all faecal samples of symptomatic patients with a positive immunoassay (Premier Cytoclone A/B or Triage Micro *C. difficile* panel) and/or a positive cytotoxicity assay were cultured for the presence of *C. difficile*. The isolated strains were investigated for the presence of *tcd*A, *tcd*B, and *erm*(B) genes. All clinical isolates were further typed using PCR-ribotyping, amplified fragment length polymorphism (AFLP) and toxinotyping.

Results. The incidence of CDAD per 1,000 admissions varied from 5.9 (2000), to 10.9 (2001), 6.9 (2002) and 4.7 (2003), respectively. Most patients were diagnosed at the Departments of Internal Medicine (57%), Pulmonology (13%), and Intensive Care (10%). Of all *C. difficile* isolates, the percentage A-B+ isolates increased from 12.5% in 2000, 58.1% in 2001, 87.9% in 2002, to 96% in 2003. Comparison of 37 patients with CDAD due to A+B+ strains with 80 patients with CDAD due to A-B+ strains, revealed no significant differences with respect to age, gender, department, underlying disease, severity of CDAD, or relapse rate of the patients. All 80 A-B+ *C. difficile* isolates belonged to toxinotype VIII and PCR-ribotype 017, and 89% were resistant to clindamycin due the presence of the *erm*(B) gene. Using AFLP, A-B+ isolates could be divided in clusters, and differed from the A+B+ strains cluster.

Conclusion. In a period of 4 years, A-B+ strains completely replaced A+B+ strains. This shift was not accompanied with a change in incidence, patient group, clinical presentation or relapse rate. All strains belonged to one PCR-ribotype and toxinotype, but could be distinguished in smaller clusters using AFLP. Clinicians should be aware of the widespread distribution of the clindamycin resistant A-B+ strains belonging to toxinotype VIII and PCR-ribotype 017.

P16

Staphylococcus aureus wound colonization in patients admitted to a burn centre: an overview

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Objectives. *Staphylococcus aureus* (*S. aureus*) colonisation of burn wounds increases morbidity and delays wound healing. Many burn wound colonizations with *S. aureus* are endogenous in nature.

The aim of this study was to study the *S. aureus* colonization rate in burn wounds in a period of 3 years and to evaluate the effect of eradication of nasal *S. aureus* with mupirocin in patients with regard to *S. aureus* burn wound colonization.

Methods. From September 2000 to March 2001 (Mup-A) and from June 2001 to January 2002 (Mup-B) all burn patients who were admitted to our 10-bed burn centre received at admission nasal mupirocin ointment. Patients were screened for *S. aureus* in nose, throats and burn wounds. Data of groups Mup-A and Mup-B were compared with 4 control groups (July 1999-January 2000 (C1), January 2000-July 2000 (C2), January 2002-July 2002 (C3) and July 2002 to January 2003 (C4) for age, total burned surface area, length of stay at the burn centre, burn wound colonization and days till colonization. Patients in all control groups did not receive mupirocin prophylaxis.

Results. Although the groups were unequal in size and time period, the patient population and admission practises were homogenous throughout this study-period. The acquired wound colonization rate among non-carriers in group Mup-A was 30%. This is lower than the 71% in the preceding control period C2.

The time-line shows that in control periods C1 and C2 the wound colonization rate among patients who were at admission not colonized with *S. aureus* (risk patients) varied between 53% and 79%. In Mup-A and Mup-B the colonization rate among risk patients varied between 55% and 56%. Control groups C3 and C4 showed a colonization rate among risk patients of 50% and 48%.

Conclusion. The results of this study suggest that application of nasal mupirocin to all patients upon admission to a burn

centre may reduce but not eliminate the risk of subsequent *S. aureus* colonization of burn wounds. Other measures including improved infection control practices and eradication of exogenous *S. aureus* reservoirs, including *S. aureus* carriage among healthcare workers, may be necessary to further reduce the incidence of *S. aureus* burn wound colonisation.

P17

Serotype distribution of *Actinobacillus actinomycetemcomitans* in Indonesia and the Netherlands

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Introduction. Periodontitis is a chronic inflammatory disease of the periodontium with involvement of anaerobic bacteria. Some bacteria as *Actinobacillus actinomycetemcomitans* (Aa), subdivided in six serotypes, have typical characteristics of primary pathogens with a strong association with persistence of disease. The aim of this study is to elucidate the distribution of serotypes of the periodontal pathogen Aa. This distribution was examined in Indonesia and the Netherlands; changes of this distribution were examined over an eight-years period in an Indonesian population.
Methods. In 1994 subgingival plaque samples were taken from 158 adult subjects from a rural village in Western-Java for microbiological analysis. From these subjects 115 were sampled in 2002 with 1-4 isolates per patient. Aa was retrieved from an Aa-selective Trypticase Soy Bacitracin Vancomycin-agar and used for serotyping. Serotypes a-f were determined by PCR reactions based on the distinct serotype-expression gene clusters. Subgingival plaque from 103 consecutively Aa-positive adult periodontitis patients from the Netherlands was analyzed for serotype distribution for comparison.
Results. In 1994 the serotype distribution of Aa in Indonesia was 18.5%, 47.7%, 16.9% and 1.5% for serotype a,b,c and e respectively and shifts to 8.5%, 30.5%, 35.6% and 10.2% in 2002. Serotypes d and f were not detected in Indonesia. The serotype distribution of Aa in the Netherlands were 60.8%, 19.6%, 13.7%, 4.9% and 1,0% for serotype a,b,c,e and f respectively. In Indonesia but not in the Netherlands patients harboring multiple clones were found. In 1994 the following combinations were found: a+b: 6.2%, a+c: 4.6% and a+e: 4.6%. This distribution changed in 2002 to a+b: 1.7%, a+c: 1.7%, a+e: 1.7%, b+c: 3.4% and c+e: 6.8%.
Conclusion. 1. Serotype a is predominant in the Netherlands whereas the serotype distribution of Aa in Indonesia seems to shift from a predominant serotype b population towards a more equally serotype distributed population. 2. The observed shift of distribution and the presence of multi-clonality suggest that Aa behaves like an opportunistic pathogen in Indonesia rather than a primary pathogen what is suggestive for the Dutch situation.

P18

Staphylococcus Aureus infections after percutaneous coronary interventions

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Purpose. Patients were studied to define the incidence, risk factors, and characteristics of staphylococcus (S.) aureus infections complicating percutaneous coronary interventions (PCI).
Methods. Between January 1999 and December 2002, we included in our hospital 7488 consecutive patients who underwent PCI with clinical follow-up in a retrospective case-control study; both univariate and multivariate analysis was performed.
Results. In total 21 *S. aureus* infections (0.28%) were documented at 1 to 16 days after the index PCI. The overall incidence of PCI-related infection was 0.08% (6 cases), 0.12% (9 cases) were i. v. catheter-related and in 0.08% (6 cases) the cause of the infection was not procedure-related. The 21 cases with *S. aureus* infections were matched with 42 controls randomly selected among patients who underwent a PCI but did not have *S. aureus* infections. Independent risk factors for *S. aureus* infections were congestive heart failure (5 cases {24%} vs 3 controls {7%}), alcohol abuse (4 {19%} vs 0), emergency PCI (8 {38%} vs 3 {7%}), one or more PCI in the preceding three months (4 {19%} vs 2 {4.8%}) and the presence of a sheath in the femoral artery and/or vein for the duration of more than 1 day after the procedure (4 {19%} vs 1{2.4%}). Among the patients with *S. aureus* infections, the duration of hospital stay was significantly increased (24 vs 5 days). The overall mortality rate in the 21 patients with *S. aureus* infections was 3/21 {14.3%} (controls 1/42 {2.4%}).
Conclusions. *S. aureus* infection is a very rare but potentially serious complication of PCI. Congestive heart failure, alcohol abuse, emergency PCI, more than 1 PCI in three months and the presence of a sheath in the femoral artery and/or vein for the duration of more than 1 day after the procedure are independent risk factors for *S. aureus* infection after PCI. Additional precautions should be considered in patients with these risk factors.

P19

High prevalence of resistant HIV in patients failing on HAART in the Netherlands

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Background. Resistance to at least one antiretroviral drug has been found in 65-76% of patients with HIV viremia under HAART. In the USA 48% of the HIV-infected population is

estimated to harbour resistant virus. The objective is to assess the prevalence of drug-resistance and therapy failure in HAART-treated patients in the the Netherlands.

Methods. HAART-treated patients were selected from the national observational cohort; 2103 were previously treated with antiretroviral drugs and 5223 were therapy naïve. A period of failure was defined by at least two consecutive plasma HIV-RNA measurements (500 after a period of suppression < 500 copies/ml. From these patients 1012 RT and protease sequences were obtained at failure and screened for primary drug-resistance mutations.

Results. Amongst pre-treated patients, the fraction failing HAART declined from 52% in 1996 to 34% in 2003; in the naïve patients it increased from 7% to 17%. Sequences were obtained in 6% of the patients in 1996 and in 18% in 2003. Resistance associated mutations were found in 92% and 71% of the sequences obtained in the pre-treated and naïve population, respectively.

In the pre-treated population the prevalence of resistance to at least one NRTI decreased from 94% in 1996 to 90% in 2003. In contrast, the prevalence of PI resistance increased from 13% in 1996 to 65% in 1998 and declined thereafter to 46% in 2003, whilst resistance to NNRTIs steadily rose from 3% in 1996 to 61% in 2003. The prevalence of resistance to 3 drug-classes was 0% in 1996 and 27% in 2003. In the naïve population prevalences were 20-40% lower.

As per 31 July 2004, in 6479 patients still in follow-up resistance to drugs from one drug-class has been found in 271 (4.2%), from two drug-classes in 382 (5.9%) and from three in 180 (2.8%) patients.

Conclusions. At present a relative small fraction of the HAART-treated patients fail on therapy. However, in failing patients the prevalence of resistance is high. Our data suggest that drug-resistance is an increasing problem in the Netherlands in both the pre-treated and the naïve population, yielding a growing reservoir for transmission of drug-resistant virus strains.

P20
Induction of sporulation in *Bacillus subtilis* continuous cultures

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The spores of *Bacillus subtilis* are extremely resistant to heat and other preservation treatments in comparison to their vegetative cell counterparts and thus are often associated with food spoilage and also on occasion safety incidence. Nutrient limitation and cell density are known factors that induce sporulation, however the specific signals of induction are more obscure. This work uses a controlled chemostat to study sporulation of *B. subtilis* 168 (lab strain) under specific nutrient limitations while monitoring cellular physiology and spore characteristics.

Sporulation could be induced under carbon (C-L), nitrogen (N-L) and phosphate limiting (P-L) conditions and it was observed that a decrease in the dilution rate resulted in an increase in the absolute number of spores produced (0.01% sporulation at D= $0.6h^{-1}$ to 20% sporulation at D=$0.05h^{-1}$). The percentage of cells that sporulate at a dilution rate of $0.3h^{-1}$ is 10, 0.3 and 2.3% in C-L, N-L and P-L chemostats respectively and was significantly lower than those obtained in corresponding batch cultures (89,52 and 59%). A GFP reporter strain under the promoter of *spoIIAA* was also used to monitor the onset of sporulation in the chemostat at varying growth rates. Remarkably in a first set of experiments we observed that virtually all cells in a C-L chemostat displayed a positive *spoIIAA* GFP signal. Clearly, however, not all cells in the culture sporulated (as assayed by heat resistance). We are currently assessing the activation stage of vegetative stress response pathways such as the Sigma B mediated pathway in individual cells.

On complete starvation no further induction of sporulation can be seen in a C-L chemostat. This was explained by the lack of glucose present which is a necessary source of energy for the sporulation process. In the N-L and P-L chemostats, glucose was still present on switching off the pumps. Some induction was seen in the N-L chemostat, however in the P-L chemostat, no increase in spore numbers is observed. It may be suggested from this data, that nitrogen limitation represses sporulation.

P21
Stress induced by weak organic acids in *Saccharomyces cerevisiae*

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Sorbic acid, a weak organic, is the most widespread food preservative used in the industry. Yeast and other fungi are, to a certain extent, able adapt to this acid and resume growth in the presence of the highest concentrations allowed in foods. This can result in product spoilage and thus create substantial economic losses. Quite a lot is known with respect to the end point of the yeasts' response to sorbic acid stress, i.e. when growth is resumed, from genome-wide transcript analyses and studies with yeast knockout mutants. However currently we still do not know why the cells initially arrest growth upon the weak-acid challenge. Also the molecular physiological events that occur during the adaptation phase and finally lead to a resumption of growth are poorly understood. Thus, to understand the mechanisms of growth limitation and adaptation we perform time-resolved studies of yeast cells exposed to sorbic acid in an integrated way. That is, we perform analyses at the level of gene expression, protein composition, and cellular metabolism. By calculating energy generating capacity, we try to map the cost and benefit of the various aspects of the stress response towards weak acids. In practice this means we determine metabolic fluxes, ATP/ADP ratios and ultimately try to construct a mathematical model of the response to the stress. Our initial results show that the growth limiting properties of sorbic acid are due to the undissociated form of the acid. It is believed that in this form the acid can diffuse freely over the plasma membrane. When we add potassium sorbate at different pH and account for the same amount of undissociated acid, this gives rise to an equal growth limitation. First experiments on measuring metabolite fluxes show an increase in many fluxes and a possible switch to a more respiratory metabolism. Currently we are also setting up an experiment to measure

intracellular pH upon sorbate stress. These results will be presented in the poster.

P22
Adaptation of yeast glycolysis to temperature changes

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Organisms are able to respond to their environment to maintain homeostasis. A well-studied environmental parameter is temperature variation, which exerts a complex combination of effects on the cell. We have studied the relation of growth rate and temperature of *S. cerevisiae* under well-defined conditions and focused on the effect on glycolysis. We selected a temperature from the supra-optimal side of the temperature growth rate graph and shifted a culture from the reference temperature to a higher temperature (38°C) to examine quantitatively the effects on glycolytic flux during adaptation. We observed a twofold increase in glycolytic flux. Now we want to find out how this flux increment is regulated; is it regulated metabolically, through temperature effects on the glycolytic enzyme reaction rates, through variations in metabolite concentrations, or is it regulated hierarchically, at the level of mRNA levels, protein levels, through the modulation of the expression alternative iso-enzymes. We will use regulation analysis to determine this for all enzymes in the glycolytic pathway. The principles of this approach are outlined in the current poster.

P23
Proteus' miracle: Self and non-self distinction in *Proteus mirabilis*

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Proteus mirabilis is a normal resident of the gut and a common causative agent of urinary tract and other infections in man. An important feature in pathogenicity of *P. mirabilis* is its ability to rapidly spread over large areas as swarmer cells.
When two different strains of *P. mirabilis* swarm on an agar plate, a macroscopic demarcation line with low cell density appears between the two swarming colonies. This demarcation line, known as the Dienes line, doesn't appear when members of two identical strains encounter each other. This implies an inherent ability to discern between self and non-self. The factors governing this phenomenon have been quite extensively researched in the past but no satisfactory explanation has been given yet.
To determine whether cell-cell contact was a prerequisite for the phenomenon to occur, two different *P. mirabilis* strains were allowed to swarm on different sides of an agar plate separated by a 60µmm thick, 0,2µmm pore-size membrane, permeable to proteins and most other molecules but not to bacteria. Five strains of *P. mirabilis* isolated from patient samples were tested against each other in this set-up. On no occasion any sign of inhibition between strains was observed.
A second goal was to visualize the initial encounter of two different strains. To make this possible, the nucleic acid-binding fluorescent dye Syto 9 was used to stain the bacteria. Strains were incubated at room temperature on blood agar. Shortly before the swarming colonies reached each other, the agar was inoculated with the dye. The area of first contact was visualised by fluorescence microscopy. A remarkable phenomenon was observed: where cell-cell contact occurred between the strains, large immotile round cells were formed. In areas where strains did not yet have contact with each other, no such effect was observed.
We conclude that: 1. Cell-cell contact, or at least close proximity, seems to be a prerequisite for the Dienes phenomenon to occur. 2. Cells of a large round morphotype seem to be associated with establishment of the Dienes line.

P24
Anaerobic methane oxidation coupled to nitrate reduction in freshwater ecosystems

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Methane is a greenhouse gas of growing importance. Over the past decades, methane emissions to the atmosphere have increased due to global warming and agricultural practices. A potential positive feedback loop exists, which can only be balanced by sufficient methane sinks. In the freshwater environment, aerobic oxidation by methanotrophic bacteria is generally considered to be the only sink for methane.
However, in these ecosystems, methane and nitrate also coexist in space and time. This has led microbiologists to debate and investigate the possible existence of an anaerobic bacterium that uses methane as the main carbon and energy source for denitrification, representing a new methane sink of presently unknown proportions. Up to now, such a bacterium has not been found.
Recently, we found evidence for the occurrence of anaerobic methane oxidation coupled to nitrate reduction in an anoxic freshwater sediment. The observed rates were 20 times higher than those observed for methane oxidation coupled to sulfate reduction in marine sediments of continental margins. This indicates that indeed an as yet unknown bacteria oxidizes methane anaerobically with nitrate as electron acceptor.
To extend this initial observation towards complete scientific understanding, the following questions need to be answered: What is the identity of the bacterium responsible for methane oxidation coupled to nitrate reduction? To what extent does it contribute as a methane sink in freshwater ecosystems, relative to aerobic methane oxidation? What is the biochemical mechanism of anaerobic methane oxidation with nitrate? This proposal aims to answer these questions by application of molecular ecological and geochemical techniques, laboratory cultivation and tracer studies.
If successful, this research will lead to the discovery of a qualitatively new mode of autotropic life, an extremely rare

event in modern Microbiology. It will also yield new insight into the responses of the freshwater environment to agricultural practices and climate change.

P25
Comparison of dominant bacterial populations from two marine sediments differing in heavy metal stress

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Sediments in coastal waters are often contaminated with various heavy metals, due to industrial discharges and antifouling paints on ship?s hulls. In this study, the microbial diversity of a pristine and a metal-polluted site along the coast of Germany were investigated. The hypothesis is that micro-organisms resistant to elevated levels of heavy metals would be detected more frequently in the bacterial community from the polluted site than in that from the pristine site.
To test this hypothesis, nearly complete bacterial 16S rRNA genes were amplified using general primers and genomic DNA extracted from the sediments. Subsequently, the amplification products were used to construct two clone libraries. Identification of 100 positive clones from each library was done by restriction analysis and sequencing of inserts. Rarefaction curves showed an estimated saturation of 30 unique clusters, of which 23 were detected by our methods, implying good library coverage. Phylogenetic analysis showed that the distribution of dominant populations in both libraries was rather similar. More than 50% of the clones belonged to the phylum Cytophaga/Flavobacterium, whose members are often detected in marine samples but whose ecological role is largely unknown. Other dominant clones were related to members belonging to the genera Roseobacter (6% and 14%), and Methylobacterium (6% and 1%), both from the alpha lineage of the Proteobacteria, and to a group of uncultured bacteria belonging to the gamma subdivision of the Proteobacteria (3% and 8%) (frequencies for the polluted and pristine site, respectively).
Unfortunately, little knowledge is available on the heavy metal tolerance and sensitivity of the detected species. Therefore, this study will be complemented with an investigation of these characteristics on a random collection of culturable isolates, in order to confirm the hypothesis.

P26
Quality control for handling of accidental blood exposures

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Introduction. A regional, round-the clock by telephone accessible, counselling centre was established to counsel all accidental blood exposures by a standardized protocol. To categorize levels of risks an algorithm was developed. During one year the procedure for adherence to this standard protocol was analysed.

Methods. In the algorithm used high risk accidents pose a risk for transmission of hepatitis B (HBV), hepatitis C (HCV) and human immunodeficiency virus (HIV) and low risk only for HBV. Medical interventions were instituted according to the risk level. During one year all accidents were registered and analysed for adherence to the standard protocol.
Results. In 2003 the centre handled a total of 454 incidents. Of these 36 (7.9%) incidents contained no risk, 329 (72.5%) contained low risk, and 67 (14.8%) high risk. Because of incomplete registration 22 (4.8%) incidents could not be further analysed.
In 396 of the remaining, fully-evaluable incidents (n=432) prevention for hepatitis B was needed in low-risk incidents and prevention for HCV and HIV in 63 high-risk incidents. A total of 36% of the incidents with risk for HBV transmission and 40% of the incidents with risk for HCV and HIV transmission were not handled according the proposed protocol. Multiple breaches occurred in 8% of the cases.
Breaches consisted of doing too much (25/396) as well as doing not enough (123/396). Under treatment potentially occurred for HIV-PEP in 12 of 63 incidents, incomplete follow-up for HCV in 11 of 63 incidents, and lack of HBIg administration in 5/396 incidents, including 3 high risk incidents. In 21 of 396 low risk cases breaches resulted from reporting late.
Conclusions. It remains difficult to achieve an acceptable level of standardised care when using standard operational procedures to handle blood exposure accidents. Documenting and evaluating the flaws is an essential element to improve the system.

P27
An *Helicobacter pylori* TolC efflux pump confers resistance to metronidazole

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Introduction. *Helicobacter pylori* infections are treated with a proton pump inhibitor in combination with amoxicillin or metronidazole (MTZ) and clarithromycin. Bacterial resistance to MTZ or clarithromycin, hamper the treatment of *H. pylori* infections. Possible mechanisms of intrinsic drug resistance involve decreased drug uptake or increased drug efflux. In *H. pylori*, the contribution of efflux proteins to antibiotic resistance is not well established. As parallel acting translocases may have overlapping substrate specificities, loss of function of one such translocase may be compensated by that of another with no effect on the susceptibility to antibiotics. Translocases, located in the inner membrane, interact via a periplasmatic efflux protein with an outer membrane efflux protein (OEP) or TolC like protein. Bacteria may have a number of different translocases, acting with only a limited number of OEPs. In *H. pylori* 26695, 27 translocases were identified, of which HP1184 was the sole representative of the multidrug and toxic compound extrusion family of translocases, which hence could have a unique substrate specificity. In addition, four TolC like proteins (HP0605, HP0971, HP1327 and HP1489) were identified. Thus, it is feasible that inactivation of a TolC like protein would affect the function of multiple translocases.

Aim. We aimed to determine whether efflux systems contribute to antimicrobial susceptibility by evaluation of the susceptibility profiles of *H. pylori* mutants in which HP1184 or TolC like proteins were inactivated.
Methods. The HP1184, HP0605, HP0971, HP1327 and HP1489 knockout mutants as well as a mutant in which both HP0605 and HP0971 were inactivated were assessed for their susceptibility to antimicrobials by Etest or disk diffusion.
Results. The HP1184 and HP1489 knockout mutants both showed an increased susceptibility to ethidium bromide, while the HP0605 knockout mutant exhibited an increased susceptibility to novobiocin and sodium deoxycholate. The HP0605/HP0971 double knockout mutant was, in addition to novobiocin and sodium deoxycholate, also more susceptible to MTZ.
Conclusion. Active efflux is eminent in resistance to antimicrobials in *H. pylori*.

P28
Isoniazid resistance and catalase in *Mycobacterium tuberculosis*

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Isoniazid (INH) has proven a valuable drug for the treatment of *Mycobacterium tuberculosis* infection. Unfortunately, the first reports of INH resistance appeared soon after its introduction on the market in 1952. INH is converted to its active metabolite by catalase and initially, resistance was linked to absent or largely reduced catalase activity. However, also catalase positive strains can be resistant towards INH. These strains have the combined phenotype of withstanding oxidative stress inside macrophages while being resistant towards INH. One of the most predominant mutations in the catalase G gene is S315T. This mutant catalase still retains significant catalase activity, but conversion of INH into its active form is largely reduced, either through decreased binding or decreased oxidation of INH. Mycobacterial strains carrying the S315T mutation have a MIC for INH of around 5-10 mg/ml, which is mainly above therapeutic blood levels.
In the Netherlands (90s), around 7% of *M. tuberculosis* isolates was INH resistant, 50% carrying the S315T mutation. Since long, it is practice to test INH resistant strains for catalase activity. INH is still considered useful as part of the treatment regimen, when the catalase reaction is positive. Given the information above, the justification of this common practice in the Netherlands is not entirely clear and should in our opinion be re-evaluated. In the present work, we give an overview of INH resistance in relation to catalase activity in order to provide arguments for this discussion.

P29
Does shortening turnaround time of microbiological procedures affect clinical outcomes? A randomised controlled trial among hospitalised patients in the Netherlands

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Introduction. Shortening the turnaround time of microbiological procedures is supposed to be associated with an improved clinical outcome. Two studies performed in the USA, in which the turnaround time was shortened by using an automated system for bacterial identification and susceptibility testing, yielded a significant decrease in mortality, morbidity and clinical costs. By conducting a single blind, randomised controlled trial we investigated whether the same clinical impact could be reported in a Northwest European (Dutch) setting.
Methods. All patients hospitalised in the Isala klinieken in Zwolle, the Netherlands, with a bacterial infection confirmed by culture were randomly assigned to a control (conventional) or to an intervention (rapid) group. The clinical microbiologists orally reported clinically relevant information to the clinician for all patients. For all patients complete culture results were reported on paper and delivered in the hospital by courier. For identification and susceptibility testing conventional overnight methods were used for the control group. For the rapid group the Vitek 2 system, which can produce same-day results, was used. To increase same-day reporting, in each of three consecutive study periods accelerating factors were added in a step-up manner to the laboratory workflow of the rapid group, such as increasing oral reporting, extending the working day and adding an extra hard-copy report delivery. Methods for the conventional group remained identical throughout the study.
Results. For the rapid groups the turnaround time was significantly shorter for oral reporting of final susceptibility results in all three study periods and for reporting on paper in the third study period. There was no significant difference between groups in any of the clinical impact outcome measures.
Conclusions. Vitek 2 results were significantly earlier available as compared to conventional testing. For the overall patient group in our hospital setting however, this had no clinical impact.

P30
Response of *Streptococcus mutans* towards environmental stresses

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Streptococcus mutans is an important pathogen in the initiation of dental caries. The acidogenic and aciduric nature of the

organism is one its important virulence determinants. We have shown that these determinants are even more distinctive when *S. mutans* is growing as a biofilm. It is known that several stress-responsive genes are involved in biofilm formation. Expression of these and several other genes in biofilms differs significantly from suspension-growth. However, the distribution of these stress-responsive gene-products in biofilms is unclear and the relation between physicochemical gradients and the (antimicrobial) resistance properties still needs to be explored.

To be able to so we have constructed several promoter GFP/DsRed fusions that allow us to study the expression of stress-responsive genes under various conditions.

In this study we aimed at determining the expression of the ClpP gene. It has been shown that the Clp family of proteinases plays an important role in *S. mutans*, both in biofilm formation as well as in adaptation to growth at low pH.

The ClpP promotor from *S. mutans* UA159 was cloned into the pVA838 shuttle vector in front of the coding sequences for the fluorescent proteins DsRED and GFP respectively. The shuttle vectors were transformed back into UA159 and these reporter strains were used to study the expression levels of ClpP by determining fluorescence levels during growth in a biofilm and in planktonic cells. Different stress situations i.e. increasing concentrations of fluoride and pH stress were evaluated under the different growth conditions. We were able to show increased resistance of *S. mutans* after growth in a biofilm, as well as a clear difference in ClpP gene expression under the different conditions.

P31
Performance of CHROMagar(r) MRSA to detect methicillin-resistant *Staphylococcus aureus*

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Introduction. *Staphylococcus aureus* is one of the most frequently isolated pathogens from clinical specimens and a common cause of nosocomial infections. An important problem at this moment throughout the world is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA). To identify MRSA in contaminated samples more easily and reliable, selective media have been developed. The purpose of this study is to evaluate the in vitro sensitivity and specificity of a recently developed medium called CHROMagar MRSA for identification of MRSA.

Materials and methods. A well-defined collection consisting of 457 *Staphylococcus aureus* strains was used. 241 were methicillin susceptible (MSSA) and 216 were MRSA. The isolates were inoculated on columbia agar plates with 5% sheep blood and incubated for 24 hours at 35 °C. From the resulting cultures a suspension of 0,5 MacFarland was made and subsequently a swab was dipped in the suspension and streaked on a CHROMagar MRSA plate. The results were read after 24 and 48 hours of incubation at 35 °C. Growth of colonies showing any pink or mauve coloration was considered to be positive (indicating MRSA).

Results. A total of 216 MRSA strains were tested. The sensitivitiy to detect MRSA after 24 hours was 95,4% (206 of 216). After 48 hours the sensitivity was 100% (216 of 216). A total of 241 MSSA was tested. The specificity was 100% after 24 and after 48 hours of incubation.

Conclusion. CHROMagar MRSA is highly sensitive and specific to differentiate between MSSA and MRSA in vitro. Since the sensitivity is optimal after 48 hours only, samples need a minimum of two days of incubation before final results can be obtained.

P32
Characterization of integrons from European *Salmonella* isolates

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Introduction. *Salmonella* is an important cause of food-borne gastroenteritis. Resistance among *Salmonella* has increased worldwide. Antibiotic resistance determinants may be transferred among bacteria via mobile genetic elements, such as plasmids and transposons. In Gram-negative bacteria integrons are often associated with these elements. The presence and distribution of integron-associated resistance among European *Salmonella* isolates was determined as well as the ability of these elements to transfer resistance by conjugation.

Methods. Fifty multi-drug resistant *Salmonella* isolates were chosen randomly from a collection of European isolates. Identification and characterization of the integrons was performed by PCR and RFLP typing. Plasmid analysis of integron-positive isolates was performed by gel electrophoresis. Conjugation of resistance genes to *E. coli* was performed. Since integrons are associated with multiresistance, they were used as a marker for resistance gene transfer.

Results. 24 of 50 isolates were integron positive and 14 among them were positive for the conserved-segment (CS) PCR, which spans the resistance encoding gene cassettes. RFLP of CS-PCR products showed the presence of integrons with *aadA2* (integron type I) and *aadA1a* (integron type V) gene cassettes, 13 and 1 isolates, respectively. Plasmid profiles of type I integrons isolates were identical. The isolates with a type I integron were obtained from 7 cities. Since type V integrons are commonly found from different sources, one isolate carrying this integron was used for conjugation to *E. coli*. After conjugation the recipient *E. coli* was resistant to tobramycin, gentamicin, amoxicillin, amoxicillin/clavulanate, and trimthoprim/sulfamethoxazole.

Conclusion. In this study, type I integrons have been shown to be the most prevalent in European *Salmonella* isolates. Only one isolate contains a type V integron and it could be transferred to *E. coli*, yielding a multi-drug resistant *E. coli*.

P33
Trimethoprim-induced filamentation in clinical isolates of the Enterobacteriaceae discovered using a porous living chip

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Introduction. Microbiology needs new growth formats to suit automation and future cell-based diagnostics. We propose a porous ceramic material (anopore) for the creation of a 'better Petri dish'. Anopore offers significant advantages: it is an inert and rigid material that can be used as a growth and imaging support. Anopore can be compartmentalized, allowing highly multiplexed microbial assays. Imaging micro-colonies by fluorescent microscopy can be performed after only a few rounds of division on anopore, enhancing the speed of AST (antibiotic sensitivity testing). In developing anopore chips for AST we noticed that the antifolate drug trimethoprim commonly induced filamentation in Enterobacteriaceae-infected urine samples. We followed up this observation with a systematic screening for the morphological effects of trimethoprim using anopore chips.
Methods. Anopore strips (36 x 8 mm, 60 microns thick, 0.2 microns pore size) were engineered to create discrete test areas printed with trimethoprim. After inoculation with clinical isolates and subsequent incubation, data capture was by fluorescence- or electron-microscopy. ImageJ software was used for analysis; analyzing 160 to 320 cells per test condition.
Results. Anopore proved an effective growth and imaging support for bacterial culture and in screening for highly elongated or damaged cells. Filamentation of up to 50x the normal cell length was discovered in clinical isolates of *Shigella sonnei, Shigella flexneri, Pantoea* species, *Enterobacter aerogenes, Hafnia alvei* and *Citrobacter koseri*.
Conclusion. This novel approach demonstrates that trimethoprim induces filamentation much more widely than has previously been reported. Anopore has potential as a multiplexed growth format for applications in clinical microbiology, and in other areas such as screening for new antimicrobials and strain improvement.

P34
Prevalence of extended-spectrum beta-lactamases in Amsterdam

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Introduction. Gram-negative pathogens harbouring extended-spectrum beta-lactamases (ESBL) are widely prevalent in many parts of Europe and their prevalence is increasing, but little is known about their prevalence in the Netherlands. We evaluated the prevalence of ESBL in Gram-negative isolates of enterobacteriaceae and non-fermenting Gram-negative bacilli in Amsterdam.
Methods. In May 2004, in a 1-week study at 5 hospitals in Amsterdam, 343 isolates were collected, consisting of 144 *Escherichia coli*, 32 *Klebsiella pneumoniae*, 15 *Klebsiella oxytoca*, 89 Gram-negative isolates of enterobacteriaceae non-*E. coli* and non-*Klebsiella spp*. and 63 non-fermenting Gram-negative bacilli. The isolates were tested with a modified double disk test (MDDT) consisting of disks of ceftazidime, cefotaxime, cefpodoxime and cefepime placed at distances of 30 and 20 mm (center to center) from a disk containing amoxicillin plus clavulanic acid. Also disks containing cefoxitin or ceftazidime plus clavulanic acid were included. ESBL positive results were confirmed by PCR and sequence analysis of the SHV, TEM and CTX-M genes.
Results. An ESBL phenotype was detected in 5.5% (8/144) *E. coli*, 3% (1/32) *K. pneumoniae*, 20% (18/89) enterobacteriaceae non-*E. coli* and non-*Klebsiella spp*. and 4.7% (3/63) non-fermenting Gram-negative isolates.
Conclusion. In comparison with a previous study concerning the occurrence of ESBLs in Dutch hospitals in 1999, our results show that ESBLs is an increasing problem in Amsterdam. Due to the serious implications of ESBLs in terms of complication and mortality, medical microbiologists and clinicians need to be familiar with the clinical significance of these enzymes and potential strategies for dealing with them.

P35
Expression of immune-evasion molecules on Staphylococcal Pathogenicity Island 5

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Introduction. Staphylococcal Complement Inhibitor (SCIN) and Chemotaxis Inhibitory Protein of *Staphylococcus aureus* (*S. aureus*) (CHIPS) are excreted proteins that play a crucial role in the staphylococcal defense against our innate immune system. Together with the genes for Staphylokinase (*sak*) and Staphylococcal Enterotoxin A (*sea*), the genes for SCIN (*scn*) and CHIPS (*chp*) are clustered on a bacteriophage-encoded Pathogenicity Island (*S. aureus* Pathogenicity Island 5 (SaPI-5)).
Methods. Transcriptional activity of all SaPI-5 encoded genes was studied after cloning their promoter sequences upstream of the gene for Green Fluorescent Protein (GFP) in pSK236. Plasmids were transferred to clinical or laboratory *S. aureus* strains (wild-type or mutated in regulatory loci) via transduction or electroporation. Excretion of SaPI-5 encoded proteins was monitored in supernatants of bacterial growth cultures by ELISA.
Results. Expression of exoproteins by *S. aureus* mainly occurs in the late exponential and stationary growth phases. Using *gfp*-constructs, we found *sak* and *sea* promoters also to be activated during these growth stages. In contrast, *chp* and *scn* promoters were transcribed immediately in the exponential phase, with a peak after one and two hours of growth respectively. During late exponential and stationary growth phase, transcription continues at a lower level for *chp* but rises again for *scn*. These unique transcription patterns were confirmed by Northern analyses. In bacterial supernatants we observed that early transcription results in the early excretion of SCIN and CHIPS. Although *scn* and *chp*

are located on a bacteriophage, their transcriptional activity was shown to be under control of known regulatory loci in *S. aureus*.

Conclusions. 1. In contrast to other staphylococcal exoproteins, the genes for *scn* and *chp* are transcribed very early during growth.

2. Early transcription results in early excretion of SCIN and CHIPS.

3. Transcription of *scn* and *chp* is under control of known *S. aureus* regulatory genes.

P36

Comparison of the efficacy of disinfectants to control microbial contamination in dental unit water systems in general dental practices across the European Union

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Water delivered by dental unit water systems (DUWS) in general dental practices may harbour high numbers of bacteria, including opportunistic pathogens. Biofilms in the tubing of DUWS provide a reservoir for these microbes and need to be controlled. This study tested and compared the efficacy of seven products in a head-to-head trial to lower the bacterial load in DUWS outflow water to below the American Dental Association's guideline of 200 CFU/ml. The products Alpron, Bioblue, Dentosept, Oxygenal, Sanosil, Sterilex Ultra and Ster4Spray were tested in DUWS (n=134) in Denmark, Germany, Greece, Ireland, the Netherlands, Spain and the United Kingdom. Weekly water samples were tested for total viable counts (TVC) and the effect of the products on biofilm presence was measured. A four week baseline measurement period was followed by eight weeks of disinfection. TVCs before disinfection ranged from 0 to 2.6 x 105 CFU/ml (0 to 5.41 log CFU/ml). During disinfection, the products achieved a reduction of the median TVC ranging from 0.69 to 3.06 log CFU/ml, although occasional high values up to 7.6 x 104 CFU/ml (4.88 log CFU/ml) were found during treatment. A reduction of biofilm TVCs ranging from 0.56 to 2.22 log CFU/ml was found. The results confirm the presence of high bacterial numbers in untreated DUWS outflow water and show the ability of disinfecting products such as Alpron, Dentosept, Sanosil and Oxygenal to reduce these values to < 200 CFU/ml. A ranking showed Alpron and the hydrogen peroxide-based products to be most effective.

P37

INH-resistant *Mycobacterium tuberculosis* strains with a mutation at amino acid position 315 of the katG are a more dangerous public health threat than other INH-resistant strains; a 10-years experience in the Netherlands

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In a previous, limited study it was shown that isoniazid (INH)-resistant *Mycobacterium tuberculosis* isolates with a mutation at amino acid position 315 of *katG* (Δ315) were high-level resistant to INH and more frequently resistant to streptomycin. In the present, much more extended study, different aspects, under which transmissibility of Δ315 strains were re-examined. Therefore, INH-resistant *M. tuberculosis* isolates in our database of 8,332 patients from the Netherlands (1993-2002) were screened for the Δ315 mutation. INH resistance was found in 592 (7.1%) isolates. Among the INH-resistant isolates 323 (54.6%) were Δ315. IS*6110*-RFLP analysis showed that Δ315 isolates were found in clusters – suggesting recent transmission – at the same frequency as INH-susceptible isolates. In contrast, the other INH-resistant isolates were significantly less frequently clustered. In addition, Δ315 isolates were significantly more often high-level INH-resistant, streptomycin-resistant, and multidrug-resistant than other INH-resistant isolates. In conclusion, Δ315 isolates pose a much more serious threat to public health than other INH-resistant isolates.

P38

An unexplained increased isolation rate of *Mycobacterium gordonae* at several laboratories in the Netherlands

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Mycobacterium gordonae is considered one of the least pathogenic mycobacteria, although it can cause opportunistic infections in immunocompromised patients. It can be isolated from soil and (potable) water and is a frequent colonizer of sputum, gastric fluid and urine. At the Jeroen Bosch Hospital, an increase in the number of *M. gordonae* isolates was noted in November 2003. The number of isolates

increased from 3 in 2003 to 21 in 2004, including 1 repeat isolate. There had been no recent changes in decontamination procedures to explain this observation. In 2004, *M. gordonae* made up 47% of non-repeat mycobacterial isolates recovered from clinical specimens. These specimens came from various inpatient and outpatient services and consisted of sputa, bronchial washings and urine. The RIVM also recognized an increased isolation rate of *M. gordonae*. The number of *M. gordonae* isolates identified at the RIVM increased 2,9 fold from 34 in 2003 to 97 in 2004. When the isolates from the Jeroen Bosch Hospital were omitted, the increase was still 2,2 fold. After inquiring, several other laboratories reported an increased isolation rate of *M. gordonae*. At the Canisius Wilhelmina Hospital, the number of isolates increased from 0 in 2003 to 8 in 2004, including 2 repeat isolates. At the St. Antonius Hospital, 3 *M. gordonae* isolates were cultered in 2003 against 11 in 2004, including 1 repeat isolate. This laboratory also reported 12 nontuberculous isolates not determined to species level. DNA fingerprinting of the isolates from the Jeroen Bosch Hospital with the polymorphic GC-rich sequence probe revealed that non-repeat isolates belonged to different strains. Two repeat isolates from one patient were identical. Thus, it was considered unlikely that the increased isolation rate resulted from laboratory cross-contamination. This is supported by the fact that the increased isolation rate was observed at se veral laboratories. Currently, studies are being undertaken to look for other explanations for this observation.

P39
PerR is a regulator of oxidative stress defense in *Helicobacter hepaticus*

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Introduction. Infection with *Helicobacter hepaticus* can lead to enteritis, hepatitis and liver cancer in mice, and is associated with an active cellular immune response with production of oxygen radicals. The ability of bacteria to cope with such oxidative stresses comprises an important virulence factor. The *H. hepaticus* genome sequence contains genes encoding homologous of several oxidative stress defense proteins and a gene encoding a homologue of the iron responsive regulatory protein PerR, which mediates regulation of peroxide stress defense. In this study we have investigated the expression and regulation of the oxidative stress defense system of *H. hepaticus*.
Methods. *H. hepaticus* ATCC51449 was grown in Brucella broth supplemented with β- cyclodextrins. Iron-restriction of growth media was established by supplementing the medium with the iron chelator desferal. Protein expression was monitored by SDS-PAGE, and selected proteins were identified using MALDI-TOF mass spectometry. Transcription of selected genes was monitored by Northern hybridization.
Results. Growth of *H. hepaticus* in iron-restricted conditions resulted in altered expression levels of six proteins. Three of these proteins displayed iron-repressed expression, while three other proteins displayed iron-induced expression. Two of the iron-repressed proteins were identified as AhpC (25 kDa) and KatA (55 kDa). Both proteins are involved in the degradation of peroxide compounds, and are known to contribute to the bacterial oxidative stress defense. Mutation of the *perR* gene resulted in high-level, iron-independent, expression of both AhpC and KatA.
Conclusions. In *H. hepaticus*, iron metabolism and oxidative stress defense are intimately connected via the PerR regulatory protein. This regulatory pattern resembles that seen in the enteric pathogen *C. jejuni*, but contrasts with the regulatory patterns observed in the human gastric pathogen *H. pylori*. Therefore, iron-dependent regulation of peroxide stress defense may be an advantage in enteric colonization

P40
Iron-repressed expression of urease in *Helicobacter hepaticus* is mediated by the transcriptional regulator Fur

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Introduction. Enterohepatic *Helicobacter* species (EHS) are being recognized as emerging pathogens in the intestinal tract and/or liver of mammals. *Helicobacter hepaticus* is a EHS that naturally infects the gastrointestinal and hepatobiliary tract of mice. The enzyme urease is an important virulence factor in the genus *Helicobacter*, allowing survival and growth in acidic conditions. We have previously shown tha, unlike in the human gastric pathogen *H. pylori*, the urease expression in *H. hepaticus* is not regulated by either pH or nickel. Here it is demonstrated that *H. hepaticus* urease is regulated at the transcriptional level by iron, and this repression is mediated by the Fur regulatory protein.
Methods. *H. hepaticus* ATCC 51449 was grown in Brucella broth supplemented with β-cyclodextrins. Iron-restriction of growth media was established by supplementing the medium with the iron chelator desferal. Urease activity was determined by a colorimetric assay, whereas protein expression was monitored by SDS-PAGE. Transcription was monitored by Northern hybridization.
Results. Growth of *H. hepaticus* in iron-restricted media resulted in a twofold increase in urease activity, when compared to growth in iron-replete medium (7.8 ± 2.0 vs 3.6 ± 1.5 U, respectively, $p < 0.001$). However, iron-responsive repression of urease activity was absent when growth medium was supplemented with the protein synthesis inhibitor chloramphenicol, suggesting transcriptional regulation of urease activity. To identify the regulatory protein mediating this process, the two genes encoding iron-responsive regulators Fur and PerR of *H. hepaticus* were mutated. Only mutation of the *fur* gene abolished iron-responsive regulation of urease activity.
Conclusion. *H. hepaticus* regulates its important virulence factor urease in response to the availability of iron. Low iron is often used by pathogenic bacteria as a signal for entering the host. Thus, it is likely that *H. hepaticus* uses this signal to switch on urease for passage of the gastric environment. This type of urease regulation has not been reported before and may allow colonization of the hepatobiliary tract by *H. hepaticus*.

P41

Staphylococcus epidermidis is cleared from biomaterial but persists in peri-implant tissue in a mouse biomaterial infection model despite rifampicin/vancomycin treatment

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Introduction. Infections associated with inserted or implanted biomedical devices (BAI), are predominantly caused by *Staphylococcus epidermidis* and other coagulase negative staphylococci. Extensive antibiotic treatment is required, and the biomedical device often needs to be removed. In an experimental model of BAI, we have observed that *S. epidermidis* persists longer in peri-implant tissue than on the implanted biomaterial itself. The aim of the present study was to investigate the effectiveness of rifampicin/vancomycin in clearance of bacteria from the implant and the peri-implant tissue.
Methods. Two polyvinylpyrrolidone-coated silicon elastomer (SEpvp) biomaterial segments (BM) were implanted s.c. in C57Bl/6 mice. The mice (9/group) were challenged with 10E7 cfu of *S. epidermidis* strain AMC5, a clinical isolate, and injected daily with 500ml of 2 mg/ml vancomycin + 1 mg/ml rifampin in 0.9% NaCl i.p., except on the day of termination. Control mice received injections with 0.9% NaCl only. Nine mice in each group were sacrificed on day 1, and 8 mice on day 8 after challenge. BM and peri-BM tissue were processed and cultured on blood agar plates and in Brewer Tween liquid medium.
Results. After 1 and 8 days cultures of BM of untreated mice (control group) were positive in 14/18 and in 5/16, respectively. The tissue biopsies of the control group were all culture positive. In the antibiotic-treated mice only 1 of the BM was culture positive after 1 as well as 8 days, whereas the tissues surrounding the BM were positive in 14/18 and 7/16 of the cases 1 and 8 days after infection, respectively.
Conclusions. Our results show that *S. epidermidis* persists in peri-implant tissue, even after treatment with vancomycin/rifampicin, an antibiotic regimen generally considered to eliminate both intracellular and extracellular microorganisms. This is in contrast with the BM themselves, which are sterilised by this treatment. These data suggest that biofilm formation on the biomaterial surface is not necessarily the only cause of persistent biomaterial-associated infection. Analysis of peri-implant tissue as well as of the biomaterial itself is required to unravel the true pathogenesis of biomaterial-associated infection.

P42

The NikR regulatory protein regulates nickel-responsive expression of the iron-uptake genes *fecA3* and *frpB3* in *Helicobacter pylori*

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Introduction. Intracellular homeostasis of the essential nutrient iron is a necessity for all living organisms, since both iron-deficiency and iron-overload will result in cell death by either growth inhibition or toxicity. The gastric pathogen *Helicobacter pylori* has three copies of the fecA and frpB iron-uptake genes, of which expression of the *fecA1/2* and *frpB1/2* genes is regulated by the Fur protein, the regulatory protein which controls iron homeostasis in most Gram-negative bacteria. Surprisingly, the *fecA3* and *frpB3* genes were not regulated by Fur and thus are thought to be constitutively expressed. *H. pylori* expresses a second metal-regulatory protein, NikR, and in this study we have investigated wether the *fecA3* and *frpB3* genes are regulated by NikR.
Methods. *H. pylori* reference strain 26695 and its isogenic *nikR* and *frpB3* mutants were grown in Brucella broth supplemented with 20 and 200 mM NiCl2. Whole cell protein was separated using SDS-PAGE. Transcription of *fecA3* and *frpB3* genes was monitored by Northern hybridization.
Results. The *fecA3* and *frpB3* genes were nickel-repressed in wild-type *H. pylori*, but constitutively expressed in the *nikR* mutant. However, the genes *fecA1/2* and *frpB1/2*, known to be Fur-dependent regulated, did not display any nickel or NikR-dependent regulation. On the translational level the nickel- and NikR-dependent regulation was confirmed for the *FrpB3* protein. Mutation of *frpB3* had no effect on growth in nickel-supplemented growth medium. However, this may be due to the compensatory expression of the two other copies of the *fecA* and *frpB* genes.
Conclusion. NikR and Fur proteins each regulate the expression of a subset of iron-transporter proteins. This allows differential expression of iron-uptake systems depending on the environmental conditions, and this may help *H. pylori* to survive the acidic conditions occurring in the gastric mucosa.

P43

GlnR-mediated regulation of nitrogen metabolism in *Lactococcus lactis* and *Streptococcus pneumoniae*

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Introduction. In *Bacillus subtilis*, the regulatory protein GlnR and its paralog TnrA play, together with glutamine synthetase GlnA, a key role in regulation of nitrogen metabolism. We studied the role of the GlnR ortholog in the human pathogen *Streptococcus pneumoniae* and the lactic acid bacterium *Lactococcus lactis*. For *L. lactis* knowledge of nitrogen metabolism is important, regarding its use in the dairy industry. From an entirely different point of view, several studies point to a direct role of nitrogen-related genes in virulence of pathogens, including *S. pneumoniae*. Therefore, investigating their action is crucial for understanding the infection process.

Methods. The regulatory role of GlnR in *S. pneumoniae* and *L. lactis* was studied by means of DNA-microarray analysis, *in silico* predictions, *lacZ* expression studies, gel mobility-shift assays and growth experiments.
Results. In both organisms, GlnR regulates its own bicistronic operon and glutamine uptake genes. In *S. pneumoniae* GlnR regulates also genes involved in glutamate biosynthesis and possibly arginine catabolism, while in *L. lactis* GlnR strongly represses a putative ammonium transport operon. The observed regulation is dependent on the medium nitrogen source and occurs via a highly conserved binding box. A similar binding box can also be found in related Gram-positive bacteria, where it is almost always present in the promoter of the *glnRA* operon and glutamine uptake genes. In addition, boxes can be found in genes involved in ammonium metabolism, glutamate metabolism, amino acid transport and carbohydrate metabolism.
Conclusions. GlnR mediates nitrogen-source dependent regulation of different and orthologous nitrogen genes in *L. lactis* en *S. pneumoniae*. In other pathogens orthologs of the targets of GlnR we identified have been shown to accomplish a task in virulence, such as adherence and protection against macrophages. This suggests that these processes and glutamine metabolism are intertwined with each other.

P44
Bactericidal activity of BP2 in a murine biomaterial-associated infection model

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Introduction. Antimicrobial peptides (AMPs) are an important first line of defense against micro-organisms. AMPs are generally small (< 50 amino acids), positively charged peptides, and contain both hydrophobic and hydrophilic domains. This results in specificity for interactions with negatively charged microbial cell membranes.
Bactericidal Peptide 2 (BP2) is a synthetic alpha-helical antimicrobial peptide based on conserved elements from various LPS-binding proteins. BP2 has broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts at micromolar concentrations, and no cross-resistance between BP2 and antibiotics has been observed. The aim of this study was to assess the efficacy of BP2 in vivo.
Methods. The efficacy of BP2 as prophylaxis was tested in a murine biomaterial-associated infection model. In this model, a catheter segment is implanted subcutaneously and an inoculum of 10E6 CFU of S*taphylococcus epidermidis* is injected along the segment. BP2 was injected along the catheter segment immediately after implantation. *S. epidermidis* was injected 3 hours later, and mice were sacrificed after 24h.
Results. The number of colonized biomaterial segments was reduced by 50% (to 5 out of 18 segments) in the group of mice that received BP2. In addition, the number of adherent bacteria per segment was significantly reduced. In the peri-implant tissues, survival of *S. epidermidis* was >10 fold reduced in the group pretreated with BP2.
Conclusion. Even with a 3 hour interval between prophylactic administration of BP2 and bacterial challenge, BP2 showed significant in vivo antimicrobial activity. The efficacy of BP2 in a treatment model for biomaterial-associated infection will be tested in the near future.

P45
Peri-implantitis and *IL-1* gene cluster polymorphisms

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Introduction. Interleukin (*IL*)-1α, *IL*-1β and their natural specific inhibitor *IL*-1 receptor antagonist (*IL*-1ra) play a key role in the regulation of the inflammatory response in periodontal tissues. Polymorphisms in the *IL-1* gene cluster have been associated with severe adult periodontitis. We aimed to investigate the *IL-1* gene cluster polymorphisms in patients with peri-implantitis.
Material and methods. The study included 120 North Caucasian individuals. A total of 71 patients (mean age 68 years, 76% smokers) demonstrating peri-implantitis at 1 or more implants as evidenced by bleeding and/or pus on probing and bone loss amounting > 3 threads on Brånemark implants and 49 controls (mean age 66 years, 45% smokers) with clinical healthy mucosa and no bone loss around the implants were recruited for the study. The titanium implants, *ad modum* Brånemark, had been in function for at least two years. Mouthwash samples were collected and genotyped for bi-allelic polymorphisms *IL-1A*$^{-889}$, *IL-1B*$^{+3953}$, *IL-1B*$^{-511}$ and a variable number of tandem repeat *IL-1RN* gene polymorphisms using PCR technique.
Results. Significant differences were found in the carriage rate of allele 2 in the *IL-1RN* gene between peri-implantitis patients and controls (56.5% vs. 33.3%, respectively, p=0.015, OR 2.6, 95% CI 1.2-5.6). Logistic regression analysis taking smoking, gender and age into account showed an association between the *IL-1RN* allele 2 carriers and peri-implantitis (p=0.020, OR 3.0, 95% CI 1.2-7.6).
Conclusions. Our results provide evidence that *IL-1RN* gene polymorphism is associated with peri-implantitis and may represent a risk factor for this disease.

P46
Functional analysis of PrmC of *Neisseria meningitidis*

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PrmC plays a key role in the accuracy of termination of translation in *Escherchia coli* K12 by methylation of glutamine of the GGQ domain of the protein release factors. So far, the function of only *E. coli* and *Chlamydia trachomatis* PrmC has been experimentally established, although PrmC homologues are well-conserved in nature. The aim of this study was to evaluate the genetic organization and functional characterization of *prmC* in *Neisseria meningitidis*.

The genomes of *N. meningitidis* MC58 and Z2491 were assessed for homologues of PrmC of *E. coli*. Potential PrmC function of neisserial genes was assessed by complementation of the growth deficiency in the *E. coli prmC* knock out strain SC5. Transcription and translation of putative neisserial *prmC* was assessed by RT-PCR and SDS-PAGE.
In *N. meningitidis* Z2491 one open reading frame (ORF) (NMA0369) of 822 bp was found, encoding a 30 kDa putative PrmC (42% identity with *E. coli* PrmC). NMA368 (462 bp) upstream of NMA369 had been annotated as a putative integral membrane protein (PIMP). The stop codon of *pimp* is located 5 nucleotides downstream of the start codon of *prmC*. Interestingly, the corresponding genome region of strain MC58 contains one large ORF (NMB2065) of 1272 bp, encoding a putative protein of 46 kDa. Here, deletion of one nucleotide near the junction of *pimp* and *prmC* created a frame shift, resulting in loss of the stop codon of *pimp*, resulting in an in-frame fusion between the *pimp* and *prmC*. Homologues of PIMP in other bacterial species, or any other fusions between *prmC* and other genes were not found. NMA0369 as well as NMB2065 could trans-complement the growth defect of SC5, indicating functionality of both putative PrmCs. RT-PCR data indicated a 1300 bp long transcript from NMB2065. However, only a 30 kDa recombinant protein was detected by SDS-PAGE, suggesting that only a part of the transcript is being translated.
In conclusion: in *N. meningitidis* MC58, PrmC is encoded by an abnormal large ORF. This ORF is transcribed into a similarly large transcript. Translation into functional PrmC of normal size is most likely from an internal ribosomal binding site.

P47
Different evolutionary histories for the two outer membrane efflux proteins of *Neisseria meningitidis*

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Introduction. In both published complete genome sequences of *N. meningitidis*, two genes containing outer membrane efflux protein domains are present (in strain MC58, NMB1714 and NMB1737, respectively). These proteins form trimeric channels that allow export of a variety of substrates, including antimicrobial agents and toxins, making the channel-tunnels possible targets for intervention. Aim
The aim of this study is to assess the diversity of the outer membrane efflux proteins and the encoding genes in *N. meningitidis*.
Methods. Thirty-eight isolates were selected from the database of the Netherlands Reference Laboratory for Bacterial Meningitis (RIVM/AMC, Amsterdam). These comprise 17 genotypes from 6 serogroups, isolated from 7 countries in three continents during four decades. Sequences of 1162 bp of both genes encoding the outer membrane efflux proteins were obtained. DNA sequences and derived protein sequences were analysed using the program MEGA.
Results. Nineteen different NMB1714 alleles with 76 polymorphic sites were identified, encoding 16 different polypeptides. In marked contrast, only 5 alleles with 5 polymorphic sites encoding 4 different proteins were found for NMB1737.
Conclusion. The difference in number of alleles and polymorphic sites between NMB1714 and NMB1737 suggest a different evolutionary history for the two genes. One possibility is a recent introduction of NMB1737 in the meningococcal population, which led to limited accumulation of polymorphisms. The absence of this gene in *Neisseria gonorrhoeae* and *Neisseria lactamica* supports a recent introduction in the neisserial genepool. Alternatively, one NMB1737 allele may have spread rapidly through the meningococcal population, but this assumes a strong selective pressure for this allele or a neighboring locus.

P48
Effects of Fluoride and Chlorhexidine on Lactate Dehydrogenase Expression in *Streptococcus mutans* biofilms

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Streptococcus mutans (Sm) is often found in human dental plaque and it has been identified as a major etiological agent of human dental caries. Sm is able to cause dental caries in various animal models. It is found in dental plaque of both caries active as well as in caries free humans. Likely, not the species but its properties, which may not be exclusively for this species, will play a role in the cariogenesis. Formation of a biofilm is one of the requirements of expression of the cariogenic properties of Sm. It possesses an efficient glycolytic pathway whereby a rapid drop in plaque pH is established. Glycolysis in a biofilm is performed under anaerobic conditions and the enzyme lactate dehydrogenase (LDH) plays a crucial role in maintaining the NADH/NAD^+ balance.
This study was aimed at determining the activity of the expression of the LDH gene by studying its promotor activity. This was done by cloning the promotor region from the LDH gene from Sm strain UA159 into a shuttle vector pVA838 in front of the coding sequences for the fluorescent proteins DsRED respectively. The shuttle vectors were transformed back into UA159 and this reporter strain was used to study the expression of the LDH gene by determining fluorescence levels during growth in a Calgary Biofilm device.
It was found that DsRed under the LDH-promotor was more strongly expressed in the biofilm model in comparison to planktonic cells. Furthermore, the effect of different stressors, such as fluoride and chlorhexidine, on the DsRed-expression were studied. From these results we conclude that the LDH gene is probably not constitutively expressed. Future studies will resolve its induction principles.

P49

Fur mediates iron-responsive gene regulation in *Helicobacter mustelae*

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Introduction. Iron-restriction at mucosal surfaces by the mammalian host is a non-specific defense mechanism against bacterial pathogens. Iron acquisition is thus considered an important virulence factor for bacterial pathogens. *Helicobacter mustelae* is a gastric pathogen of ferrets, and pathogenesis of *H. mustelae* infection mimics many aspects of human infection with *H. pylori*, and thus is used as animal model for *H. pylori* infection in humans. However, little is known about general metabolism of *H. mustelae*. Here we have compared iron metabolism of *H. mustelae* and *H. pylori* using a combination of comparitive genomics and transcriptomics.
Methods. The partial genome sequence of *H. mustelae* (http://www.sanger.ac.uk/Projects/H_mustelae/) was screened for genes encoding orthologs of iron-metabolism-related proteins.To study regulation of the iron-metabolism genes by iron and the Ferric Uptake Regulator (Fur), an isogenic *fur* mutant was created in *H. mustelae* strain ATCC 43772. The wild-type strain and its *fur* mutant were grown under iron restricted and sufficient conditions, and RNA isolated from these cultures was used for Northern hybridization.
Results. The *H. mustelae* genome sequence contained 5 orthologs of outer membrane receptors for iron uptake (IROMPs), and a ferritin ortholog, probably involved in iron storage. Transcription of the IROMP orthologs HmFecA, HmFrpB1A and HmCfrA was repressed by iron, while transcription of one ferritin homolog was iron-induced in the wild-type strain. For all genes, regulation was absent in the *fur* mutant, suggesting that Fur mediates this iron-responsive regulation. In contrast, the HmFrpB1B protein was not regulated by iron nor by *fur*.
Conclusions. Iron metabolism is of importance to *H. mustelae*, as its genome sequence contains several orthologs of iron acquisition and iron storage systems. Most of the genes encoding these factors are regulated both by iron and Fur, similar to *H. pylori*. This confirms the close relationship between *H. mustelae* and *H. pylori*, making *H. mustelae* a good candidate for studying pathogenesis of *Helicobacter* infection in the natural host.

P50

Selective isolation of anomalous DNA from an unsequenced Neisseria

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Introduction. Horizontal gene transfer contributes largely to the shape and evolution of prokaryotic genomes. Horizontally transferred DNA can be identified by its anomalous nucleotide composition. The meningococcal genome sequences have revealed many regions of anomalous DNA (aDNA), suggesting frequent events of DNA acquisition. Previous studies showed that frequent DNA transfer of housekeeping genes occurs between commensal *Neisseriae* and *N. meningitidis*. It is unknown whether aDNA regions are also transferred between these related species.
Aim. To obtain more insight in the neisserial gene pool, we explored the presence of aDNA in *Neisseria lactamica*, a commensal inhabiting the human nasopharynx.
Methods. aDNA in *N. lactamica* was identified using the same set of endonucleases of which the recognition sites are overrepresented in regions of aDNA in the known genome sequences of the closely related *N. meningitidis*. Using a newly developed online tool, ??-web, we were enable to place individual sequences in a selectable genomic context.
Results. We identified 8 fragments of which 7 were considered anomalous in composition. The other fragment appeared to be a plasmid. aDNA fragments display sequence similarities to virulence-associated genes *N. meningitidis* encoding various factors. In addition, we identified a sequence encoding a novel putative adhesin like autotransporter, located in the genome at the same position as a similar gene in the *N. meningitidis* genome. Two contiguous fragments encode a putative chaperonin and a putative protease. Homologs of these genes, encountered simultaneously and in the same order, implying an operon, are distributed amongst phylogenetically distinct respiratory inhabitatants.
Conclusion. The identification of aDNA in *N. lactamica* with similarity to meningococcal DNA is consistent with the notion of genetic exchange between commensal and pathogenic *Neisseriae*. The discovery in *N. lactamica* of aDNA homologous to a locus in other pathogens substantially expands the neisserial gene pool.

P51

Comparison of five different methods of isolation of DNA of *M. tuberculosis* in sputum and its quantitation by using real-time PCR

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Introduction. DNA loss of *M. tuberculosis* during isolation and inhibition of PCR are investigated by quantitative real-time PCR. Detection limits of PCR reactions will decrease if DNA isolation is optimised. Five different DNA isolation methods were compared for the isolation of *M. tuberculosis* DNA in sputum samples; Generation Capture Column (Gentra Systems, Inc.), QIAamp (Qiagen), MagNA Pure LC Isolation Kit III (Roche), MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) and miniMAG (BioMerieux).
The DNA output was quantified with two real-time PCR systems: ABI Prism® 7000 Sequence Detection System (Applied Biosystems) and LightCycler 2.0 Instrument (Roche).
Methods. A pooled sputum sample was treated with the N-acetyl-L-Cysteïne (NaLC) method and was divided into equal portions. Before (method A) and after (method B) DNA isolation two different concentrations of purified DNA of *M. tuberculosis* H37Rv was added to these samples. The products, and series of *M. tuberculosis* DNA dilutions, were tested for the IS*6110* element (ABI prism 7000) and the *Kat*G codon 315 (LightCycler).
The difference in measured DNA between method B and method A is caused by loss of DNA due to the DNA isolation. The difference in measured DNA between method B and

the series of dilutions of purified DNA of *M. tuberculosis* H37Rv is caused by inhibition in the PCR.
Results. The miniMAG is not appropriate to isolate NaLC-homogenized sputum samples, because of the clots forming between NaLC and the miniMAG reagents.
PCR analysis of the samples from the QIAamp (Qiagen) shows the best results, followed by the MagNA Pure LC Isolation Kit III (Roche) and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche). The most unreliable results were obtained from the Generation Capture Column (Gentra Systems, Inc.), especially the amount of DNA loss.
Conclusion. Optimising the DNA isolation shows decrease of the detection limit of an already optimised real-time PCR. The QIAamp (Qiagen) gives the highest DNA output, causes no extra PCR inhibition and is the best choice for the tested DNA isolation methods.

P52
Comparison of three broad range bacterial real-time PCR sets for quantification of oral micro-organisms

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Introduction. specific real-time PCR assays have been used for the identification of *Porphyromonas gingivalis* (Pg), *Actinobacillus actinomycetemcomitans* (Aa), *Tannerella forsythensis* (Tf), *Peptostreptococcus micros* (Pm), *Prevotella intermedia* (Pi) and *Fusobacterium* spp. (Fuso spp.) in periodontitis patients. The broad range PCR could give an estimation of total bacterial load in clinical samples derived from subgingival plaque from periodontitis patients. The present study describes the comparison of three broad range real-time PCR assays used for determination of the anaerobic bacterial load. The results of these three sets were also compared to the proportion of anaerobic bacteria in the samples as determined by specific real-time PCR and anaerobic culture.
Materials & methods. Plaque samples (n=32) from periodontal pockets were collected from subjects with periodontitis. Aliquots were evaluated by both broad range {Set 1: Yang *et. al.* 2002; Set 2. : Nadkarni *et. al.* 2002; Set 3: Huijsdens *et. al.* 2005} and species-specific real-time PCR (ABI 7000 system) and quantitative anaerobic culture. After primer and probe optimisation, specificity and sensitivity tests were performed. The total bacterial load and the amount of cultured oral anaerobic bacteria were then calculated and compared with both techniques as well as with specific PCR for Pg, Aa, Pi, Pm, Fuso spp. and Tf.
Results. The results of the three different broad range sets showed large differences. Set 1 was not universal in contrast to what is publishedwhereas set 2 and 3 showed a reduced sensitivity and reduced reproducibility compared to the micro-organism specific real time sets. As a consequence the results of the broad range real-time PCR showed no correlation with the culturing results due to variation in the calculated cfu equivalents of the PCR.
Conclusions. The published broad range real-time PCR sets show large variation in sensitivity and reproducibility. In general, these sets are not yet reliable for quantification of the total bacterial load in subgingival plaque samples and for quantification of oral pathogens.

P53
Comparison of automated and manual extraction methods for detection of *Salmonella enterica* in fecal samples

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Introduction. Increasing demand for molecular diagnostics has made efficient methods for nucleic acid extraction mandatory, especially for difficult clinical samples like feces. Automated extraction systems are an alternative to labour-intensive manual methods. In this study we compared the performance of DNA recovery of a manual BOOM-Feces (BOOM-F) method, the semi-automated NucliSens miniMAG and the automated MagNA Pure LC DNA III Kit direct from fecal samples. Also, we evaluated the PCR performance of these three methods with real time detection of *Salmonella enterica* in comparison to conventional culture.
Methods. For studying the recovery of DNA a known amount of *Hin*dIII digested phage lambda (_*Hin*dIII) DNA together with the fecal sample was processed according to the manufacturer's instructions. The DNA recovery with the three methods was estimated by agarose gel electrophoresis. A real-time PCR assay for *S. enterica* was validated with 69 culture positive and 67 culture negative fecal samples.
Results. A panel of 136 fecal samples was analysed. The BOOM-F method performed with a high average recovery (97%) compared to miniMAG (70%) and MagNA Pure (64%). No samples showed low recovery with BOOM-F (i.e. recovery < 50%), whereas 17% and 27% scored low recoveries (0-38%) with miniMAG and MagNA Pure respectively. BOOM-F had the highest PCR sensitivity (91%) as compared to miniMAG (86%) and MagNA Pure (87%). No PCR inhibition was observed with miniMAG, whereas with both BOOM-F and MagNA Pure 4 samples were detected with moderate to severe inhibition. This resulted in 3 and 2 samples that were not interpretable for BOOM-F and MagNA Pure respectively. Average processing time for miniMAG was slightly shorter (120 min) compared to MagNA Pure (135 min), whereas BOOM-F proved to be most labour intensive (180 min).
Conclusion. Although there are major differences in DNA recovery, all methods seem to have a comparable PCR performance. Regarding flexibility and inhibition miniMAG might be preferred when more automation is required in the laboratory. The choice of nucleic acid extraction method will also depend on user convenience, total processing time and throughput of samples.

P54

Evaluation of an internally controlled, real-time PCR targeting the OspA gene for detection of *Borrelia burgdorferi* (sensu lato) DNA in cerebrospinal fluid

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Introduction. Molecular detection has successfully been used in diagnosis of erythema migrans and Lyme arthritis. In neuroborreliosis, however, the sensitivity of standard PCR tests on CSF samples is only 10-30%. The aim of the study was to evaluate the sensitivity of the real-time PCR for CSF samples.
Methods. An internally controlled real-time PCR targeting the OspA gene was developed using *Borrelia burgdorferi* (sensu lato) specific primers and Taqman probe. DNA from *B. burgdorferi* and an internal control (Phocine herpes virus) were amplified and detected simultaneously using probes labelled with 6-carboxy-fluorescein (FAM) and indodicarbocyanine (Cy5) respectively. Analytical sensitivity was evaluated on DNA extracted from 33 *B. burgdorferi* sensu lato strains, including *B. burgdorferi sensu stricto* (n=6), *B. garinii* (n= 12), *B. afzelii* (n=9), *B. valaisiana* (n=4), *B. japonica* (n=1) and strain A14S. Specificity was evaluated with DNA extracted from relapsing fever (RF) *Borrelia* sp. (n=4), and on 31 viral, bacterial and fungal samples. CSF samples from culture-positive patients (n=3) as well as CSF samples from patients with neuroborreliosis in which culture was not done (n=13), and cerebrospinal fluid (CSF) samples spiked with limiting dilutions of freshly cultured *B. garinii* (1.25 10E7 spirochetes/ml) were tested in the assay. DNA was extracted from CSF samples by QIAamp DNA mini blood kit columns.
Results. The real-time PCR detected DNA of all B. *burgdorferi* (sensu lato) species and was negative for DNA from RF *Borrelia* sp. and all other specimens. The sensitivity of the assay in CSF was 1-5 spirochetes. Two out of three culture-positive CSF samples were positive in the PCR, whereas the third sample was inhibited. Two out of 13 samples from other patients with neuroborreliosis were positive in the PCR.
Conclusion. The real-time PCR assay developed in this study provides sensitive and specific detection of all *B. burgdorferi* sensu lato species tested. The QIAamp mini blood kit columns are suitable to extract *Borrelia* DNA from CSF, and combination with real-time PCR provides a sensitive assay. However, even using this assay the yield from CSF samples from patients with neuroborreliosis in this series is low.

P55

Validation of the Roche Amplicor® HPV Test for the screening of high-risk hpv genotypes in cervical scrapes; a comparison with the INNO-(SPF_{10})-LiPA HPV detection/genotyping assay

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Background. Certain high-risk human papillomavirus (HPV) types are a necessary cause for the development of cervical disorders. Women with persistent hr-HPV infections have an increased risk of developing high-grade cervical lesions, compared with those who have no, or low-risk HPV infections. Therefore, implementation of HPV detection into cervical screening programs might identify women at risk of cervical cancer. Several HPV detection methods with different sensitivities and specificities are available. Recently, a new PCR-based technique, the Roche AMPLICOR® HPV Test, was developed. This test recognizes a group of 13 hr-HPV types simultaneously.
Aim. This study was undertaken to validate and compare HPV detection in 573 cervical scrapes, by the AMPLICOR HPV Test and the INNO-LiPA HPV detection/genotyping assay (SPF10-LiPA system version 1).
Results. Human â-globin was not detected in nine specimens, which were therefore excluded from the comparison. Eleven scrapes containing HPV type 53 or 66 were also excluded from the comparison because these (probably) hr-HPV types can not be detected by the AMPLICOR HPV Test. The results of the HPV detection by the Roche AMPLICOR HPV Test was confirmed by INNO-LiPA HPV detection/genotyping assay in 539/553 cases, showing an absolute agreement of 97.5% with a Cohens kappa of 0.9327, indicating almost complete similarity of the two tests.
Conclusion. Like the INNO-LiPA HPV detection/genotyping assay, the AMPLICOR HPV Test was sensitive, specific, feasible and easy to handle. The value of the Roche AMPLICOR® HPV Test with a broad spectrum hr-HPV detection has to be determined in prospective clinical studies (Journal of Clinical Microbiology, 2005; In press.).

P56

Improved rapid detection of *Salmonella enterica* DNA in feces with real-time PCR by addition of an enrichment procedure

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Introduction. *Salmonella enterica* is a major cause of bacterial gastro-enteritis in the Netherlands. Conventional diagnosis, based on detection of *S. enterica* in feces, consists of enrichment, selective culture and identification and can take 3-5 days. Cost-effective PCR screening of feces is feasible (ICAAC 2004 poster O-1626) and will decrease the turn around time significantly. However in this approach a sensitive PCR is mandatory. Previously (NVMM 2003/04) we showed

that 9% of the fecal samples culture positive for *S. enterica* remained PCR negative, presumably due to the low numbers of bacteria. This study describes the validation of enrichment prior to PCR for the detection of *S. enterica* in feces.
Methods. The real-time PCR assay for *S. enterica* targets the *invA* gene (considered specific for *S. enterica*) and is combined with a very efficient 'in-house' DNA extraction from feces. Enrichment consisted of overnight incubation in a selenite enrichment broth at 35 °C. A total of 291 culture positive and 240 culture negative fecal samples for *S. enterica* were used for validation of the direct approach. A sub-panel of 36 *S. enterica* culture positive and 21 *Campylobacter jejuni* culture positive were fecal samples used to validate the enrichment approach.
Results. The sensitivity and specificity of the direct detection of *S. enterica* in feces were 90% and 99% respectively. PCR inhibition was observed in 3.4% of the fecal specimens resulting in 1.3% non-interpretable results. In the sub-panel sensitivity and specificity of the enrichment approach were both 100%, compared to 83% and 96% for the direct approach respectively. No PCR inhibition was observed in the enriched samples.
Conclusion. Addition of enrichment prior to DNA extraction improves the detection of *S. enterica* in feces. Including an enrichment step will not necessarily lead to an increase in the total processing time for detecting *S. enterica* in feces compared to direct PCR on feces, due to the batch wise nature of performing PCR. This makes rapid, sensitive and cost effective molecular screening of feces for *S. enterica* feasible. The direct detection may be valuable in out-break management.

P57
Whipple's disease in the Netherlands: An overview of PCR confirmed cases.

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Introduction. Whipple's disease is a rare systemic disease with a multitude of different symptoms, including diarrhea, severe weight loss, arthralgia, myalgia, fatigue, low-grade fever and myocarditis. Not all patients experience all symptoms and therefore diagnosis is usually delayed. The disease is caused by the Gram-positive bacillus *Tropheryma whipplei*, belonging to the family of *Actinomycetes*. The aim of the study was to obtain an overview of the Whipple's disease in the Netherlands diagnosed during the past 7.5 years by PCR at the VUMC.
Methods. Data from all PCR analyses performed in the period January 1997 until June 2004 were collected. The PCR positive cases for Whipple's disease were identified. After informed consent, medical information was collected from medical specialists and general practitioners including a questionnaire that was sent to the patients.
Results. During 7.5 years 352 PCR analyses were performed, 31 of which were positive, mostly on duodenal biopsies, but in some cases on blood and liquor. Whipple's disease was diagnosed or confirmed in 21 cases. The mean age of diagnosis was 53 years, among which 11 males and 10 females. Medical data were traceable from 18 Whipple patients. From these data arthralgia was presented as the first appearing symptom before diagnosis. After diagnosis the main symptoms were weight loss, arthralgia and diarrhoea. Most patients, 14 out of 18, were treated with cotrimoxazole, being the recommended therapy. There were 5 relapses and in contrast to earlier literature no patient had been working as a farmer or with animals, neither in the countryside nor in nature.
Conclusion. Most results of Whipple's disease in the Netherlands correspond with known facts about Whipple's disease in literature, except the male female ratio and the lack of the disease among farmers. It is remarkable that out of 18 people from whom data were traceable, 3 have some kind of mental retardation, 2 of which with positive PCR in the blood.

P58
Influence of dietary calcium on the composition of the fecal microflora in human volunteers

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The composition of the human fecal microflora is relatively stable in time, but can be altered by specific dietary components such as non-digestible fibers. Dietary calcium may be an additional food component able to influence the microflora, since non-absorbed calcium can lower the soluble bile acid concentrations in the colon. Many intestinal bacteria cannot withstand high bile acid concentrations.
The influence of calcium on the human fecal microflora was examined in a cross-over study with 12 healthy volunteers. The subjects were asked to consume 1 liter of calcium depleted (120 mg Ca/l) or regular (1200 mg Ca/l) dairy products/day on top of their habitual but further dairy-free diet. Fecal samples were collected at days 5-7 of each study period. Terminal Restriction Fragment Length Polymorphism (T-RFLP)-analysis and quantitative real-time PCR were used to analyze the fecal microflora composition.
The study showed that each individual has its own characteristic fecal microflora, as was seen in a typical T-RFLP-profile. Moreover, shifts in bacterial communities were observed by variation in T-RFLP-profiles that were likely due to changes in diet. Four terminal fragments can be found in almost all samples representing dominant intestinal bacterial species. Differences between individuals can be found in the number and sizes of terminal fragments and in the intensity of the bands.
Shifts in specific groups of bacteria were assessed using quantitative real-time PCR assays. During the high calcium diet, fecal *Lactobacillus* numbers were found to increase in 10 out of 12 individuals, *E. coli* numbers were found to decrease in 8 out of 12. *Bifidobacterium*, *Propionibacterium*, and *Fusobacterium* numbers did not change.
We conclude that dietary intake of calcium results in qualitative and quantitative differences in bacterial microflora between the samples taken from one individual. The increase in lactobacilli and decrease in *E. coli* numbers is an indication of a beneficial quality of calcium that deserves further attention.

P59

Automated DNA extraction influences the sensitivity of PCR-based molecular diagnostics

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Introduction. Previously (NVMM 2004) we showed impaired DNA recovery from PBS with the MagNA Pure Total Nucleic Acid Kit (M-extraction) compared to the method of Boom (Si-extraction). This study was conducted to establish the consequences of this impaired DNA recovery with regard to PCR sensitivity.

Methods. DNA recovery experiments were conducted with PBS, CSF, plasma and whole blood (4-8 replicates for each sample matrix) for both methods. Other reconstruction experiments were performed with positive control DNA's for EBV and VZV (2 dilutions, tested in 5 replicates) in PCR negative blood and analyzed by PCR. Also serial diluted CMV was spiked in CMV negative blood (4 dilutions tested in 8-fold) and analyzed by CMV PCR. Finally clinical blood samples were tested for EBV (n=23) and CMV (n=16) by both methods.

Results. Recovery experiments showed reduced recovery for M-extraction (~20%) compared to Si-extraction (~80%) for all matrices tested. Part of the missing DNA from the M-extraction could be retrieved from binding (~20%), washing (~5%) and silica matrix (~15%), leaving ~40% of the DNA irretrievable. PCR control DNA's showed between 7.6 and 14.2 and 1.9 and 7.9-fold lower PCR signals after M-extraction, compared to Si-extraction for EBV and VZV respectively. PCR analysis of spiked CMV showed reduced recovery and loss of sensitivity for M-extraction, resulting in 0% (0/8) and 88% (7/8) hit rates for the lowest spiked CMV loads, whereas Si-extraction obtained 50% (4/8) and 100% (8/8) hit rates. In clinical blood samples this resulted in 11 false negative and 1 invalid result(s) for M-extraction, compared to 1 false negative result for Si-extraction. Similar results have been obtained with other lots of the M-extraction reagents over a 4 year time period and with 2 instruments.

Conclusion. The M-extraction seems to have a substantial lower DNA recovery compared to Si-extraction, leading to reduced sensitivity and possible false negative results with low microbial loads in PCR methods. Before implementing the M-extraction the clinical consequences of the loss in sensitivity should first be considered, especially when maximal sensitivity is required.

P60

Genotypic determination of Fungi using the MicroSeq™ D2 LSU kit

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Introduction. Traditional methods of determination of fungi rely on their phenotypic properties, an important aspect of which is micro- and macroscopic morphology. The subjective interpretation of this morphology requires much experience and even then does not always produce unequivocal results. On the other hand, molecular methods do not rely on the experience of the technician performing the test. Applied Biosystems offers a kit to sequence a part of the D2-region of the large subunit ribosomal DNA(LSU rDNA) of fungi.

Here we describe our experience comparing this sequencing method for genotypic determination of fungi with the results of conventional methods.

Methods. Using Applied Biosystem's MicroSeq™ D2 LSU rDNA Fungal Sequencing Kit we sequenced part of the LSU rDNA fragment of 43 strains of yeasts and fungi according to the manufacturer's instructions, without prior knowledge of the name of the species in question. The resulting sequence was compared with database sequences (Genbank) using BLASTN or FASTA. If the strain sequence matched at least 99% with the database sequence it was considered to be the same species. A match between 97% and 99% was regarded as the same genus.

Results. For 22 'unknown' strains sequencing provided the same species name as conventional methods. In 13 other cases the same genus was found. In 5 of these a match at species level was not possible because either conventional methods or sequencing did not provide a species name. In another 2 of the 13 cases the species name found by conventional methods was found in the genbank-database with a match over 99%, but in these two cases it was not the best matching species.

In 8 cases sequencing and conventional methods resulted in different names. In 2 of these the species name found by conventional methods was found in the genbank-database with a match over 99% (but less than the best matching species). In another 2 of the 8 cases no matching species or genus was found in the genbank-database.

Conclusion. Sequencing and subsequent genetic database analysis is relatively easy to perform and a valuable additional tool in determining fungi.

P61

Epidemiological background of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands: results from six months' MRSA isolates

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Introduction. All first MRSA isolates from the Netherlands are sent to the National Institute for Public Health (RIVM) for confirmation and typing. In addition to the typing, epidemiological data characteristics of the patient, the care setting and the culture and risk factors for MRSA-carriage are collected through a questionnaire. In the spring of 2004, both the questionnaire and the procedures were improved importantly. We present the first results of this renewed surveillance covering the period from 1 May to 1 November 2004.

Methods. Questionnaires were sent together with the MRSA-isolates. A random sample of 178 isolates was drawn

from a total of 847 unique isolates. Senders of questionnaires in the random sample were recalled by telephone if questionnaires had not been returned within three weeks or if questionnaires were not complete. For the analysis, we only considered questionnaires with sufficient information related to isolates that were *mecA* gene positive.

Results. In total, 460 questionnaires were received of which 444 met the inclusion criteria. The random sample contained 157 MRSA isolates with 115 questionnaires containing sufficient information. The resulting response of 73% was significantly higher than the overall response ($p < 0.05$). Out of the 444, 343 isolates (77%) were from patients and 101 (23%) from personnel. 287 isolates (65%) were found by MRSA-directed screening and 155 isolates (35%) by routine microbiological examination. Most laboratories (70%) used enrichment broth. 87 MRSA patients (25%) were contaminated in the institution where they were nursed. 64 patients (19%) had recently stayed in a foreign hospital. Common risk factors were wounds, abscesses or furuncles (37%). Most of the personnel (60%) was contaminated in their own care setting. Eleven employees (11%) were contaminated during their work in a foreign care setting. 6% of those detected with MRSA had skin disorders

Conclusion. 1. Recalls significantly increased the response to the questionnaire. 2. A minority of isolates was related to stay in foreign hospitals.

P62

Rapid identification of (methicillin-resistant) *Staphylococcus aureus* (MRSA) strains by TaqMan Martineau/Coagulase Duplex real-time PCR

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Rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) is a key aspect of infection control in hospitals to limit the spread of this organism. Primary isolates are identified on the basis of morphology, Gram staining, catalase activity and slide agglutination reaction (Staphaurex Plus). However, these methods can produce both false-positive and false-negative results.

We therefore set out to develop an easy and quick real-time PCR to detect MRSA on a TaqMan platform. Mec-A detection was carried out as described previously. The DNA sequence used to detect *S. aureus* is a specific genomic fragment described by F. Martineau. Recently *S. aureus* strains have been identified that are negative in the Martineau PCR. Therefore we added the detection of the coagulase-gene as duplex-PCR to the *S. aureus* test.

The Accuprobe *S. aureus* identification test (Gen-Probe Incorporated) was used as described in the documentation accompanying the test kits. For the real-time PCR, one fresh colony was resuspended in 0.5 ml of Tris-EDTA and incubated at 100 degree Celsius for 15 minutes. 5 microliter was used in the PCR (2 hours of cycling in the standard TaqMan program). Of 71 Gram positive bacterial strains, 35 strains were Accuprobe-, Martineau- and coagulase positive, while strains of *S. capitis* (n= 1), *S. simulans* (n=1), *S. intermedius* (n=1), *S. haemolyticus* (n=2), *S. hominis* (n=1), *S. lugdunensis* (n=3), *S. warneri* (n=1), *S. saprophyticus* (n=1), *S. schleiferi* (n=13) -kind gifts from Dr. J. Kluytmans and Dr. W. Wannet- and 12 other strains were negative in all three tests, indicating a 100% agreement between the Accuprobe-test and the Martineau/coagulase duplex PCR.

Although the one *S. intermedius* strain that we tested was negative in the coagulase PCR, it has been described that a coagulase gene can be present in non-aureus Staphylococci such as *S. intermedius* and *S. hyicus*. More of these strains will be analysed to establish whether our coagulase PCR does cross react with these species.

We conclude that we have developed a simple TaqMan *S. aureus* test that, in combination with the TaqMan Mec-A detection test, can be employed to quickly identify MRSA.

P63

Is Ciprofloxacin resistance in *Staphylococcus aureus* a biological marker for methicillin-resistant *Staphylococcus aureus* in the Netherlands?

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Introduction. Ciprofloxacin, a drug from the class of the fluoroquinolones, can be used to treat *S. aureus* infections. In *S. aureus*, ciprofloxacin resistance is caused by 3 mechanisms: 1) mutations in the DNA topoisomerase IV gene (*grlA* and *grlB*), 2) mutations in the DNA gyrase gene (*gyrA* and *gyrB*) and 3) the NorA efflux pump. Resistance to methicillin is caused by the *mecA* gene, which is situated on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). It is known that less than 5% of the methicillin-susceptible *S. aureus* (MSSA) strains and over 95% of the MRSA strains are resistant to ciprofloxacin. In addition, it was reported that exposure to ciprofloxacin was a significant risk factor for the isolation of MRSA, but not for the isolation of MSSA (Weber *et al*, 2003). The objective of this study was to investigate the predictive value of ciprofloxacin resistance of *S. aureus* for the isolation of MRSA in the Netherlands.

Methods. Between 1995 and 2002, 868 *S. aureus* strains were isolated from intensive care units and urology wards in fifteen academic and general hospitals in the Netherlands. A total of 117 randomly selected ciprofloxacin susceptible *S. aureus* and all 109 ciprofloxacin resistant *S. aureus* strains from individual patients were included. Polymerase Chain Reaction was used to determine the presence of *mec*A and to identify the SCC*mec* type. Furthermore, Pulsed-Field Gel Electrophoresis analysis was performed to establish genetic relationships between MRSA strains.

Results. Nine of 109 (8.3%) ciprofloxacin-resistant *S. aureus* strains were *mecA* positive and were thus classified as MRSA. Among the 117 ciprofloxacin-susceptible *S. aureus* strains the *mecA* gene was not found. Of the nine MRSA isolates, which were classified into different clonal groups, four harbored SCC*mec* Type I, one Type II, three Type IV and one a not typeable cassette.

Conclusions. 1. In the Netherlands, ciprofloxacin-resistance in *S. aureus* is not a marker for MRSA. 2. There is no relation between ciprofloxacin-resistance and a specific SCC*mec* type of MRSA strains.

P64

Dissemination of methicillin-resistant *Staphylococcus aureus* clones in the Euregio Meuse-Rhine

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Background. The Euregio Meuse-Rhine (EMR) is formed by the border regions of Belgium, Germany and the Netherlands. In this region, cross-border health care requires infection control measures, in particular since the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) differs considerably between the three countries of the EMR (23.6%, 13.8% and 0.6%, respectively). Consequently, the cross-border transfer of patients may have an important impact on the dissemination and prevalence of MRSA, in particular in cases where patients are transferred from countries with a high prevalence to a country with a low prevalence.

Methods. To investigate the dissemination of MRSA, a total of 152 MRSA isolates (63 from Dutch, 40 from Belgian and 46 from German hospitals), obtained between 1999 and 2004 in the EMR, were characterized by Pulsed Field Gel Electrophoresis (PFGE), SCC*mec* typing and multilocus sequence typing (MLST). In addition, the presence of Panton Valentine leukocidin (PVL) was detected by real-time PCR and MIC determinations were performed.

Results. Four major clonal groups (A, G, L and Q) were identified by PFGE. A large majority of the strains from group A harbored SCC*mec* type III. MLST of these isolates revealed the presence of ST241-MRSA-III, which is a novel finding in Germany and the Netherlands. The majority of the strains from clonal group G harbored SCC*mec* type I, whereas most strains from group L carried either SCC*mec* type IV or I. Within group L, isolates belonging to different clonal complexes (CC5 and CC8) were found. Major clonal group Q included mainly SCC*mec* type II strains. Isolates from this group were typed as ST225-MRSA-II, which is a novel finding in the EMR countries. One isolate was found to contain both SCC*mec* type V and PVL. Finally, a correlation of approximately 85% was observed between the antibiotics susceptibility pattern and the SCC*mec* type.

Conclusions. 1. MRSA clones from CC5 and 8 are disseminated in the EMR.

2. Two new MRSA clones (ST225-MRSA-II and ST241-MRSA-III) were found in the EMR.

3. The first report of MRSA harbouring both SCC*mec* type V and PVL.

P65

Spa-typing of MRSA isolates from hospital outbreaks in the Netherlands

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Introduction. Typing of methicillin-resistant *Staphylococcus aureus* (MRSA) is essential to detect outbreaks and to set up surveillance programs. To date, Pulsed Field Gel Electrophoresis(PFGE) is the method of choice for typing MRSA isolates. However, typing based on banding patterns, are only partially successful since speed, nomenclature, and interchange of results between different laboratories can be difficult. The *spa*-typing method can circumvent these problems.

Method. *Spa*-typing of MRSA is based upon the DNA-sequence of the *Staphylococcus* aureus Protein A (*spa*) gene. Lysates have to be prepared, followed by PCR and sequencing. The X-region in the *spa* gene contains repeats which can differ in sequence and length. A *spa*-type is deduced from the number and order of these repeats. To date (10-01-05) there are 70 different repeats. These repeats and the *spa*-types are determined by Ridom StaphType software. There are many different *spa*-types (to date: 554), therefore this typing method is highly discriminatory. To determine the suitability of spa-typing for the detection of MRSA outbreaks, ten hospital outbreaks were investigated. Different outbreaks from different parts of the country with different time frames were included.

Results. *Spa*-typing of MRSA, after optimization of the PCR and sequence reactions, is a relatively easy and fast sequence-based typing method. All isolates could be typed, and results could be compared with an international database. All of the isolates within each of the outbreaks had the same *spa*-type (tested up to a nine months time frame).

Conclusion. *Spa*-typing appears to be suited for the detection of MRSA outbreaks. The technique is highly reproducible, it has an unambiguous nomenclature and it enables an easy interlaboratory exchange of results.

P66

Comparison of three selective media for the isolation of methicillin-resistant *Staphylococcus aureus* (MRSA)

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Introduction. Accurate methods to detect methicillin-resistant *Staphylococcus aureus* (MRSA) are useful, because early identification of MRSA in carrier patients enables the implementation of specific measures to prevent dissemination of these bacteria. Several culture media can be used to isolate MRSA from microbial beds, in particular from the nose, throat and perineum. In this study we investigated the usefulness of three MRSA isolation media.

Methods. We tested the performance of ORSAB (Oxoid), MRSAselect (Bio-Rad) and CHROMagar MRSA (ITK) using 23 isolates of MRSA, 30 isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) and 27 isolates of methicillin-resistant coagulase negative *Staphylococcus* (MRCNS). All strains were isolated from clinical specimens. A standard inoculum (10 ml of a suspension of 0.5 McFarland) was streaked onto each of the media. MRSAselect was incubated at 37 °C for 24 hours, ORSAB and CHROMagar MRSA for 24 hours and 48 hours. Preparation of the media and interpretation of bacterial growth were done according to the manufacturers instructions.

Results. MRSAselect was successful in detecting all 23 isolates as MRSA after 24 hours incubation. However, with ORSAB and CHROMagar MRSA, respectively 4 and 5 isolates were not detected after 24 hours. After 48 hours the ORSAB medium detected all isolates, on CHROMagar MRSA 1 isolate was not detected.

Calculated sensitivity, specificity and positive predictive value after 24 hours were respectively 100%, 95% and 88% for MRSAselect, 83%, 95% and 86% for ORSAB and 78%, 100% and 100% for CHROMagar MRSA. After 48 hours for ORSAB and CHROMagar MRSA this figures were respectively 100%, 91% and 82% and 96%, 100% and 100%.

Conclusions. All three tested media have high sensitivity and specificity, however the result of MRSAselect medium is obtained faster (within 24 hours) than with the other media.

P67

Comparison of real-time PCR, rapid immunoassay, and microscopy for the detection of *Giardia lamblia* in human stool specimens

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Introduction. Infectious gastro-enteritis is still a major public health burden in developed countries, although the related mortality is low. The intestinal protozoan *Giardia lamblia* is the most frequent pathogenic parasite involved in infectious gastro-enteritis. Classically, diagnosis of giardiasis is conducted by microscopically analyzing multiple stool specimens for the presence of *G. lamblia* cysts or trophozoites. The sensitivity of the microscopy is largely dependent on the skill of the microscopist, making it time-consuming and expensive. Recently, direct fluorescent-antibody staining and enzyme immunoassays have shown to be sensitive and cost-effective alternatives for microscopic examination of stools. Also, real-time PCR detection of *G. lamblia* directly from stools has been described with similar or improved sensitivity in single fecal specimens, compared to microscopy and antigen detection.

Methods. For this study, 103 *G. lamblia* positive and 97 negative fecal specimens were selected by microscopy. Rapid immunoassay (ImmunoCard STAT!) and real-time PCR were performed in a blinded study. ImmunoCard STAT! was used according to the manufacturer's instructions. PCR was targeted at the SSU rRNA gene as described by Verweij (JCM 42:3317-3320) and fecal DNA was extracted with a very efficient 'in-house' DNA extraction.

Results. The extended gold standard was defined as true positive with 2 out of 3 test positive. Microscopy and ImmunoCard STAT! showed 99 and 98% sensitivity and 97 and 100% specificity respectively. PCR showed 100% sensitivity, but only 92% specificity. When the cut off was set at a Ct of 35, specificity increased to 99%. False positive results in PCR were most likely caused by high *G. lamblia* DNA levels present in feces, since average Ct value of the true *G. lamblia* positive feces was 21.92 ± 4.32.

Conclusion. Microscopy, ImmunoCard STAT! and PCR all offer good sensitivity. Specificity of PCR can be increased by adjusting the threshold cycle for positive results to < 35. Microscopy will remain the primary tool for diagnosing parasitical gastro-enteritis, however the ImmunoCard STAT! may prove a valuable asset for detecting *G. lamblia* due to its speed and simplicity.

P68

Identifying genes involved in pore plugging of mechanically stressed *Rhizoctonia solan*i by subtractive hybridization

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Rhizoctonia spp. genus is a plant pathogenic fungus belonging to the basidiomycotina and has a wide host range, including more than 500 genera of higher plants. This fungus causes diseases in several important crops worldwide, infecting the seeds, roots, leaves, stems and fruits. The dolipore septum and the septal pore cap (SPC) of Basidiomycetes have become an organelle of taxonomic and phylogenetic interest. The objective of our research was the analysis of the SPC complex-related genes in *R. Solani*, which we think are involved in plugging the pore upon stress. As a first step towards a better understanding of the molecular basis of the pore-blocking mechanism, we applied the subtractive hybridization technique on mechanically stressed and non-stressed hyphae of *Rhizoctonia*. The RNAs were extracted immediately as well 15 minutes after mechanical stress. The differential clones generated by the subtractive hybridization technique were sequenced. Fifty two contigs and 155 singletons sequences were obtained in the first treatment (immediately) and 20 contigs and 40 singletons in the second treatment. Most of the sequences were hypothetical proteins in both treatments. Sequences homologous to ubiquitin, actin, glutathione transferase and glutamine synthetase were obtained after the first treatment and glyceraldehyde-3-phosphatase dehydrogenase and volvatoxin after the second treatment.

P69

Mutational changes in the pbp1A gene mediate amoxicillin resistance in *Helicobacter pylori*

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Introduction. Amoxicillin-based therapies are highly effective for the treatment of *Helicobacter pylori* infections, but this may decline as the incidence of amoxicillin resistance is currently increasing. So far, the molecular mechanism underlying amoxicillin resistance has been identified only for three amoxicillin-resistant (Amx^R) chinical *H. pylori* isolates. In these Amx^R *H. pylori* isolates the resistance is mediated by mutations in penicillin-binding protein 1A (PBP1A). In this study we have established the molecular mechanism underlying amoxicillin resistance in 11 additional Amx^R clinical isolates.

Methods. Clinical Amx^R *H. pylori* isolates (MIC 2-8 μg/ml) were obtained between 2000 and 2003 from dyspeptic

patients from Brazil (n=8) and the Netherlands (n=3). Amoxicillin-sensitive (Amx^S) *H. pylori* reference strain 26695 (MIC 0.125 µg/ml) was naturally transformed with total DNA and pbp1A PCR-products from the Amx^R *H. pylori* isolates, and the MIC of amoxicillin and *pbp1A* gene sequence of the obtained Amx^R transformants were determined.

Results. For all 11 Amx^R isolates, amoxicillin resistance could be transferred to Amx^S *H. pylori* strain 26695 by transformation with total DNA as well as with DNA of the pbp1A gene. Both DNA sources resulted in equal numbers of transformants with an MIC of amoxicillin ranging from 0.5 and 1.0 µg/ml. Sequence analysis of the smallest pbp1A gene fragments required to transfer amoxicillin resistance resulted in the identification of several mutational changes that are responsible for the resistance.

Conclusion. In naturally occurring Amx^R *H. pylori* isolates, amoxicillin resistance is mediated by various mutational changes in the pbp1A gene. Although we cannot exclude the role of the other genes in amoxicillin resistance, it is likely that multiple mutational changes in the pbp1A gene are the major contributing factor to this resistance. This currently precludes the detection of amoxicillin resistance in *H. pylori* by molecular tests.

P70
The effect of low level transient HIV viremia on the outcome of HAART

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Background. Transient low level viremia (TLLV) in patients treated with HAART may be a result of HIV release from latent reservoirs and ongoing low level virus production. We describe the effects of TLLV up to 1000 HIV RNA copies/ml plasma on the outcome of HAART.

Methods. From 1730 adult HIV-infected patients participating in the Dutch ATHENA national observational cohort with > 365 days follow-up and > 3 visits per year and initiating HAART in 1997-2003, 87 patients with TTLV, defined as 1-2 plasma viral load measurements between 50-1000 copies/ml, preceded by ¡Ã2 and followed by ¡Ã1 measurement ¡Â50 copies/ml were selected. 134 patients without TLLV were identified, maintaining a viral load ¡Â50 copies/ml continuously for three years. All selected patients had suppressed viral replication to ¡Â50 copies/ml within 26 weeks and were followed for at least 3 years. Mixed effects and Cox proportional hazards models were used to explore differences between groups.

Results. Among the 1730 patients, 6.3 blips per 100 person-years on HAART were found, with the highest rates (11.6) occurring in the first 2 years. Patients with and without TLLV were similar with regards to age, gender, transmission group, year of start HAART, time since diagnosis, pre-treatment, drug combination, $CD4^+$ T-lymphocyte (CD4) count, viral load and AIDS status at baseline. In 3 years, median CD4 counts rose from 140 (IQR: 40-310) to 430 cells/mm² (315-615) in patients with and from 180 (70-300) to 410 cells/mm² (310-580) in patients without TLLV. After 3 years, 4 patients (3%) without and 3 patients (4%) with TLLV showed viral rebounds to >1000 copies/ml. The hazard of a new AIDS-defining event did not differ between groups, when AIDS prior to start of HAART and baseline CD4 count were taken into account (HR=1.4, CI:0.60-3.4). All patients were still alive at the end of follow-up (205 weeks, IQR:177-242).

Conclusions. Patients who experienced low level transient viremia had a similar clinical outcome after three years of HAART as patients who maintained HIV-RNA levels < 50 copies/ml continuously.

P71
Evaluation of a commercial Pulsed Field Gel Electrophoresis kit compared with Amplified Fragment Lenght Polymorphism

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At present the Regional Public Health Laboratory for Groningen and Drenthe (SLGD) uses the Pulsed Field Gel Electrophoresis (PFGE) and Amplified Fragment Lenght Polymorphisms (AFLP) techniques during outbreaks. In the past the typing of gram negative bacteria, especially the typing of *Pseudomonas spp*. led to poor results. Even after optimization steps the results with *Pseudomonas* strains did not improve. To resolve this typing problem we evaluated Bio-Rads PFGE Genepath kit 3. This commercial kit is optimized for the typing of *Pseudomonas spp., Enterobacter spp.* and it is recommended for the typing of *Neisseria gonorrhoeae, Burkholderia cepacia* and *Serratia marcescens*. This kit has not been previously used in the Netherlands. To evaluate this kit we used isolates from clusters of hospital infections with *Pseudomonas spp., Enterobacter spp.* and *Serratia spp*. The relatedness of involved isolates was previously determined by AFLP, performed at an university hospital laboratory.

Isolates with proven involvement in clusters of HOSPITAL infection in the past were collected from our database. The samples comprised 12 isolates of *Pseudomas aeruginosa*, 5 isolates of *Enterobacter cloacae* and 6 isolates of *Serratia marcescens*. The isolates were processed according to factory instructions. The genomic restriction fragments were generated by the restriction enzyme SpeI and stained with an ethidiumbromide solution.

The guidelines proposed by Tenover et al. were used to interpret the results. The gels showed distinctive banding patterns with minimal smears. Differentiation of the banding patterns to identify the isolates could be realized without the need of statistical methods and equipment to digitize patterns. The PFGE showed the same relatedness between isolates as previously determined with AFLP. Furthermore

the costs to type a single isolate by AFLP and PFGE is respectively 75 and 60 Euro.
The implementation of Bio-Rads Genepath PFGE kit 3 proved to be both a discriminatory and reproducible strain typing technique for *Pseudomonas aeruginosa, Enterobacter cloacae* and *Serratia marcescens* in our laboratory and is a cost effective method compared to AFLP.

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