NEDERLANDS TIJDSCHRIFT VOOR

Medische Microbiologie

SUPPLEMENT BIJ ELFDE JAARGANG, APRIL 2003

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

Papendal, 15-16 april

Programma-overzicht

Abstracts

Auteursindex

Omsl 2

Advertentie

De voorjaarsbijeenkomst van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) vindt plaats op dinsdag 15 en woensdag 16 april 2003 te Papendal.

Gezien de positieve reacties van vele leden is gekozen voor een opzet die veel overeenkomst vertoont met de voorgaande bijeenkomst: een algemeen symposium (plenair) op dinsdagochtend met als thema 'Evolution', gevolgd door parallelsessies die overwegend thematisch zijn ingedeeld. Nieuw is een plenaire voordracht op de dinsdag voor de borrel: Co-evolution of fungi with other (micro-)organisms door J.W. Taylor van de Universiteit van California, Berkeley, Verenigde Staten. Tevens wordt in samenwerking met de Nederlandse Werkgroep Klinische Virologie een multidisciplinaire sessie georganiseerd rond ziekten van het centraal zenuwstelsel.

Ook dit jaar kan gebruik worden gemaakt van de 'Young Investigators Grant'.
AIO's die een presentatie houden worden vrijgesteld van het betalen van inschrijfkosten. Voor alle duidelijkheid: het betreft uitsluitend de presenterende auteur van een poster of een voordracht en alleen de inschrijfkosten komen te vervallen.

De Voorbereidingscommissie heeft zich unaniem uitgesproken voor het thema 'Evolution' en dit is niet verwonderlijk. Evolutie speelt een cruciale rol voor alle levende (micro)organismen. De evolutionaire processen hebben geresulteerd in de onmetelijke biodiversiteit zoals we deze dagelijks ervaren. Evolutie omvat het continue proces van aanpassing van micro-organismen aan hun dynamische omgeving. Deze omgeving kan de mens zijn die een infectie doormaakt, het rioolwater tijdens microbiologische zuivering, maar ook het fermentatievat voor de productie van levensmiddelen. Kortom, evolutie is een proces dat alle aanwezigen zal boeien.

Voorbereidingscommissie
Dr. P.W.M. Hermans, voorzitter
Dr. T. Boekhout
Dr. C.H.E. Boel
Prof. dr. S. Brul
Dr. R.J.A. Diepersloot
Mw. dr. L. Dijkshoorn
Prof. dr. J.M.D. Galama
Mw. drs. L.M. Kortbeek
Prof. dr. H.J. Laanbroek
Dr. J.A.G. van Strijp
Dr. P.E. Verweij
Prof. dr. E.J.H.J. Wiertz

Jury Yakult posterprijzen
Dr. J.G. Kusters, voorzitter
Dr. W. Crielaard
Mw. drs. L.M. Kortbeek
Dr. A.C.T.M. Vossen

De NVMM organiseert deze bijeenkomst in samenwerking met
Nederlandse Vereniging voor Microbiologie
Secties Algemene en Moleculaire Microbiologie, Microbiële Ecologie, en Technische Microbiologie en Mycologie
Sectie Algemene Virologie
Sectie Levensmiddelenmicrobiologie
Nederlandse Vereniging voor Medische Mycologie
Werkgemeenschap Microbiële Pathogenese
Werkgroep Epidemiologische Typeringen
Werkgroepen Oost, West en Noord Medische Microbiologie
Nederlandse Werkgroep Klinische Virologie
Stichting Kwaliteitsbewaking Medische Microbiologie

Congressecretariaat
Congress Care
Postbus 440
5201 AK 's-Hertogenbosch
Tel.: 073-683 12 38
Fax: 073-690 14 17
E-mail: info@congresscare.com
Internet: www.congresscare.com

Accreditatie
De Wetenschappelijke Voorjaarsvergadering 2003 is door de NVMM geaccrediteerd met 5 punten per dag.

Sponsors en exposanten

3W Informed

Abbott

Aventis

Bayer Diagnostica

Becton Dickinson Diagnostics

Bio Rad

Bio Trading Benelux

Biomedical Diagnostics

bioMerieux

Biotest Seralco

Bipharma Diagnostics

Clean Air Techniek

Clindia Benelux

Dade Behring

ELISA kits + accessoires
- Adenovirus
- Amplified Astrovirus **NIEUW**
- Rotavirus
- Norwalk Like Virus **NIEUW**
- Helicobacter Pylori Antigeen **NIEUW**
- PCE Chlamydia Antigeen
- Herpes Simplex Virus [HSV]
- Lyme Borrelia IgG/M/Neuro

BLOT's
- Lyme Borrelia IgG
- Lyme Borrelia IgM

Specifieke Antilichamen

Direct Antigeen Detectie [IF=IMAGEN]
- Adenovirus
- Chlamydia
- Chlamydia Pneumoniae
- Cytomegalovirus [CMV]
- Enterovirus
- Herpes Simplex Virus [HSV]
- Influenza virus A an B
- Parainfluenza Groep
- Parainfluenza typering 1, 2 en 3
- Respiratory Screen [7 virussen]
- Respiratory Syncytial Virus [RSV]

DakoCytomation Benelux – Interleuvenlaan 12B – 3001 Heverlee [België]
Tel.: +31 20 42 11 100 – Fax: +31 20 42 11 101 – E-mail: info@dakocytomation.nl – www.dakocytomation.nl

Diagnostics Products Corporation

Fornix Theranostics

Gilead Sciences

IQ Products

ICN Pharma

ITK Diagnostics

Lucron Bioproducts

Mediphos Medical Supplies

Merck Sharp & Dohme

Meridian Bioscience

Micronic

Minigrip

MP Products

Necontra

Oxoid

Pharmacia

Roche Diagnostics

Snijders Scientific

Technidata

Tritium Microbiologie

Uniprom

VIATRIS

VIATRIS B.V.
Verrijn Stuartweg 60
1112 AX DIEMEN
Tel: 020 – 5198300
Fax: 020 – 6906435
E-mail: info@viatris.nl
www.viatris.nl

VIATRIS B.V. is de producent van o.a. Betadine®, Ascal®, MS Contin®, Tramadol®, Sevredol®, X-Praep®, Inhibin® en Dagravit®.

Wyeth

ZIRBUS technology

Productenpakket
o – Autoclaven
o – > 100 liter Autoclaven
o – Draai Autoclaven
o– Hogedrukautoclaven
o – Testautoclaven
o – Stoomgenerators
o – Mediabereiders
o – Pasteurisatie systemen
o – Retro-fit en specials

o – Laboratorium Vriesdrogers
o – Productie Vriesdrogers
o – Sterilisatoren
o – (Vacuüm)droogsterilisatoren
o – Vacuümdroogstoven
o – Roterendevacuümconcentrator
o – Temperatuur kasten (met ramping)
o – Expl.-vrije koelkasten, vriezers
o – Retro-fit en specials

o – Miele professional desinfectieautomaten
o – Miele professional reinigingsautomaten
o – Neodisher reinigingsmiddelen

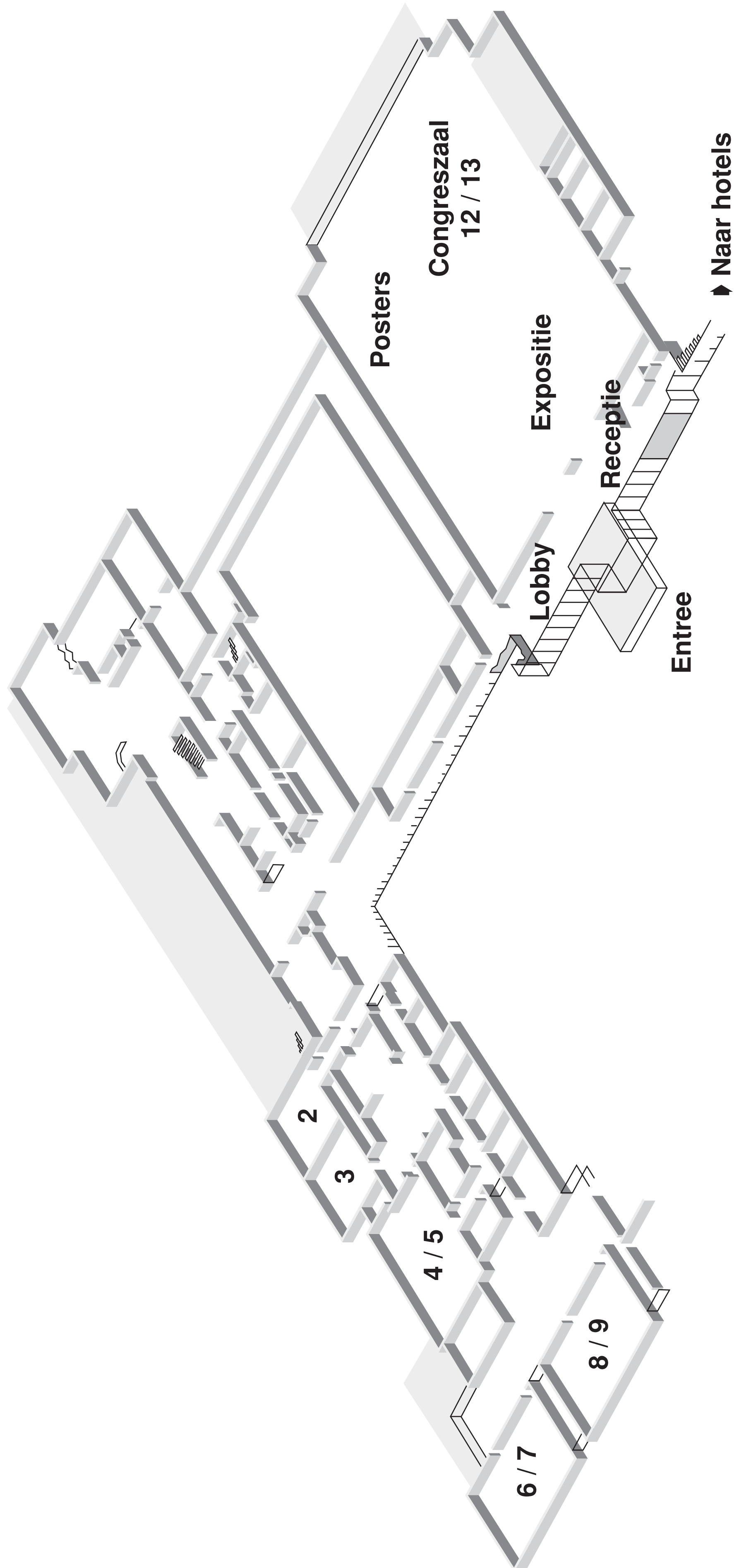
Korte beschrijving:
Zirbus Technology heeft een uitgebreid standaard productenpakket van goede kwaliteit. Naast het standaardpakket worden er producten geleverd die aansluiten bij specifieke wensen. Zirbus Technology kan in onderling overleg u een oplossing op maat bieden.

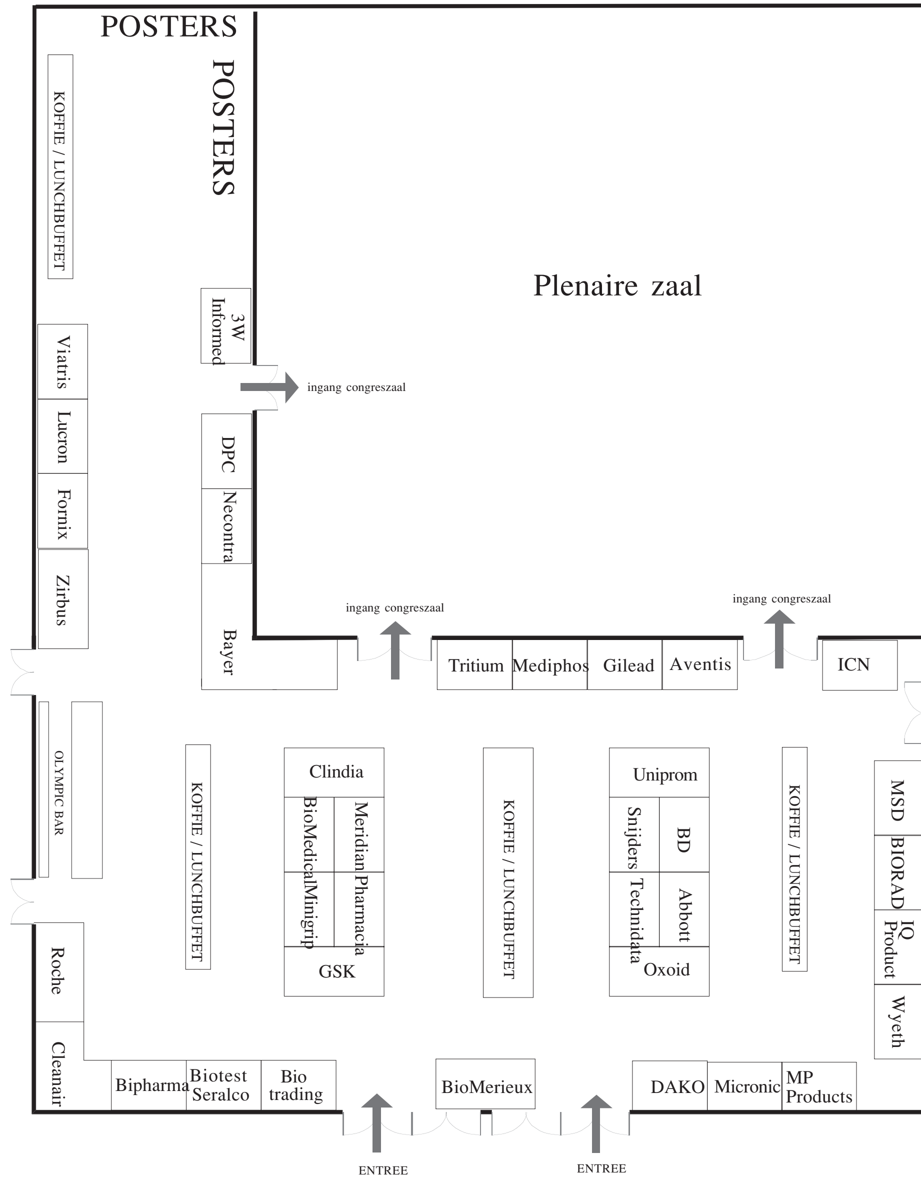
Zirbus Technology Benelux BV
Postbus 22
4153 ZG BEESD
Tel. 0345 684975
Fax 0345 684975
Website: WWW. Zirbus.DE

Sponsor posterprijzen

Yakult

Sponsors


Congreszaal
12 / 13
Posters
Expositie
Receptie
Lobby
Entree
Naar hotels
2
3
4 / 5
6 / 7
8 / 9


POSTERS
POSTERS
KOFFIE / LUNCHBUFFET
Plenaire zaal
3W Informed
ingang congreszaal
Viatris
Lucron
Fornix
Zirbus
DPC
Necontra
Bayer
ingang congreszaal
ingang congreszaal
Tritium
Mediphos
Gilead
Aventis
ICN
OLYMPIC BAR
KOFFIE / LUNCHBUFFET
Clindia
BioMedical
Meridian
Minigrip
Pharmacia
GSK
KOFFIE / LUNCHBUFFET
Uniprom
Snijders
BD
Technidata
Abbott
Oxoid
KOFFIE / LUNCHBUFFET
MSD
BIORAD
IQ Product
Wyeth
Roche
Cleanair
Bipharma
Biotest Seralco
Bio trading
BioMerieux
DAKO
Micronic
MP Products
ENTREE
ENTREE

DINSDAG 15 APRIL 2003

Tijd	Programma		Zaal
08:30 - 09:30	Registratie en koffie/thee		
09:30 - 11:00	Symposium 'Evolution'	A	12/13
11:00 - 11:30	Koffie/thee		
11:30 - 13:00	Vervolg symposium	A	12/13
13:00 - 14:00	Lunchsymposium Fornix Theranostics 'Extended spectrum beta-lactamases'		12/13
	Beroepenbelangencommissie niet-registerleden		2
14:00 - 15:15	Parallelsessies:		
	Epidemiology 1	B	4/5
	Diagnostics 1	C	6/7
	Pathogenesis 1	D	8/9
	Microbiology in progress 2003	E	12/13
	Medical mycology	F	2
	Food microbiology	G	3
15:15 - 15:45	Koffie/thee		
	Vergadering werkgroepleiders Microbiële Pathogenese		8/9
15:45 - 17:15	Parallelsessies:		
	Pathogenesis 1 (vervolg)	D	8/9
	Microbiology in progress 2003 (vervolg)	E	12/13
	Medical mycology (vervolg)	F	2
	Food microbiology (vervolg)	G	3
	Virology	H	4/5
	Case presentations	J	6/7
17:15 - 18:00	Plenaire sessie	A	12/13
18:00 - 18:30	Borrel		
18:30 - 20:00	Diner		
20:00 - 22:00	Postersessie en uitreiking Yakult posterprijzen	P	11
	Vergadering werkgroepleiders NVvM		2

WOENSDAG 16 APRIL 2003

			ZAAL
07:30 - 08:45	Ontbijtsymposium Roche Diagnostics		12/13
08:30 - 09:00	Algemene vergadering SKMM	Q	3
09:00 - 10:30	Parallelsessies:		
	Workgroup epidemicity markers and bacterial typing (WET)	K	2
	Diseases of the central nervous system	L	6/7
	Education	M	8/9
	Microbiology in progress 2003	N	12/13
	SKMM	Q	3
10:30 - 11:00	Koffie/thee		
11:00 - 12:15	Parallelsessies:		
	Workgroup epidemicity markers and bacterial typing (WET)	K	2
	Diseases of the central nervous system (vervolg)	L	6/7
	Education (vervolg)	M	8/9
	Microbiology in progress 2003 (vervolg)	N	12/13
	Therapy	R	3
	Pathogenesis 2	S	2
12:15 - 14:00	Lunchsymposium Pharmacia		12/13
14:00 - 15:30	Parallelsessies:		
	Diseases of the central nervous system (vervolg)	L	6/7
	Microbiology in progress 2003 (vervolg)	N	12/13
	ICT in de medische microbiologie	T	4/5
	Epidemiology 2	V	8/9
	Diagnostics 2	W	2
15:30 - 16:00	Koffie/thee		
16:00 - 18:00	Ledenvergadering NVMM		12/13
	Vergadering bestuur NVvM		10a
18:00	Sluiting		

DINSDAG 15 APRIL 2003

A Zaal 12/13	**Symposium 'Evolution'**	
	Voorzitter: S. Brul	
09:30 - 10:15	G. S. Wain-Hobson (Paris, France) Virus variation and evolution	
10:15 - 11:00	G.S. de Hoog (Utrecht) Selection of potential fungal agents of bioterrorism using evolutionary criteria	A02
11:00 - 11:30	Koffie/thee	
	Voorzitter: F.R. Mooi	
11:30 - 12:15	A. Buckling (Bath, UK) Experimental coevolution	A03
12:15 - 13:00	N.A. Moran (Tucson, USA) Genome reduction in bacterial pathogens and symbionts	A04
Zaal 12/13	**Lunchsymposium Fornix Theranostics 'Extended spectrum beta-lactamases'**	
12:45 - 13:05	T.R. Walsh ESBLs, AmpC metallo beta-lactamases and other enzymes mocking beta-lactam therapy	
13:05 - 13:25	J.P. Arends Extended spectrum beta-lactamases in het Academisch Ziekenhuis Groningen	
13:25 - 13:45	M.A. Leverstein-van Hall Evaluation of the Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 Automated Instruments for Detection of Extended-Spectrum Beta-lactamases (ESBLs) in Multiresistant *Escherichia coli* and *Klebsiella* species	
B Zaal 4/5	**Epidemiology 1**	
	Voorzitter: A. Voss	
14:00 - 14:15	F.R. Mooi, C. Heuvelman, A. King, M. Hijnen, H.G.J. van der Heide, I. van Loo, L.M. Schouls, G. Berbers Evolution of the human pathogen *Bordetella pertussis*: the role of vaccination	B01
14:15 - 14:30	D.A. Diavatopoulos, M. Arnold, H.G.J. van der Heide, M.C. Maiden, F.R. Mooi Evolution and host adaptation of the *Bordetella* genus	B02

14:30 - 14:45	H.F.L. Wertheim, M.C. Vos, A. Ott, A. Voss, J.A.J.W. Kluytmans, C.M.J.E. Vandenbroucke-Grauls, M.H.M. Meester, P.H.J. van Keulen, H.A. Verbrugh Low prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in hospital admissions in the Netherlands	B03
14:45 - 15:00	H.L. Zaaijer, M.H.G.M. Koppelman Genetic diversity and origin of hepatitis B virus in Dutch blood donors	B04
15:00 - 15:15	A. Troelstra, L.A.M. de Graaf-Miltenburg, H.E.M. Blok, T.W.J. Schulpen Prevalence of multiresistant micro-organisms among adopted children in the Netherlands	B05

C Zaal 6/7 — Diagnostics 1

Voorzitter: J. Verhoef

14:00 - 14:15	A.M.C. Bergmans, L.M. Schouls, R.G.F. Wintermans Validation of a fast molecular method for detection and identification of dermatophytes in nail and skin samples	C01
14:15 - 14:30	I.J.B. Spijkerman, J.J. Verweij, B. van Hoek, L. van Lieshout Real-time polymerase chain reaction (PCR) for diagnosis and monitoring of *Toxoplasma gondii* infection	C02
14:30 - 14:45	H.R. van Doorn, E.C.J. Claas, K.E. Templeton, A.G.M. van der Zanden, A. te Koppele-Vije, M.D. de Jong, J. Dankert, E.J. Kuijper A real-time PCR using 3'-minor groove binder-DNA probes for detection in clinical samples of an SNP associated with high level isoniazid resistance in *Mycobacterium tuberculosis*	C03
14:45 - 15:00	B. Roerig, H. Klip, M.C.J. Persoons, A.A. van Zwet The use of Procalcitonin (PCT) serum level for the diagnosis of bacteraemia	C04
15:00 - 15:15	C.M.C. van Herk, I.M. Slootjes, G.H.W. Onland, A.J.C. van den Brule, C.H.E. Boel Evaluation of four confirmation assays for AMPLICOR *Neisseria gonorrhoeae* PCR using conventional and real-time PCR with *cppB* and 16S rRNA as targets	C05

D Zaal 8/9 — Pathogenesis 1

Voorzitter: J.G. Kusters

14:00 - 14:15	A.H.M. van Vliet, A. Heijens, S.W. Poppelaars, J. Stoof, E.J. Kuipers, J.G. Kusters *Helicobacter pylori* is sensitive to nickel only at acidic pH	D01
14:15 - 14:30	J. Gooskens, A.J. de Neeling, R.J.L. Willems, E.J. Kuijper Streptococcal toxic shock syndrome caused by a MLS resistant M type 77 *Streptococcus pyogenes* carrying the *erm*TR gene: a retrospective analysis among M77 isolates collected in the Netherlands	D02

14:30 - 14:45	C.A. Bruggeman, R.R. Ezzahiri, F.R.M. Stassen, M.P.J. de Winther, H.A.J.M. Kurvers, S.B. Herngreen, P.J.E.H.M. Kitslaar Dissemination of *Chlamydia pneumoniae*: a role for bone marrow-derived monocytes/macrophages?	D03
14:45 - 15:00	M.P. Bergman, A. Engering, J.M. Maaskant, B. de Goeij, J. Stoof, H.P. Wirth, C.M.J.E. Vandenbroucke-Grauls, Y. van Kooyk, B.J. Appelmelk *Helicobacter pylori* escapes DC-SIGN mediated dendritic cell function by phase variation in lipopolysaccharide Lewis x and y blood group antigens	D04
15:00 - 15:15	K. Huijsdens-van Amsterdam, A. van der Ende (Amsterdam) *Helicobacter pylori ylxH* (HP1034) is essential for motility	D05
15:15 - 15:45	Koffie/thee	
	Voorzitter: S.A.J. Zaat	
15:45 - 16:00	A. Meijer, J.H. Siekman, G. Celik, S.K. Gielis-Proper, M.C. Burger, P.J.M. Roholl, J.M. Ossewaarde Characterisation of an *in vitro* model for chronic infection with *Chlamydophila pneumoniae*	D06
16:00 - 16:15	J. Piet, A. van der Ende, J. Dankert, A. Bart Diversity of *vex* and *vnc* genes in streptococci	D07
16:15 - 16:30	F.D. Ernst, J. Stoof, B. Waidner, A.H.M. van Vliet, M. Kist, J.G. Kusters, S. Bereswill, G. Homuth Identification of fur- and iron-regulated genes of *Helicobacter pylori* using whole-genome DNA array analysis	D08
16:30 - 16:45	A.H.M. van Vliet, J. Stoof, S.W. Poppelaars, A. Heijens, E.J. Kuipers, J.G. Kusters Acid- and nickel-responsive transcriptional induction of ammonia-producing enzymes in *Helicobacter pylori*	D09
16:45 - 17:00	S. Kuipers, P.C. Aerts, T. Harmsen, H. van Dijk Micro-organism-induced mannose-binding lectin (MBL) activation	D10
17:00 - 17:15	M.W.J. van Passel, A. Bart, A. van der Ende Identification of virulence-related genes in commensal *Neisseriae*	D11

E Zaal 12/13 **Microbiology in progress 2003**

	Voorzitter: R. Laanbroek	
14:00 - 14:15	I. Schmidt, K.T. van de Pas-Schoonen, M. Strous, H.J.M. op den Camp, J.G. Kuenen, M.S.M. Jetten Anaerobic ammonia oxidation in the presence of nitrogen oxides by two different lithotrophs	E01
14:15 - 14:30	H.J.E. Beaumont, H.V. Westerhoff, R.J.M. van Spanning The denitrification enzymes of the nitrifying bacterium *Nitrosomonas europaea*: familiar players in a new game	E02
14:30 - 14:45	W.A. van Winden, J.C. van Dam, C. Ras, W.M. van Gulik, J.J. Heijnen Direct analysis of mass isotopomers of most primary metabolites in *Saccharomyces cerevisiae* using LC-MS	E03

14:45 - 15:00	M.J.M. Wagemaker, C. van der Drift, L.J.L.D. van Griensven, M.S.M. Jetten, H.J.M. op den Camp Biochemical and molecular characterisation of arginase from *Agaricus bisporus*	E04
15:00 - 15.15	W.C. van Heeswijk, D.J. Kiviet, K.J. Hellingwerf Hierarchy in the adaptation of *Bacillus subtilis* to nitrogen starvation	E05
15:15 - 15:45	Koffie/thee	
	Voorzitter: R. Laanbroek	
15:45 - 16:00	L.A. van Niftrik, M. Strous, J.G. Kuenen, M.S.M. Jetten, J.A. Fuerst Compartmentalisation in *Candidatus* 'Brocadia anammoxidans'	E06
16:00 - 16:15	M.H.J. Sturme, E.E. Vaughan, M. Kleerebezem, A.D.L. Akkermans, W.M. de Vos Identification and analysis of quorum sensing two-component regulatory systems in the human isolate *Lactobacillus plantarum* WCFS1	E07
16:15 - 16:30	B. Boxma, J. Tjaden, F. Voncken, J.H.P. Hackstein The evolution of hydrogenosomes	E08
16:30 - 16:45	Y. Li, A. Felske, W.M. de Vos, E.E. Vaughan, A.D.L. Akkermans Characterisation of the predominant *Bacillus* BACREX cluster species in soil by cultivation and 16S rDNA analyses	E09
16:45 - 17:00	L. Wu, H.H.J. Bloemen, W.M. van Gulik, M.H.G. Verhaegen, J.J. Heijnen Reconstruction of the O2 uptake rate and CO2 evolution rate on a time scale of seconds	E10
17:00 - 17:15	W.M. van Gulik, L. Wu, M.R. Mashego, J.C. van Dam, C. Ras, A. Proell, J.L. Vinke, W.A. van Winden, J.J. Heijnen Development of methods for the *in vivo* kinetic modelling of the metabolism of *Saccharomyces cerevisiae*	E11

F Zaal 2 — Medical mycology

	Voorzitter: S. de Hoog	
14:00 - 14.15	J. Dijksterhuis Confocal microscopy of Spitzenkörper dynamics during growth and differentiation of rust fungi	
14:15 - 14:30	F.B.J.M. Thunnissen, G.S. de Hoog, J.F.G.M. Meis, H.E. Viëtor The systemic mycosis array test (SMART)	F02
14:30 - 14:45	G. Haase, H. Stender Labelled peptide nucleic acids (PNA) as species-specific probes for fluorescence in situ hybridisation (FISH) enabling an easy-to-perform identification of fungi in blood cultures	F03
14:45 - 15:15	P.M. Ellerbroek, A.M. Hoepelman, F. Wolbers, F.E.J. Coenjaerts Cryptococcal glucuronoxylomannan (GXM) inhibits adhesion of polymorphonuclear leukocytes (PMN) to stimulated endothelium *in vitro* by affecting both PMN and endothelial cells in both static and dynamic adhesion models	F04-05
15:15 - 15:45	Koffie/thee	

15:45 - 16:00	T. Boekhout, V. Robert, J. Stalpers, G. Gijswit, C.P. Kurtzman, J.W. Fell, I. Roberts Yeasts of the world, an interactive CD-ROM	F06
16:00 - 16:15	H.J. Deelstra, R. Bohlmann, J. Dijksterhuis, R. Kahmann, H.A.B. Wösten Repellents of the phytopathogenic fungus *Ustilago maydis*	F07
16:15 - 16:45	M.C. Fisher Population genetics of the AIDS-associated fungus, *Penicillium marneffei* in South East Asia	F08-09

G Zaal 3 **Food microbiology** G01-09

Voorzitter: S. Brul

14:00 - 14:30	M. Zwietering How can quantitative methods be used for the control of the safety of food?	
14:30 - 15:00	S. Brul Genomics: new possibilities in food microbiology	
15:15 - 15:45	Koffie/thee	
15:45 - 16:15	T. Abee The impact of stress on the activity (survival, virulence) of unwanted food-borne micro-organisms	
16:15 - 16:45	S. Notermans New developments in the production of safe food products	

H Zaal 4/5 **Virology**

Voorzitter: J.M.D. Galama

15:45 - 16:00	M.P.G. Koopmans, B. Lopman, E. Kohli, E. Schreier, B. Bottiger, L. Svensson, C. von Bonsdorff, Y. Duijnhoven, E. van Strien, H. Vennema Recent surge in outbreaks of viral gastro-enteritis in Europe may be related to epidemic spread of a new Norovirus variant	H01
16:00 - 16:15	I. Vliegen, S.B. Herngreen, G. Grauls, S. Stevens, C.A. Bruggeman, F.R.M. Stassen Genotype differences determine *cytomegalovirus* dissemination in the mouse	H02
16:15 - 16:30	K. Waar, M.P.G. Koopmans, C.A. Benne Hepatitis E in the north-eastern part of the Netherlands, a retrospective study	H03
16:30 - 16:45	J. Schinkel, M.J.D. van Tol, A.C.M. Kroes, W. Dinkelaar, C.M. Jol-van der Zijde, J.M. Vossen Risk factors for adenovirus infection and death in paediatric stem cell transplant recipients	H04
16:45 - 17:00	M. Nijhuis, R. Schuurman, D. de Jong, P. Schipper, C.A.B. Boucher Fitness and evolution of *Human Immunodeficiency Virus* during antiretroviral treatment	H05

17:00 - 17:15	A. Meijer, E.E.H.M. van de Kamp, G. Koch, T.G. Kimman Cell-ELISA for antiviral susceptibility testing of influenza virus: performance depends on the compatibility of virus strain and type of MDCK cells	H06

J Zaal 6/7 **Case presentations**

Voorzitter: J. Degener

15:45 - 16:00	M.W.H. Wulf, X.R. Bakker, P.H.M. Spauwen, T. Schülin Actinomycetoma of the thumb caused by Gordona terrae	J01
16:00 - 16:15	F. Bosma, M.W.H. Wulf, C. Smeets, M. Pruszczynski, C.M.E. Weemaes, J.M.D. Galama Pseudotumor of the right upper arm due to *Bartonella henselae* infection	J02
16:15 - 16:30	E. van Duijkeren, A.T.A. Box, W. Wannet, J.A.H. Smit, A.C. Fluit First report on methicillin resistant staphylococci from animals in the Netherlands	J03
16:30 - 16:45	F.G.C. Heilmann, H.M. Bruns, H. van Dessel, J. Kissing, J.F.P. Schellekens *Borrelia burgdorferi*-associated lymphocytoma cutis of the glans penis simulating a primary cutaneous B-cell lymphoma	J04
16:45 - 17:00	J.A. Wagenaar, C. Appels, A.H.W. Schoormans, E.A.P.M. Thewessen, T. Koster Brucellosis in a game butcher: need for reliable subspeciation to trace the infection	J05
17:00 - 17:15	J.A. Jacobs, P.L.J.M. Leroy, A.W.D. Gavilanes, N. London, C. Driessen, C. Vink Clonal identity of *Staphylococcus aureus* isolates in repeat bacteraemia, demonstrated by pulsed-field gel electrophoresis	J06

A Zaal 12/13 **Plenaire sessie**

17:15 - 18:00	J.W. Taylor (Berkeley, California, USA) Co-evolution of fungi with other (micro-)organisms	A05

P Zaal 11 **Postersessie en uitreiking Yakult posterprijzen**

20:00 - 20:45	Posterpresentaties oneven posternummers
20:45 - 21:30	Posterpresentaties even posternummers
22:00	Uitreiking posterprijzen

WOENSDAG 16 APRIL 2003

Zaal 12/13 07:30 - 08:45 — **Ontbijtsymposium Roche Diagnostics**

K Zaal 2 — **Workgroup epidemicity markers and bacterial typing (WET)** K01-10

Voorzitters: L. Dijkshoorn

09:00 - 09:45 P. Vandamme (Universiteit Gent)
Polyphasic taxonomy of *Burkholderia cepacia* complex

09:45 - 10:15 P. Savelkoul (Vrije Universiteit)
Database construction & the need for exchance of microbial typing information

10:15 - 10:30 B. Duim
Book presentation

10:30 - 11:00 Koffie/thee

Voorzitter: P. Hermans

11:00 - 11:30 M. Koopmans (RIVM)
Molecular tools to understand enteric virus transmission: methods and their application

11:30 - 12:00 J. Rademaker (NIZO)
Diversity and indentification screens using pcr-fingerprinting and ribosomal PNA sequening

L Zaal 6/7 — **Diseases of the central nervous system: a multidisciplinary approach**
Interactieve sessie in het Nederlands

Voorzitters: P.H. Rothbart en C.M.A. Swanink

09:00 - 09:45 M. Keuter
Presentatie van een casus waarbij uw actieve deelname wordt gevraagd. U kunt door het uitbrengen van uw stem uw visie geven op vragen die gesteld worden naar aanleiding van diagnostiek en behandeling van de casus

09:45 - 10:30 P. Portegies
Ontsteking binnen het Centraal zenuwstelsel en de klinische differentiaaldiagnose voor infectieziekten

10:30 - 11:00 Koffie/thee

11:00 - 11:20 J. Galama
Serologische liquor diagnostiek

11:20 - 11:40 P.M. Schutten
Moleculaire liquor diagnostiek

11:45 - 12:00 M.W.H. Wulf, R. van Crevel, A. van der Ven, J.M.D. Galama L10
Primary toxoplasmosis in a renal transplant patient

12:00 - 12:15	S. Kuipers, J.J.W. Prick, H.D.R. Kuipers, J.H.R. Vliegen, G.J.J. van Doornum West Nile virus encephalitis in an elderly Dutch patient	L11

Zaal 12/13 — **Lunchsymposium Pharmacia**
12:15 - 14:00

L Zaal 6/7 — **Diseases of the central nervous system: a multidisciplinary approach Interactieve sessie in het Nederlands (vervolg)**

14:00 - 14:15	M.P.G. Koopmans, K. van den Wijngaard, W. van Pelt, A. Bosman Response to the West Nile virus threat in the Netherlands	L12
14:15 - 14:30	J.H. van Zeijl, B. Wilbrink, J.M.D. Galama Febrile seizures and viral infections: a case presentation	L13
14:30 - 14:45	A. van Griethuysen, A. Rolink, C. Richter, L. Verschoor, C. Swanink A patient with pain in the back. Pain everywhere	L14
14:45 - 15:00	B.U. Ridwan, C.A.B. Boucher, R. Schuurman, M. Schneider A HIV patient with neurological deterioration and discrepant viral load between plasma and CSF	L15
15:00 - 15:15	F. Bosma, S. van Assen, L.M.E. Staals, W.J.G. Melchers, B.J. Kullberg, M. Lammens, P. Vos, B.G. Fikkers, J.M.D. Galama *Borrelia burgdorferi*-associated acute disseminated encephalomyelitis	L16

M Zaal 8/9 — **Education** — M01-11

Voorzitter: L. Dijkshoorn

09:00 - 09:30	L. van de Grint (Vrije Universiteit) Nieuwe onderwijsvormen en ICT: Implementatie van een digitale leeromgeving bij faculteit aard- en levenswetenschappen
09:30 - 10:00	N. Harms (Vrije Universiteit) Samenwerkend leren en ICT in de cursus celbiologie
10:00 - 10:30	E. de Groot, E. Langewis (Universiteit Utrecht) Inzet ICT bij het veterinair en biologisch onderwijs
10:30 - 11:00	Koffie/thee
11:00 - 11:30	P. Schaap (Wageningen Universiteit) Bioinformation Technology, a hands on course at Wageningen University
11:30 - 12:15	De sessie eindigt met een spel, 'Genomics, wie wint de biologie Nobelprijs?'

N Zaal 12/13 — **Microbiology in progress 2003**

Voorzitter: L. Dijkhuizen

09:00 - 09:15	D. Claessen, D. Jager, C. Leeflang, F. Goedegebuur, H.J. Deelstra, N.A. Penninga, C. Bormann, J. Salas, L. Dijkhuizen, H.A.B. Wösten Rodlins are involved in but not sufficient for assembly of the streptomycete rodlet layer	N01

09:15 - 09:30	M.R. Mashego, M.L.A. Jansen, W.M. van Gulik, J.T. Pronk, J.J. Heijnen Long-term aerobic glucose-limited chemostat cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D	N02
09:30 - 09:45	R.J.H.M. van der Straaten, C.M. Janssen, D.T. Mevius, J.T. van Dissel The *ramA* gene is not involved in multidrug-resistant *Salmonella typhimurium*	N03
09:45 - 10:00	I. Cirpus, M.C. Schmidt, I. Schmidt, H.J.M. op den Camp, D. Lepaslier, J. Weissenbach, M. Wagner, J.G. Kuenen, M. Jetten, M. Strous Cytochromes *c* of *Candidatus* 'Kuenenia stuttgartiensis'	N04
10:00 - 10:15	K. Ben-Amor, I.G.A. Heikamp-de Jong, S. Verhargh, A.D.L. Akkermans, W.M. de Vos, E.E. Vaughan Assessment and evaluation of the effects of probiotics on faecal microbiota of patients with inflammatory bowel disease	N05
10:15 - 10:30	S. Brul, K.J. Hellingwerf, W. Crielaard Development and use of a *pspA*-based reporter system for screening the mode of action of natural preservatives	N06
10:30 - 11:00	Koffie/thee	
11:00 - 11:15	P.W.J.J. van der Wielen, J.K. Brons, H. Bolhuis Novel archaeal and bacterial divisions from mediterranean deep hypersaline anoxic basins	N07
11:15 - 11:30	A.P.H.M. Hermans, T. Abee, H.J.M. Aarts Gene expression profiles of acid tolerant *Salmonella typhimurium* DT104 isolates	N08
11:30 - 11:45	I. Janse, M. Meima, M.P. Kamst-van Agterveld, E. Kardinaal, G. Zwart Population dynamics, toxin induction and early detection of toxic cyanobacteria (DYNATOX)	N09
11:45 - 12:00	T. Kaper, M. Habets, J. van Munster, T. Ettema, M.J.E.C. van der Maarel, L. Dijkhuizen Extreme thermoactive amylomaltase from *Pyrobaculum aerophilum* IM2	N10
12:00 - 12:15	S. Kralj, G.H. van Geel-Schutten, M.J.E.C. van der Maarel, L. Dijkhuizen Characterisation of a glucansucrase gene from *Lactobacillus reuteri* strain 121	N11

Zaal 12/13 12:15 - 14:00 — **Lunchsymposium Pharmacia**

N Zaal 12/13 — **Microbiology in progress 2003 (vervolg)**

Voorzitter: W. de Vos

14:00 - 14:15	R. ten Have, G. Straatsma, P.J. Schaap Analysis of the extracellular proteome of *Agaricus bisporus*	N12
14:15 - 14:30	A. ter Beek, K.J. Hellingwerf, S. Brul Molecular characterisation of the general stress response of *Bacillus subtilis*	N13

14:30 - 14:45	G. Zwart, M.P. Kamst-van Agterveld, E. van Hannen, K. van der Gucht, E.S. Lindström, T. Lauridsen, S. Declerck Typical freshwater bacteria	N14
14:45 - 15:30	G. Muyzer The need of a polyphasic approach in microbial ecology	N15-17

Q Zaal 3 **Stichting Kwaliteitsbewaking Medische Microbiologie (SKMM)**

Voorzitter: L. Sabbe

08:30 - 09:00	Algemene vergadering	
09:00 - 09:45	F. Vlaspolder Richtlijn mycobacteriële diagnostiek	
09:45 - 10:30	D. van Soolingen De secundaire laboratoriumdiagnostiek van tuberculose	Q04-06

R Zaal 3 **Therapy**

Voorzitter: C.M Verduin

11:00 - 11:15	H.F. Berg, J.H.T. Tjhie, E.E. Stobberingh, M.F. Peeters, P.H.J. van Keulen, J.A.J.W. Kluytmans Effects of clarithromycin on oropharyngeal and nasal flora: a double-blind placebo-controlled study	R01
11:15 - 11:30	M.I.A. van der Kraan, R. de Bruijn, J. Groenink, J.G.M. Bolscher, E.C.I. Veerman, A.V. Nieuw Amerongen Antimicrobial peptides derived from bovine milk proteins	R02
11:30 - 11:45	R.P. Rietveld, J.H. Sloos, H.C.P.M. van Weert, P.J.E. Bindels Low susceptibility to antibiotics of the causing agents of bacterial conjunctivitis in primary care	R03
11:45 - 12:00	I.H. Bartelink, G.J. van Asselt, J.W.P.M. Overdiek, P.A.M.M. Boermans, P.M. Oostvogel, H.J. ter Horst, R.J.M. Brüggemann Evaluation and optimisation of antibiotic treatment in a Dutch teaching hospital	R04
12:00 - 12:15	M.M. Gerrits, M. Berning, A.H.M. van Vliet, E.J. Kuipers, J.G. Kusters Effects of 16S rRNA gene mutations on tetracycline resistance in *Helicobacter pylori*	R05

S Zaal 2 **Pathogenesis 2**

Voorzitter: J.A.G. van Strijp

11:00 - 11:15	A. van Diepen, J.S. van de Gevel, H. Beekhuizen, R. Janssen, J.T. van Dissel Chronic persistence and reactivation of *Salmonella typhimurium* infection in mice	S01
11:15 - 11:30	M. Llamas, W. Bitter Trans-cell envelope signalling in *Pseudomonas aeruginosa*	S02

11:30 - 11:45	R. de Jonge, R.G.J. Pot, R.J.L.F. Loffeld, A.H.M. van Vliet, E.J. Kuipers, J.G. Kusters The functional status of the putative *Helicobacter pylori* adhesin *sabB* as a marker for clinical outcome	S03
11:45 - 12:00	B.J. Appelmelk, T.L. Lowary, T. Geijtenbeek, C.H. Hokke, Y. van Kooyk, R.R. Gadikota, W. Bitter, C.M.J.E. Vandenbroucke-Grauls The mycobacterial surface glycolipid lipoarabinomannan (LAM) binds to dendritic cells through its mannose caps and downregulates DC via the DC-SIGN	S04
12:00 - 12:15	A.M. van der Sar, R.J.P. Musters, F. van Eeden, C.M.J.E. Vandenbroucke-Grauls, W. Bitter Zebrafish embryos as a model for the real time analysis of *Salmonella typhimurium* disease development	S05

T Zaal 4/5 — ICT in de medische microbiologie

Voorzitter: C.H.E. Boel

14:00 - 14:30	G.D. Krediet Medische microbiologie en EPD: nieuwe kansen in een virtuele wereld	
14:30 - 15:00	P.A. de Clercq, C.H.E. Boel, H.H.M Korsten Toepassing van beslissingsondersteuning in de medische microbiologie: ontwikkeling van een aanvraagmodule	T03-04
15:00 - 15:30	F.A. van Lierop Spraakherkenning en medische microbiologie	

V Zaal 8/9 — Epidemiology 2

Voorzitter: H.A. Verbrugh

14:00 - 14:15	N. Al Naiemi, B. Duim, J.E.M. de Bruijn, P.H.M. Savelkoul, L. Spanjaard, E. de Jonge, J. Dankert, A. Bart, M.D. de Jong Increased prevalence of multiresistant *Enterobacteriaceae* during an *Enterobacter cloacae* outbreak: coincidence or transfer of resistance genes?	V01
14:15 - 14:30	W.C. van der Zwet , A.M. Kaiser, R.M. van Elburg, W.P.F. Fetter, C.M.J.E. Vandenbroucke-Grauls Nosocomial infection (NI) in a neonatal intensive care unit (NICU): results from a surveillance study with definitions for infection specifically designed for neonates	V02
14:30 - 14:45	J. Manniën, M.E.E. van Kasteren, I.C. Gyssens, B.J. Kullberg, J.C. Wille, A.S. de Boer The effect of timing of antibiotic prophylaxis on the incidence of surgical site infections after total hip replacements	V03
14:45 - 15:00	A.M.D. Kooistra, S.R. van Dijk, G.I.J.M. Beerthuizen, W.H.M. Vogels, A.A. van Zwet, H.A. Verbrugh Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn centre	V04

15:00 - 15:15	E.M. Mascini, K.P. Jalink, T.E.M. Kamp-Hopmans, H.E.M. Blok, J. Verhoef, M.J.M. Bonten, A. Troelstra Risk factors for carriage of an epidemic vancomycin-resistant *Enterococcus faecium* strain	V05
15:15 - 15:30	C.H.W. Klaassen, J.A. de Valk, C. Neeleman, J.W. Mouton Determination of epidemiological relationships between pneumococci: pulsed field gelelectrophoresis versus amplified fragment length polymorphism	V06

W Zaal 2 **Diagnostics 2**

Voorzitter: E.J. Kuijper

14:00 - 14:15	K.E. Templeton, S.A. Scheltinga, H. Goossens, E.C.J. Claas Development and application of a multiplex real-time PCR for diagnosis of *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Bordetella pertussis*	W01
14:15 - 14:30	L. Schinkel, J.J. Verweij, D. Laeijendecker, A.M. Polderman Development and assessment of a real-time PCR for *Giardia lamblia*	W02
14:30 - 14:45	T. Schuurman, M.C. Scholts, A.M.D. Kooistra, A.A. van Zwet Real-time detection of *Salmonella* DNA in faeces without culture enrichment	W03
14:45 - 15:00	T. Schuurman, R.F. de Boer, A.M.D. Kooistra, A.A. van Zwet 16S rDNA PCR and sequencing of cerebrospinal fluid in the diagnosis of bacterial meningitis: results of a multicentre study	W04
15:00 - 15:15	K.E. Templeton, S.A. Scheltinga, M.F.C. Beersma, A.C.M. Kroes, E.C.J. Claas Multiplex real-time PCR detection of respiratory viral targets	W05
15:15 - 15:30	A.H. Brandenburg, B.C. Meijer, E. Steendam, A.M. van Elsacker-Niele Evaluation of eight commercially available EIA kits and two immunoblots for the serodiagnosis of Lyme disease	W06

A02
Selection of potential fungal agents of bioterrorism using evolutionary criteria

G.S. de Hoog
Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands

The list of potential agents of bioterrorism issued by the USA Department of Occupational Safety and Environmental Health (OSEH) presently contains a single fungus, *Coccidioides immitis*. This is the agent of Valley Fever, a disseminating disease which commonly infects humans but exceptionally takes a fatal course, then mainly in patients with impaired acquired immunity. *C. immitis* is not the only fungal species known to cause fatal disease; the question then arises whether other fungi should be put on the list. Agents should combine host-specific pathogenicity with a high degree of virulence. The probability that species display these criteria optimally in terms of bioterrorism is determined by the evolutionary history of the group at hand. The fungal kingdom is reviewed in search of clades with (1) shared virulence factors including species with (2) mammal host-dependence with dual life cycles and (3) production of zoodemes while (4) fitness is increased. In addition (5) the degree of adaptation is discussed. About 400 fungal species have been reported from humans; this is less than 0.5% of the fungi known to date. Suitable criteria are encountered in only a small fraction, the remaining species being occasional opportunists or superficial pathogens. Required properties are combined in only two species. Among these is not *C. immitis*.

A03
Experimental coevolution

A. Buckling
University of Bath, Department of Biology and Biochemistry, UK

Host-parasite antagonistic coevolution, the reciprocal evolution of host defence and parasite counter-defence, is believed critical to the evolution of diversity, sex and pathogen virulence, and driving host-parasite population dynamics. Experimentally addressing the causes and consequences of coevolution is however difficult, largely because of the time scales required and lack of experimental control. These difficulties can be overcome using experimental populations of microbes, specifically the bacterium *Pseudomonas fluorescens* and an associated phage. Here I present work that experimentally addresses the role of coevolution in the generation of biodiversity and host-parasite specificity.

A04
Genome reduction in bacterial pathogens and symbionts

N.A. Moran
Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona, USA

When bacterial lineages make the transition from free-living or facultatively parasitic life cycles to permanent associations with hosts, they undergo a major loss of genes and DNA. Complete genome sequences are providing a detailed view of which genes are lost, and it is now apparent that relatively few genes are universally preserved. Analysis of genome sequences indicate that gene loss occurs largely as the result of mutations fixed through genetic drift, resulting in deletion, inactivation and erosion of genes. High levels of genetic drift are expected to result from the reduction in genetic effective population size that accompanies obligate dependence on hosts. These high levels of genetic drift underlie some other characteristic features of small bacterial genomes, including rapid sequence evolution, biases in nucleotide composition and amino acid content of encoded polypeptides, and thermal instability of secondary structures of gene products. Some or all of these features have been documented for phylogenetically diverse groups of host-dependent bacteria, including mycoplasmas, rickettsiae, chlamydeae, and gammaproteobacterial endosymbionts of insects. The latter, which include *Buchnera* and *Wigglesworthia*, are relatively closely related to well-known bacteria with larger genomes, such as *Escherichia coli* and *Yersinia pestis*; comparative analyses allow more exact reconstruction of the process of genome reduction in these groups. Continued completion of more genome sequencing projects will allow more detailed understanding of the evolutionary processes that underlie the radical genome changes observed in pathogenic and symbiotic bacteria.

A05
Coevolution of fungi with other (micro-)organisms

J.W. Taylor
University of California, Department of Plant and Microbial Biology, Berkeley, California, USA

Coevolution can be defined broadly or narrowly. The advent of phylogenetic theory and methods of assessing nucleic acid variation made it possible to narrow the definition by rigorously investigating claims of coevolution. Some of the earliest such studies now are classics, including studies of gophers and their parasitic lice[1]. With microbes, similarly rigorous studies of co evolution have been conducted with aphids and the bacterial genus *Buchneria*[2]. Fungi, members of a very large kingdom of complex and widely distributed eukaryotic microbes, certainly coevolve in the broad sense with other microbes as well as with macroscopic plants and animals. However, few of the many possible instances of coevolution have been rigorously investigated. I will touch on the possibilities for fungal coevolution with virus, bacteria, algae, animals and plants, and spend more time on examples where careful tests for coevolution could be, or have been,

applied. For virus, the interaction of double stranded RNA virus with fungi in the plant pathogen, *Cryphonectria parasitica*, stands out[3]. With bacteria, the presence in arbuscular mycorrhizal fungi of intracellular bacteria in the genus *Burkholderia* is remarkable.[4] With microscopic plants, i.e., algae, studies aimed at recognising species in the two partners of species of the lichen *Letharia* show cases of coevolution, as well as host jumps[5]. With plants, studies of the coevolution of mycorrhizae fungi and their vascular plant partners show the same pattern of coevolution and host jumping[6]. One of the best-understood systems of fungal coevolution involves the fungi farmed by attine ants[7], and recently these studies have grown to include weedy fungi and bacteria used to control the 'weeds'. Finally, there are cases where fungi are likely to be coevolving with humans. Here, commensal fungi, such as *Candida* or *Malassezia*[8] species should be very interesting, as well as the fungi causing superficial skin infections[9]. Recent studies of systemic fungi causing deep mycoses would seem to rule out long-standing co-eveolution, however there appear to be events in the recent evolution of *Histoplasma* species[10] and *Coccidioides* species[11] that may be best explained by the actions of migrating humans.

References

1. Hafner MS, Nadler SA. Systematic Zoology 1990;39:192-204.
2. Clark MA, Moran NA, Baumann P, Wernegreen JJ. Evolution 2000;54:517-25.
3. Dawe AL, Nuss DL. Annu Rev Genetics 2001;35:1-29.
4. Minerdi D, Bianciotto V, Bonfante P. Plant & Soil 2002;244:211-9.
5. Kroken S, Taylor JW. Bryologist 2000;103:645-60.
6. Bidartondo MI, et al. Nature 2002;419:389-92.
7. Green AM, Mueller UG, Adams RMM. Molecular Ecology 2002;11:191-5.
8. Gueho E, et al. Medical Mycology 1998;36:220-9.
9. Graser Y, Kuijpers AFA., El Fari M, Presber W, Hoog GS de. Medical Mycology 2000;38:143-53.
10. Kasuga T, Taylor JW, White TJ. J Clinical Microbiology 1999;37:653-63.
11. Fisher MC, et al. PNAS (USA) 2001;V98:4558-62.

B01

Evolution of the human pathogen *Bordetella pertussis*: the role of vaccination

F.R. Mooi, C. Heuvelman, A. King, M. Hijnen, H.G.J. van der Heide, I. van Loo, L.M. Schouls, G. Berbers
RIVM, Laboratory for Vaccine Preventable Diseases, Bilthoven, the Netherlands

Introduction. *Bordetella pertussis* is the agent of pertussis or whooping cough, a major cause of child mortality before vaccination. Subsequent to the introduction of vaccination in the 1950s, pertussis nearly disappeared. However, the 1990s witnessed a resurgence of pertussis in many countries, including the Netherlands. The aim of our studies is to elucidate the causes for the resurgence of pertussis.
Results. We studied changes in the *B. pertussis* population in the period 1953 (when vaccination was introduced) to 2002 in the Netherlands. The *B. pertussis* population was found to be highly dynamic, showing temporal changes in strain and gene frequencies. Three, temporally distinct, clonal expansions were observed associated, respectively, with the emergence of strains carrying non-vaccine type variants of pertussis toxin (Ptx), pertactin (Prn), and strains with a novel Ptx promoter (ptxP3). The role of Prn in immune escape was studied. The variable region of Prn was immunodominant, and antibodies against it were protective. Further, the human immune response against Prn was type-specific. Finally, we observed that variation in Prn affected vaccine efficacy in a mouse model. The effect of Prn variation on strain fitness was studied in a mouse model. Results indicated that non-vaccine type variants were less fit in naive mice. The expansion of strains carrying the PtxP3 allele coincided with the recent resurgence of pertussis and we speculate that these strains are more virulent.
Conclusions. Adaptation of *B. pertussis* to vaccination is one of the causes for the resurgence of pertussis in the Netherlands, and possibly also in other countries. Adaptation occurred in at least 3 discrete steps, 2 of which involved the emergence of strains carrying non-vaccine type Ptx and Prn variants, respectively. Variation in Prn involves a trade-off between increased fitness in vaccinated individuals and decreased ability to colonise naïve individuals. Prn and Ptx are components of the recently introduced new generation of pertussis vaccines therefore careful monitoring of the long-term efficacy of these vaccines is called for.

B02

Evolution and host adaptation of the *Bordetella* genus

D.A. Diavatopoulos[1], M. Arnold[1], H.G.J. van der Heide[2], M.C. Maiden[2], F.R. Mooi[3]
[1]*Eijkman-Winkler Centre for Microbiology, Infectious Diseases and Inflammation, University Medical Centre Utrecht, Utrecht, the Netherlands,* [2]*Laboratory for Vaccine Preventable Diseases, RIVM, Bilthoven, the Netherlands,* [3]*Department of Zoology, University of Oxford, Oxford, UK*

Most members of the *Bordetella* genus are associated with respiratory diseases. The genus can be roughly divided in species that either infect mammalian or avian hosts. The former comprise the 'classical' members, *B. pertussis, B. parapertussis* and *B. bronchiseptica*. It is widely believed that *B. pertussis* and *B. parapertussis* descended from *B. bronchiseptica*, and should be regarded as subspecies of *B. bronchiseptica*. This is confirmed by phenotypical and 16S rRNA analysis (>99% similarity). In contrast to their apparent close relationships, these species show very distinct host tropisms; *B. pertussis* is a truly human pathogen, whereas *B. parapertussis* is only isolated from human or ovine hosts. *B. bronchiseptica* is capable of infecting a wide range of mammalian species, although human infections are rare. The aim of our research was to further elucidate the phylogenetic relationships between these species using multilocus sequence typing (MLST).
A collection of 69 *B. bronchiseptica,* 19 *B. pertussis* and 13 *B. parapertussis* strains was analysed, representing a broad host range and geographic distribution. We identified 25 different sequence type's (STs), of which 11 were unique. *B. bronchiseptica* strains constituted the vast majority of all STs (18). Very little genetic diversity was observed within especially *B. pertussis* and *B. parapertussis* (both ovine and human). Our results show that *B. pertussis* and *B. parapertussis* do not comprise a distinct species, but are *B. bronchiseptica* strains adapted to humans and sheep. Surprisingly, we found a number of mostly human *B. bronchiseptica* isolates to be closer related to the *B. pertussis* cluster than to the main *B. bronchiseptica* cluster.
We conclude that MLST is a useful method to study *Bordetella* phylogenetic relationships. Our data suggest, in contrast to previous findings, that host adaptation to humans already

occurred within an ancestral *B. bronchiseptica* lineage, after which further speciation of *B. pertussis* and *B. parapertussis* took place.

B03

Low prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in hospital admissions in the Netherlands

H.F.L. Wertheim[1], M.C. Vos[1], A. Ott[1], A. Voss[2], J.A.J.W. Kluytmans[3], C.M.J.E. Vandenbroucke-Grauls[4], M.H.M. Meester[4], P.H.J. van Keulen[3], H.A. Verbrugh[1]
[1]*Erasmus Medical Centre, Medical Microbiology and Infectious Diseases, Rotterdam, the Netherlands,* [2]*University Medical Centre St Radboud, Medical Microbiology and Infectious Diseases, Nijmegen, the Netherlands,* [3]*Amphia Hospital, Medical Microbiology and Infectious Diseases, Breda, the Netherlands,* [4]*Free University Medical Centre, Medical Microbiology and Infectious Diseases, Amsterdam, the Netherlands*

Less than 1% of clinical *Staphylococcus aureus* strains is methicillin-resistant (MRSA) in the Netherlands. To prevent MRSA becoming endemic, there is a national 'search and destroy' policy. Repatriated and other risk patients are screened for MRSA carriage at admission. Recently, some MRSA outbreaks could not be related to repatriated patients. This study is designed to measure the prevalence of MRSA nasal carriage at admission of not repatriated patients.
From April 1999 to April 2000 9,859 patients admitted to non-surgical departments were screened for MRSA nasal carriage in 4 Dutch hospitals. Nasal swabs were streaked on 5% sheep blood agar (BA) and subsequently submerged in a selective broth and incubated for 2-3 days at 35°C. Colonies suspected for *S. aureus* were identified with an agglutination test (StaphaurexPlus). Susceptibility testing was performed by an automated system (MicroScan Walk-a-Way, Gram-positive panel) and oxacillin disk diffusion according to NCCLS criteria. Strains suspect for Methicillin resistance were confirmed by a hybridisation test (AccuProbe) and MecA PCR. MecA positive strains were typed by pulsed field gel electrophoresis (PFGE).
Twenty-five percent (2,471/9,859) of the patients were *S. aureus* nasal carrier. Only 3 (0.03%) patients were MRSA carrier. These patients were not repatratriated and not known to be MRSA carrier before admission. These patients were not admitted to the same department, nor were any other relationships found. No outbreaks were recorded on the departments were the patients were admitted, in the first few months after their admission. Characteristics of the three patients and their corresponding MRSA strains are shown in the tables. The PFGE patterns show that all three stains are unique.
We conclude that the MRSA prevalence is still low (0.03%) at admission in the Netherlands due to our 'search and destroy' policy and restrictive antibiotic use. Since none of the three strains resulted in an outbreak, they are possibly non-epidemic.

B04

Genetic diversity and origin of hepatitis B virus in Dutch blood donors

H.L. Zaaijer[1], M.H.G.M. Koppelman[2]
[1]*Free University Medical Centre, Medical Microbiology and Infection Control, Amsterdam, the Netherlands,* [2]*Sanquin-CLB, Virusdiagnostiek, Amsterdam, the Netherlands*

Introduction. The screening for presence of hepatitis B virus (HBV) relies on immunological detection of the a-determinant of the surface antigen (HBsAg-a) of HBV. Which HBsAg-a variants are present in HBV infected blood donors? Contrary to WHO recommendations universal HBV vaccination has not been implemented in the Netherlands, leaving future generations of donors vulnerable to HBV infection. What is the origin of HBV strains infecting donors?
Methods. Samples from 60 of the 64 blood donors, testing positive for HBV in 2000 or 2001, were available for amplification and sequencing of the 498-749 bp section of the HBV S-gene. Sequences were subjected to phylogenetic analysis and compared with HBV-prototypes and with HBV-sequences (reported by van Steenbergen *et al.*) from specific Dutch groups at-risk for HBV.
Results. The 60 HBV isolates belong to various HBV genotypes (and deduced serotypes): 21x genotype A (adw2); 6x B (4x adw2, 2x ayw1); 1x C (adrq+); 31x D (1x ayw1, 25x ayw2, 5x ayw3), 1x F (adw4). The amino acid squences of HBsAg-a show 11 variations on 8 positions; all are classical variants unrelated to vaccine or immunoglobuline escape mutants, except for 1 hitherto unreported mutation in 2 donors. The majority of the strains is related or identical to isolates from persons at-risk for HBV: 19 donors (A-adw2) cluster with strains from homosexual men; 24 donors (D-ayw2) cluster with strains from Dutch-Moroccan persons; and 5 donors (D-ayw3) cluster with strains from intravenous drug users. Strains endemic in South America, Asia and the Far East were found respectively in 1 (F-adw4); 1 (C-adrq+) and 6 (B-adw2 and B-ayw1) donors.
Conclusion. 58/60 donors carry classical HBsAg-a variants. 56/60 donors carry HBV strains from abroad, or strains closely related or identical to HBV strains found in local at-risk groups, illustrating the importance of stringent donor selection procedures; and implying that an endemic Dutch (heterosexual/non-ivd/non-Mediterranean) HBV strain does not exist.

B05

Prevalence of multiresistant micro-organisms among adopted children in the Netherlands

A. Troelstra[1], L.A.M. de Graaf-Miltenburg[1], H.E.M. Blok[1], T.W.J. Schulpen[2]
[1]*Eijkman Winkler Centre for Microbiology, Infectious Diseases and Inflammation, Utrecht, the Netherlands,* [2]*University Medical Centre Utrecht, Department of Social Pediatrics, Utrecht, the Netherlands*

Introduction. We wanted to investigate colonisation with multiresistant bacteria in Dutch children who were adopted from foreign countries.
Methods. All recently adopted children that were referred to the outpatient clinics of the University Medical Centre Utrecht were evaluated by a paediatrician. Physical exami-

nation was performed and cultures were taken from nose and stools that were screened the presence of resistant micro-organisms.
Results. During the period 1998-2000, 333 children were evaluated and included in our study. At least 15% of the children were admitted to a hospital during their first year of life. *Staphylococcus aureus* was found in 63 cultures from the nose, of which isolates from 11 were identified as MRSA (17.5%). Birth in Taiwan was identified as a significant risk factor for colonisation of the child with MRSA (RR=24.2 {8.7-67.5}), the same was true for schisis (RR=8.2 {1.3-49.6}), and skin lesions (RR=4.7 {1.5-14.8}). Fifty-six children were colonised with *Streptococcus pneumoniae*. 18 isolates were not susceptible for penicillin (32.1%); 2 isolates had an MIC ≥2 ìg/ml; 8 isolates were lost for MIC-testing, 25 of these isolates were resistant for erythromycin (49%). *Enterobacteriaceae* were isolated from 29 children, 10.3% of these were gentamicin resistant. Eleven of these isolates were evaluated for the presence of ESBL; two were found positive. Fifteen children were admitted to our hospital, at a certain time. From these, 4 children (26.6%) were colonised with resistant micro-organisms.
Discussion. In our study, the prevalence of MRSA among adopted children was 3.3%. This warrants implementation of standardised screening and isolation precautions for these children, as part of the Dutch MRSA 'search and destroy' policy. At least 3.5% of all *S. pneumoniae* strains were resistant for penicillin. The prevalence of gentamicin resistance among Gram-negative was 10.3%. If standardised screening for resistant micro-organisms will be included for these children at admission, unexpected introduction in the hospital of these micro-organisms can be prevented.

C01

Validation of a fast molecular method for detection and identification of dermatophytes in nail and skin samples

A.M.C. Bergmans[1], L.M. Schouls[2], R.G.F. Wintermans[1]
[1]*Franciscus Hospital, Laboratory of Medical Microbiology, Roosendaal, the Netherlands,* [2]*RIVM, Laboratory of Infectious Diseases Research, Bilthoven, the Netherlands*

Dermatophytes are keratinophilic fungi that cause superficial infections such as ringworm, favus, or onychomycosis. The dermatophytes comprise three genera, *Trichophyton*, *Microsporum* and *Epidermophyton*. Current laboratory diagnosis relies on direct microscopic examination of KOH-treated clinical samples, micro- and macroscopic observation of *in vitro* cultures, and on metabolism tests. With direct examination of samples, identification of fungi is impossible. Culturing is insenstive and time-consuming (2-4 weeks), and identification of strains is difficult because of overlapping characteristics, variability and pleomorphism.
Our aim was to develop a fast and sensitive molecular method for detection and identification of dermatophytes directly in clinical material.
Recent work has shown that internal transcribed sequences (ITS) between rRNA genes are sufficiently polymorphic for identification of dermatophytes to the species level. We developed a dermatophyte-specific PCR-reversed line blot (PCR-RLB) assay based on ITS sequences, and a DNA extraction procedure for nail and skin samples. Both genus- and species-specific probes were developed, which allowed the identification of nine species within three genera.
Approximately 200 nail and skin samples were used to validate three diagnostic methods: direct microscopic examination, culture, and PCR-RLB. Sensitivities of these methods showed to be 89, 72, and 100%, respectively. Specificities were 88, 96, and 96%, respectively. Identification results of approximately 50 (PCR- and culture-) positive samples showed two discrepancies between culture and PCR-RLB.
PCR-RLB was also used to identify 10 dermatophyte strains with determination problems. Three of them gave discrepant results. DNA sequence analysis of ITS regions of all discrepant samples confirmed PCR-RLB-based identification results.
The PCR-RLB method showed extremely suitable for routine detection and identification of dermatophytes directly in nail and skin samples, because of its fast, sensitive, specific and accurate performance.

C02

Real-time polymerase chain reaction (PCR) for diagnosis and monitoring of *Toxoplasma gondii* infection

I.J.B. Spijkerman[1], J.J. Verweij[2], B. van Hoek[3], L. van Lieshout[2]
Leiden University Medical Centre, [1]*Medische Microbiologie,* [2]*Parasitologie,* [3]*Maag-, darm- en leverziekten, Leiden, the Netherlands*

Diagnosing toxoplasmosis by serology can be compromised in particular in immunocompromised patients or congenitally infected new-borns. A number of studies showed successful diagnosis by specific DNA detection in several types of body fluids. Here we further optimised and implemented a multiplex real-time PCR with internal controles using primers and double-labelled probes based on the B1 gene of *Toxoplasma gondii*. The simultaneous isolation and detection of the Phocid herpes virus was used as an internal control of inhibition. The sensitivity of the PCR was established by testing a serial dilution of DNA obtained from a known number of *T. gondii* tachyzoites (TZ). Subsequently several human specimens spiked with TZ were tested. Finally, multiple sequential samples of a patient with fatal disseminated toxoplasmosis after orthotopic liver transplantation were tested retrospectively.
After optimising the magnesium, primer and probe concentrations PCR efficiency reached 97%. The sensitivity of the PCR was 0.5 to 5 TZ per reaction using DNA isolated from the serial dilution, whole blood, plasma or cerebral spinal fluid. Clinical samples were obtained from a patient with unexplained renal failure, diarrhoea and respiratory failure after liver transplantation. Prior to transplantation no toxoplasma antibodies were detected in the patient while the donor was seropositive. On the day the patient died many TZ were seen in the Giemsa stain of a tracheal aspirate (TA). Toxoplasma PCR on the TA resulted in a load of 230,000 TZ per 200 ìl. PCR on sequential plasma samples showed an increasing load from 1 week before the positive stain up to 7200 TZ per 200ìl. Moreover, DNA isolated from lung, liver and spleen obtained during autopsy were strongly positive.

In conclusion, a sensitive quantitative real-time PCR for *T. gondii* with internal control was established. This PCR can be used both as a tool for diagnosing *T. gondii* infections and, carried out on blood samples, to monitor individuals at risk for fatal *T. gondii* infections (e.g. transplant patients).

C03

A real-time PCR using 3'-minor groove binder-DNA probes for detection in clinical samples of a SNP associated with high level isoniazid resistance in *Mycobacterium tuberculosis*

H.R. van Doorn[1], E.C.J. Claas[2], K.E. Templeton[2], A.G.M. van der Zanden[3], A. te Koppele-Vije[3], M.D. de Jong[1], J. Dankert[1], E.J. Kuijper[2]

[1]*Academic Medical Centre, Medical Microbiology, Amsterdam, the Netherlands,* [2]*Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands,* [3]*Gelre Hospitals, Medical Microbiology, Apeldoorn, the Netherlands*

Tuberculosis remains one of the leading infectious causes of death worldwide. The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a serious public health threat. Resistance to isoniazid (INH) is the most prevalent form of resistance in *M. tuberculosis* and is mainly caused by mutations in the catalase-peroxidase gene (*katG*). Among high-level resistant isolates (MIC ≥2) 89% is associated with a mutation at amino acid 315 of *katG*. A single tube Real-time PCR, using a novel kind of probe (3'-minor groove binder-DNA probes), was developed to detect either the wild-type or the mutant codon directly in sputum samples. The detection limit of the assay for purified DNA was 5 fg per well (1 mycobacterial genome) and for artificially infected sputum samples 20 copies per well, corresponding to 10e3 mycobacteria per ml sputum. Sputum samples from 21 patients living in Kazakhstan or Moldova infected with mono- or multidrug resistant *M. tuberculosis* and 20 sputum samples from patients infected with INH-susceptible *M. tuberculosis* were tested. The sensitivity and specificity of the probes were 70 and 100% for the wild-type probe, and 88 and 100% for the mutant probe, respectively. The binding to either probe was non-ambiguous. This Real-time PCR allows the rapid identification of a mutant *katG* allele and can easily be implemented in a clinical microbiology laboratory.

C04

The use of Procalcitonin (PCT) serum level for the diagnosis of bacteraemia

B. Roerig[1], H. Klip[2], M.C.J. Persoons[3], A.A. van Zwet[3]

[1]*Scheperhospital, Internal Medicine, Emmen, the Netherlands,* [2]*Isala Clinics, Research Bureau, Zwolle, the Netherlands,* [3]*Regional Public Health Laboratory Groningen/Drenthe, Drenthe, Emmen, the Netherlands*

Introduction. Several studies have been published indicating an increase in PCT serum levels in patients suffering from severe bacterial or fungal infections. In contrast to C-reactive protein, PCT allows differentiation between infectious and non-infectious systemic inflammatory response syndrome in critical ill patients. The gold standard for a systemic bacterial infection (bacteraemia) is the finding of a positive blood culture. However, the main drawback of a blood culture is the time factor, whereas PCT serum levels can be available in a very short time. In this prospective multi centre study the clinical use of PCT serum levels in patients undergoing blood cultures was assessed.

Patients and methods. In 310 consecutive patients a total of 398 blood cultures were collected. At the same time a blood sample was taken for the detection of PCT. Blood cultures were processed using the BactAlert 120 automate following standard procedures. Positive blood cultures, most probably due to contamination were considered culture negative. PCT levels were determined using a rapid test kit for semi-quantitative determination and an immunoluminometric assay for quantitative detection.

Results. Forty-three blood cultures were positive and 353 were culture negative. High serum PCT levels (>10 ng/ml) were significantly associated with the appearance of a positive blood culture. Negative PCT results (<0.5 ng/ml) were most often seen in patients with a negative blood culture. Using a cut-off value of 2 ng/ml, the sensitivity, specificity, positive and negative predictive values of PCT for bacteraemia were 47, 86, 29 and 93% respectively.

Conclusion. PCT serum levels are strongly associated with bacteraemia and this parameter can be helpful to diagnose bacterial sepsis promptly in a considerable number of patients. We suggest that PCT detection should be included in the diagnosis strategy of critical ill patients. On the other hand, in spite of a high negative predictive of the test, ruling out bacteraemia solely on the basis of a negative PCT result should not be encouraged.

C05

Evaluation of four confirmation assays for AMPLICOR *Neisseria gonorrhoeae* PCR using conventional and real-time PCR with *cppB* and 16S rRNA as targets

C.M.C. van Herk, I.M. Slootjes, G.H.W. Onland, A.J.C. van den Brule, C.H.E. Boel

Stichting PAMM, Laboratory for Medical Microbiology, Veldhoven, the Netherlands

The Roche COBAS AMPLICOR is an automated PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Certain strains of *Neisseria subflava* and *Neisseria cinerea* have been reported to produce false-positive results with the AMPLICOR *N. gonorrhoeae* PCR. Therefore, positive results should be confirmed by another more specific PCR.

In this study we evaluated four PCR assays for their use as a confirmation test. We used the *cppB* gene and 16S rRNA as targets, both in a conventional in-house EIA PCR and in a real-time PCR format (Lightcycler, Roche Diagnostic Systems).

A total of 579 AMPLICOR *N. gonorrhoeae* PCR-positive specimens were obtained from 14 different laboratories throughout the Netherlands. Of these 165 samples (28.5%) were positive and 362 samples (62.5%) were negative in all four assays.

Thirteen samples tested positive in both 16S rRNA assays and negative in both *cppB* PCR assays. These 13 samples most probably contain strains of *N. gonorrhoeae* lacking the 4.2 kb cryptic plasmid on which the *cppB* gene is found.

Four samples were negative in the lightcycler 16Sr RNA PCR only, probably due to the fact that the lightcycler 16Sr RNA PCR was less sensitive than the other three assays.

In the *cppB* EIA PCR 32 samples were found positive, that could not be confirmed in one of the other assays. Of these 23 were swabs from respiratory sites. Most probably, this assay is not specific and detects other Neisseria species. Indeed, homology between the sequence of the *N. gonorrhoeae* cryptic plasmid and the sequences of *N. meningitidis* and *N. lactamica* plasmids has been reported.
Three (0.5%) of the 579 samples gave inconsistent results.
Based on the results of these four assays, the in house 16S rRNA EIA PCR performed best. Because this assay takes more hands-on-time and is more contamination prone, the lightcycler assay might be preferred. However, the 16S rRNA assay is less sensitive and the *cppB* assay will not detect *N. gonorrhoeae* missing the cryptic plasmid.

D01

Helicobacter pylori is sensitive to nickel only at acidic pH

A.H.M. van Vliet, A. Heijens, S.W. Poppelaars, J. Stoof, E.J. Kuipers, J.G. Kusters
Erasmus Medical Centre, Department of Gastroenterology and Hepatology, Rotterdam, the Netherlands

Objective. As most antibiotics do not perform well in the acidic environment of the stomach, antibiotic treatment of *Helicobacter pylori* infection is often combined with proton pump inhibitors (PPIs) to increase the gastric pH. Transition metals can have antimicrobial properties, and their biological availability is known to be affected by pH. Therefore the sensitivity of *H. pylori* to a range of metals was determined at neutral pH (7.0) and compared with the sensitivity at acidic pH (5.5).
Methods. *H. pylori* reference strain 26695 was grown in Brucella media supplemented with 1% β-cyclodextrins. Medium pH was adjusted to either pH 7.0 and pH 5.5 using hydrochloric acid, and subsequently metal chlorides were added to final concentrations ranging from 1-1000 μM. Metals tested were bismuth, cadmium, cobalt, copper, iron, manganese, nickel and zinc.
Results. Bismuth and cobalt displayed the highest level of toxicity to *H. pylori*

D02

Streptococcal toxic shock syndrome caused by a MLS resistant M type 77 *Streptococcus pyogenes* carrying the *erm*TR gene: a retrospective analysis among M77 isolates collected in the Netherlands

J. Gooskens[1], A.J. de Neeling[2], R.J.L. Willems[2], E.J. Kuijper[1]
[1]*Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands,* [2]*National Institute of Public Health and the Environment (RIVM), Research Laboratory for Infectious Diseases, Bilthoven, the Netherlands*

Background. An increasing number of group A streptococci (GAS) with a constitutive or inducible resistance to macrolide-lincosamide-streptogramin B antibiotics (cMLS or iMLS phenotype; mediated by the *erm*B or *erm*TR gene) is reported in Europe, but not in the Netherlands. In 2002 we reported a patient with a streptococcal toxic shock syndrome (STSS) caused by an iMLS resistant T28 M77 GAS (*Streptococcus pyogenes*) carrying the *erm*TR gene, and capable of producing streptococcal pyrogenic exotoxin C (SpeC) but not SpeA. Emerging MLS resistant GAS could have important therapeutic consequences, since clindamycin has a crucial role in the treatment of STSS.
Methods. We performed a retrospective analysis among M77 GAS strains (associated with invasive infections) collected at the 'National institute of public health and environmental protection' (RIVM) from January 2000 to December 2001. The isolation site of the M77 GAS strains was noted and their MIC for erythromycin was determined by the E-test. We screened for phenotypic MLS and erythromycin resistance (using double-disk testing). PCR was used to detect the presence of genes encoding MLS (*erm*B, *erm*TR) and erythromycin (*mef*A) resistance. We also investigated the presence of streptococcal pyrogenic exotoxin (Spe) genes (SpeA and SpeC) and analysed the clonal relationships of the strains using multilocus sequence typing (MLST).
Results. Of 654 GAS bacteria collected at the RIVM, 17 (2.6%) were M type 77. Of these 17 M77 GAS isolates, two isolates were MLS resistant due to the *erm*TR gene. MLST typing revealed that all 17 isolates had identical sequences in all fragments of 7 housekeeping loci and belonged to sequence type 63.
Conclusion. We conclude that all collected M77 GAS isolates belonged to the same clonal complex and were associated with invasive disease and STSS. The fact that only 12% of the strains of this clonal complex carried the *erm*TR resistance gene, indicates that this gene is acquired through horizontal spread.

D03

Dissemination of *Chlamydia pneumoniae*: a role for bone marrow-derived monocytes/macrophages?

C.A. Bruggeman[1], R.R Ezzahiri[2], F.R.M. Stassen[1], M.P.J. de Winther[3], H.A.J.M. Kurvers[2], S.B. Herngreen[1], P.J.E.H.M. Kitslaar[2]
University Hospital Maastricht, [1]*Medical Microbiology,* [2]*Surgery,* [3]*Molecular Genetics, Maastricht, the Netherlands*

Introduction. In mice, *Chlamydia pneumoniae* (*Cpn*) is disseminated systemically following primary infection. Monocytes/macrophages (M/M) have been postulated to play an important role in this dissemination process. As M/M are mainly derived from bone marrow (BM) we investigated whether (i) *Cpn* is able to infect BM cells after primary infection, (ii) *Cpn* is viable in BM cells and (iii) infected BM cells are able to disseminate *Cpn* to other organs.
Methods. C57Bl/6J mice were infected intraperitoneally with 5.10^7 IFU *Cpn* and sacrificed at 2, 4 and 6 days post infection (p.i., n=3 for each time point). BM cells were isolated from tibia and femur for *Cpn* detection by PCR. Viability of *Cpn* in BM cells was studied by culture using Hep-2 cells. To examine whether infected BM cells are able to disseminate *Cpn* to distinct organs, we used a BM transplantation model. Six recipient mice were subjected to 10-Gy lethal total body γ-irradiation the day before BMT to eliminate endogenous bone marrow cells. BM cells were obtained from 3 donor mice, infected 4 days earlier. Approximately 4.10^{10}/l BM cells per mouse were isolated and injected intravenously in the recipient mouse at a dosage of 1.10^7. Four days after transplantation, bone marrow cells, blood and internal organs

(lung, heart, liver, vascular tree, and kidneys) were isolated from recipient mice for detection of *Cpn* DNA by PCR.
Results. Bone marrow cells were *Cpn* DNA positive in all infected mice at every time point p.i. Culture showed viable *Cpn* in BM cells at 4 days p.i. At 4 days after BM transplantation all BM samples in all recipient mice were positive for *Cpn* DNA. However, blood and internal organs remained negative for *Cpn* DNA.
Conclusion. This study demonstrates that viable *Cpn* can be detected in bone marrow cells from C57BL/6J mice up to 6 days after primary infection. Nonetheless, transfer of infected BM does not result in dissemination of *Cpn* to distinct organs in the recipient at 4 days post transfer. These findings suggest that infected BM cells may not be a permanent source of infected monocytes/macrophages.

D04

Helicobacter pylori escapes DC-SIGN mediated dendritic cell function by phase variation in lipopolysaccharide Lewis x and y blood group antigens

M.P. Bergman[1], A. Engering[2], J.M. Maaskant[1], B. de Goeij[1], J. Stoof[1], H.P. Wirth[3], C.M.J.E. Vandenbroucke-Grauls[1], Y. van Kooyk[2], B.J. Appelmelk[1]
Free University Medical Centre, [1]Medical Microbiology and Infection Control, [2]Molecular Cell Biology, Amsterdam, the Netherlands, [3]Vanderbilt University School of Medicine, Division of Infectious Diseases, Nashville, USA

Introduction. *H. pylori* is a Gram-negative pathogen colonising the human gastric mucosa where it is able to persist for life, leading to gastric and duodenal ulceration, MALT lymphoma and gastric cancer. Antigenic diversity in lipopolysaccharide (LPS) of *H. pylori* is very restricted and expression of LPS Lewis x (Le^x) and Le^y blood group antigens is highly conserved. $Le^{x/y}$ expression is subjected to phase variation, resulting in Le^x and/or Le^y positive ($Le^x/^y$+) and negative ($Le^x/^y$-) bacteria within a single strain. Despite extensive research, the biological roles of both Lewis antigen expression and phase variation in *H. pylori* are not clear.
Dendritic cells (DC) play a key role in initiating adaptive immune responses to pathogens. DC-SIGN (dendritic-cell specific ICAM-3 grabbing non-integrin), a C-type lectin specifically expressed on DC, functions as pathogen-recognition receptor and mediates antigen-presentation to T cells. We investigated the role of Lewis antigens in binding of *H. pylori* to DC-SIGN, and studied whether phase variation in $Le^x/^y$ influenced *H. pylori*-DC interaction and DC functions.
Methods. DC-SIGN binding of *H. pylori* clinical isolates (n=55), LPS and glycoconjugates was studied in ELISA. $Le^x/^y$+ and $Le^x/^y$- isogenic phase variants from one *H. pylori* clinical isolate were characterised in detail by serology, AFLP and C-tract sequencing, and studied for binding to DC-SIGN-transfected cells and monocyte-derived DC by FACS analysis. *H. pylori*-induced DC cytokine production was analysed by ELISA.
Results. *H. pylori* bound to DC-SIGN via LPS $Le^x/^y$. $Le^x/^y$+ bacteria bound DC via DC-SIGN, leading to internalisation and induction of IL-10 and IL-12, whereas $Le^x/^y$- bacteria did not bind to DC-SIGN. DC functions mediated by DC-SIGN-binding of $Le^x/^y$+ *H. pylori* could be blocked by anti-DC-SIGN monoclonal antibodies.
Conclusion. Our data suggest that phase variation in LPS O-antigenic $Le^x/^y$ provides *H. pylori* with a mechanism to escape DC mediated adaptive immunity, and hence, may play an important role in the ability of *H. pylori* to persist for life in the gastric mucosa.

D05

Helicobacter pylori ylxH (HP1034) is essential for motility

K. Huijsdens-van Amsterdam, A. van der Ende
Academic Medical Centre, Medical Microbiology, Amsterdam, the Netherlands

Helicobacter pylori colonises the human gastric mucous layer, leading to a superficial gastritis. Moreover, *H. pylori* is an important aetiological agent in peptic ulceration, and infection is strongly associated with the development of gastric cancer. To establish an infection in the viscous mucous layer of the gastric epithelium, bacterial motility is required. *Helicobacter pylori ylxH* (HP1034) was previously shown to be differentially expressed in the presence of human gastric epithelial HM02 cells. *H. pylori ylxH* is found between *fliA* and *flhF* in the sequenced genomes of *H. pylori* 26695 and J99. Also, *H. pylori* YlxH showed homology to flagella biosynthesis proteins known in other bacterial species. The aim of this study was to determine the role of *H. pylori* YlxH in motility. A *H. pylori ylxH* knock-out mutant was less motile than its wt strain on soft-agar plates. Moreover, electron microscopy showed that the *H. pylori ylxH* knock-out had no flagella, whereas the wild type *H. pylori* strain possessed 2 to 3 flagella. Complementation of the *ylxH* gene will be performed to restore *H. pylori* motility. Moreover, a possible regulating role of *ylxH* on *H. pylori* genes involved in the biosynthesis of flagella will be assessed by RT-PCR. In conclusion, *H. pylori ylxH* is involved in the flagella biosynthesis.

D06

Characterisation of an *in vitro* model for chronic infection with *Chlamydophila pneumoniae*

A. Meijer[1], J.H. Siekman[1], G. Celik[1], S.K. Gielis-Proper[2], M.C. Burger[2], P.J.M. Roholl[2], J.M. Ossewaarde[3]
National Institute of Public Health and the Environment, [1]Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, [2]Laboratory for Pathology and Immunobiology, Bilthoven, the Netherlands, [3]Erasmus Medical Centre, Department of Medical Microbiology and Infectious Diseases, Rotterdam, the Netherlands

Chlamydophila pneumoniae is a causative agent of acute and chronic infections. Knowledge about the factors that characterise these infections and the transition from an acute to a chronic state will enhance our insight in the pathogenesis and diagnostics. Therefore, an *in vitro* model to study the course of infection with *C. pneumoniae* was developed using HEp2 cells cultured under microgravity conditions. The model was sustained 112 days and characterised by quantitative PCR and RT-PCR (DNA and mRNA of MOMP, GroEL, KDO transferase, and 16S rRNA), immunocytochemistry (ICC) (membrane protein, heat shock protein 60 {HSP60} and lipopolysaccharide {LPS}), *in situ* hybridisation (ISH) (MOMP

DNA and 16S rRNA), and ultrastructural analysis using electronmicroscopy (EM). Up to day 22-27 the levels of chlamydial DNA, mRNA, and 16S rRNA were steady. The DNA level decreased over time but did not reach a zero level. The mRNA levels were always lower than that of DNA. The GroEL and KDO transferase mRNA levels decreased to zero at day 27 and 41, respectively. The MOMP mRNA level sharply decreased at day 84 but was still positive at day 112. Up to day 41 the 16S rRNA level was higher than that of the 16S rRNA gene, but decreased to a steady level at day 84-112. Using ICC and ISH, the staining pattern changed gradually from large inclusions to a more granular staining dispersed throughout the cytoplasm of cells. After day 57, only sporadically staining for membrane protein and LPS was observed. MOMP gene ISH was positive up to day 84 while 16S rRNA ISH was positive throughout the chronic infection. Using EM, elementary bodies were observed at all time points in vital cells of mostly aberrant morphology. In conclusion, an acute infection with *C. pneumoniae* is characterised by detection of DNA, RNA, and antigen in large inclusions, while a chronic infection is characterised by a granular staining pattern for membrane protein and LPS, only just detectable levels of DNA and RNA, and absence of HSP60. This model closely resembles the results obtained in human studies of acute and chronic infections with *C. pneumoniae*

D07

Diversity of *vex* and *vnc* genes in streptococci

J. Piet, A. van der Ende, J. Dankert, A. Bart

Academic Medical Centre, Medical Microbiology, Amsterdam, the Netherlands

Introduction. The Vex123 ABC transporter and VncRS two component regulatory system in *S. pneumoniae* were recently suggested to form a quorum sensing system responding to the secreted Pep27 peptide. Inactivation of this system was reported to result in tolerance to several antibiotics, but this is currently under debate. To determine potential streptococcal cross-talk and the evolutionary history of this system, the occurrence and sequence of homologs of this quorum sensing system in other streptococcal species was investigated.

Methods. Primers based on conserved parts of the genes were used for PCR amplification and sequencing of *vex, vnc* and *sodA* genes from oral streptococci. Phylogenetic analyses were performed using the MEGA program, using *sodA* sequences as a reference.

Results. Sequences homologous to *vex* and *vnc* genes of *S. pneumoniae* were obtained from *S. mitis, S. oralis*, and *S. peroris* isolates. In *S. mitis* and *S. oralis*, the *vex-vnc* intergenic region was interrupted by copies of putative mobile elements, the BOX-sequences. No *pep27* gene was detected in streptococci other than *S. pneumoniae*, whereas the *vex* and *vnc* genes and the gene order of these genes were conserved. Moreover, no transcription and translation signals were detected in front of the pneumococcal *pep27*, arguing against expression of the product of this open reading frame. Phylogenetic trees for all genes were congruent, indicating that these genes are not horizontally transferred between species. Phylogenetic analysis showed that although VncS was less conserved than any of the other protein sequences, the sensing part of the protein was conserved.

Conclusion. Homologs of the Vex123 ABC transporter and VncRS two component regulatory system are conserved in *S. mitis, S. oralis*, and *S. peroris*. We postulate that the signal sensed by VncS is not the Pep27 peptide. The conservation of the sensory domain of VncS indicates that the different streptococcal species sense an identical signal via this two-component regulatory system and cross-talk between species might be possible.

D08

Identification of fur- and iron-regulated genes of *Helicobacter pylori* using whole-genome DNA array analysis

F.D. Ernst[1-3], J. Stoof[1], B. Waidner[3], A.H.M. van Vliet[1], M. Kist[3], J.G. Kusters[1], S. Bereswill[3], G. Homuth[2]

[1]*Erasmus Medical Centre, Department of Gastroenterology and Hepatology, Rotterdam, the Netherlands,* [2]*University of Greifswald, Institute of Microbiology and Molecular Biology, Greifswald, Germany,* [3]*University Hospital of Freiburg, Institute of Medical Microbiology and Hygiene, Department of Microbiology and Hygiene, Freiburg, Germany*

Objective. Intracellular homeostasis of the essential nutrient iron is a necessity for all living organisms, since both iron-deficiency and iron-overload can cause cell death. The Fur protein is known to control bacterial iron homeostasis. The gastric pathogen *Helicobacter pylori* contains few regulators, but is able to adapt efficiently to the variable environment present in the human stomach. The aim of this study was to identify *H. pylori* genes regulated by iron and Fur.

Methods. Gene expression was monitored with (i) the Eurogentec *H. pylori* DNA Array and (ii) Northern hybridisation using RNA isolated from *H. pylori* 26695 wild-type and *fur* mutant cells grown under iron-restricted and iron-sufficient conditions. Protein expression was analysed using 2-dimensional (2D)-protein gel electrophoresis, and binding of Fur was monitored using a gel shift assay.

Results. 12 genes were identified that were repressed by Fur under iron-sufficient conditions. These include genes that are involved in metal metabolism (*fecA1, fecA2, frpB1* and *hp1432*), nitrogen metabolism (*amiE*), motility (*fliP*), cell wall synthesis (*murB*), cofactor synthesis (*pdxAJ, bioB*). Conversely, 14 genes were repressed by Fur under iron-restricted conditions. These include genes that are putatively involved in iron storage (*pfr*), respiration (hydrogenase genes, cytochromes), tRNA synthesis (*trpS*), chemotaxis (*cheV*), oxygen scavenging (*sodB*) and finally the *fur* gene itself. The Fur- and iron-regulation of the *sodB* gene was confirmed using a gel shift assay, with recombinant *H. pylori* Fur, and was also present at the protein level on 2D-protein gels.

Conclusion. When compared to other bacteria, *H. pylori* Fur seems to have acquired novel functions in regulation of several metabolic pathways essential for gastric colonisation. This suggests that Fur is a central regulator of *H. pylori* pathogenicity, and thus proteins regulated by Fur may represent targets for the development of antimicrobial prevention and intervention therapies.

D09

Acid- and nickel-responsive transcriptional induction of ammonia-producing enzymes in *Helicobacter pylori*

A.H.M. van Vliet, J. Stoof, S.W. Poppelaars, A. Heijens, E.J. Kuipers, J.G. Kusters
Erasmus Medical Centre, Department of Gastroenterology and Hepatology, Rotterdam, the Netherlands

Objective. The human pathogen *Helicobacter pylori* colonises the acidic environment of the gastric mucosa. The response of *H. pylori* to acid shocks consists of the production of large amounts of ammonia via urease-mediated degradation of urea. However, the adaptive mechanisms allowing growth at mildly acidic conditions (pH~5.5) are still poorly understood. Therefore the diverse effects of medium acidification and metal supplementation on the expression of the *H. pylori* ammonia-producing enzymes urease, amidase and formamidase was analysed.
Methods. *H. pylori* strain 26695 was grown in Brucella media at pH 7.0 and pH 5.5 or in media supplemented with increasing concentrations of $NiCl_2$ for 16-24 hours, and transcription and activity of the urease, amidase and formamidase enzymes was determined.
Results. Only low levels of urease, amidase and formamidase activity were detected when *H. pylori* was grown at pH 7.0. However, when *H. pylori* was grown at pH 5.5, the activity of all three enzymes was increased three- to tenfold. The increase in enzyme activity levels corresponded with increased mRNA levels for the respective genes. At pH 7.0, medium acidification was mimicked by supplementation of growth media with $NiCl_2$, as this led to similar induction of urease, amidase and formamidase activity. Mutation of the *nikR* gene, encoding the nickel-responsive activator of urease expression, resulted in reduction of acid-responsive induction of all three enzymes. The NikR regulator did not mediate regulation of amidase and formamidase directly, but affected these enzymes indirectly via a regulatory cascade, which includes the iron-responsive regulator Fur.
Discussion. In the acidic conditions to which *H. pylori* is exposed in the human stomach, an increase in ammonia production may take place depending on urea and amide substrate availability. The use of a regulatory cascade to sense acidification of the environment allows fine-tuning of the *H. pylori* response to acid, and this modulation of acid resistance may constitute a key factor in the chronicity of *H. pylori* infection.

D10

Micro-organism-induced mannose-binding lectin (MBL) activation

S. Kuipers, P.C. Aerts, T. Harmsen, H. van Dijk
University Medical Centre Utrecht, Eijkman-Winkler Centre for Microbiology, Infectious Diseases, and Inflammation, Utrecht, the Netherlands,

Aim of the study. To determine if a functional MBL assay can be used to compare the MBL-activating capacities of a variety of micro-organisms.
Background. MBL is an ancient complement component involved in the antibody-independent activation of the lectin pathway, which leads to the opsonisation of micro-organisms with a high content of mannose, fucose and/or N-acetylglucosamine. This results in the microbial ingestion by cells of the mononuclear phagocytic lineage, or in killing by terminal complement complexes.
Material and methods. A functional MBL assay, based on the principle of bystander haemolysis of chicken erythrocytes, was used to study microbial activation in dilutions of human pooled serum. Serum of a known MBL-deficient subject was employed as reagent serum. Different concentrations of (myco)bacteria and yeasts, as MBL activators, were tested to find a Z value of 0.6. A standardised amount of *Saccharomyces cerevisiae* was used as a control. Classical pathway activation was ruled out by the addition of MBL-specific saccharides to the serum. The micro-organisms were ranked according to the concentration needed to achieve MBL-mediated haemolysis in the assay.
Results. *Neisseria meningitidis* groups B and C, *Nocardia farcinica*, *Neisseria gonorrhoeae*, *Salmonella typhimurium* and *Mycobacterium bovis* BCG showed the most potent MBL-activating capacities, as shown by the amount of CFUs needed to achieve a Z value of 0.6. Less than one CFU of meningococci was needed to find MBL activation. New findings were that *Legionella pneumophila* and *Pseudomonas aeruginosa* activate MBL quite strongly.
Discussion. The impressive ability of meningococci to activate MBL is likely to be ascribed to bleb formation, or to another submicroscopic bacterial factor. MBL activation by *P. aeruginosa* may explain why MBL-deficient cystic fibrosis patients have a shortened life span. The role of MBL in legionellosis is under current investigation. In conclusion, the functional MBL assay is very useful in providing mew insight in the role(s) of MBL in genetic susceptibility to infectious diseases.

D11

Identification of virulence-related genes in commensal *Neisseriae*

M.W.J. van Passel, A. Bart, A. van der Ende
Academic Medical Centre, University of Amsterdam, Medical Microbiology, Amsterdam, the Netherlands

Introduction. Commensal *Neisseriae* share a common gene pool with *Neisseria meningitidis*, the causative agent of meningococcal disease. Between these species, horizontal transfer of genes encoding transferrin binding proteins and lipooligosaccharide biosynthesis enzymes has been reported. So far, these data have been obtained by techniques aimed specifically at these loci. We developed a tool, specific adaptor-linked PCR, which allows isolation of different DNA sequences that deviate in nucleotide composition. Such sequences encode many virulence-related genes in *N. meningitidis* MC58. To examine to what extent the commensal neisseriae form a reservoir of virulence-related genes, specific adaptor-linked PCR was applied to *N. sicca* and *N. lactamica*.
Methods. Specific adaptor-linked PCR comprises the digestion of genomic DNA, in this case from *N. sicca* and *N. lactamica*, with a restriction endonuclease followed by the selective amplification of small (<5kb) fragments. Endonucleases with an underrepresented number of recognition sites were selected from the *N. meningitidis* MC58 and Z2491 genomes, because sequence data of genomes of

commensal neisseriae is limited. The DNA fragments obtained are subsequently identified by sequence analysis.
Results. A number of fragments contained sequences showing significant homology with genes present in *N. meningitidis*. These genes, *vapD*, *tonB* dependent receptor and *frpA*, are factors involved in pathogenesis, intracellular survival and an RTX cytotoxin related protein, respectively. Some fragments lack homology on DNA level with any entry in the databases, although they do display an atypical nucleotide composition with respect to GC-percentage.
Conclusions. Specific adaptor-linked PCR is an easy tool to examine to what extent the commensal neisserial gene pool forms a reservoir of virulence genes. In this preliminary study, *N. lactamica* and *N. sicca* isolates were shown to contain sequences similar to *vapD*, *tonB* dependent receptor and *frpA* and can therefore form a broad reservoir of virulence-related genes.

E01

Anaerobic ammonia oxidation in the presence of nitrogen oxides by two different lithotrophs

I. Schmidt[1], K.T. van de Pas-Schoonen[1], M. Strous[1], H.J.M. op den Camp[1], J.G. Kuenen[2], M.S.M. Jetten[1]
[1]KU Nijmegen, Microbiology, Nijmegen, the Netherlands,
[2]University of Technology Delft, Biotechnology, Delft, the Netherlands

The influence of nitric oxide and nitrogen dioxide on the ammonium oxidation of the anaerobic planctomycete *Candidatus* Brocadia anammoxidans and the more versatile beta proteobacterium *Nitrosomonas eutropha* were tested (Schmidt *et al.* 2002 AEM 68, 5351-5357). The anaerobic ammonium-oxidising activity of B. anammoxidans was not inhibted by unusually high NO concentrions up to 600 ppm, and NO2 conentrations up to 100 ppm. B. anammoxidans was able to convert NO which might explain the high NO tolerance of this organism. Addition of NO2 to a mixed culture of B. anammoxidans and *N. eutropha* induced simultaneous specific anaerobic ammonium oxidation activities of up to 92 nmol per mg protein per min and up to 25 nmol per mg protein per min by N. eutropha. The stoichiometry of the ammonium to nitrite ratio, and the microbial community structure were strongly influenced by the nitrogen dioxide additions. The combined activity of B.anammoxidans and N. eutropha might be of great relevance in natural environments and for technical applications in nitrogen removal from waste water (Schmidt *et al.* FEMS Microbiology Ecology 2002;39:175-81).

E02

The denitrification enzymes of the nitrifying bacterium *Nitrosomonas europaea*: familiar players in a new game

H.J.E. Beaumont, H.V. Westerhoff, R.J.M. van Spanning
Free University, Faculty Earth and Life Sciences, Molecular Cell Physiology, Amsterdam, the Netherlands

Classically, the inorganic N-metabolism of nitrifying bacteria is thought to involve only the aerobic oxidation of the free energy sources ammonia and nitrite. The identification of nitrite and nitrate as substrates for the production of nitric oxide (NO), nitrous oxide (N2O) and dinitrogen provided conclusive evidence for the occurrence of denitrification in these bacteria. This anaerobic mode of respiration involves the use of N-oxides as terminal electron acceptors. NO and N2O production by nitrifying bacteria in natural and industrial habitats contributes to the increase of these undesirable gases in the earth's atmosphere. Thus far, the molecular biology and physiological significance of nitrifier-denitrification have remained elusive. We have addressed these issues by construction and analysis of several mutants of the ammonia oxidising bacterium *Nitrosomonas europaea* that are unable to express genes that may be involved in denitrification. This established the presence of genes encoding a copper-type nitrite reductase (NirK), a nitric oxide reductase (Nor) and a regulatory protein involved in the expression of NirK. The NirK-deficient strain still produced NO while having a three-fold increased N2O production rate. Disruption of the norCBQD gene cluster had no effects on N2O and NO production. The NirK-deficient strain had a decreased tolerance to nitrite (NO2-), while the Nor-deficient strain was more sensitive to NO. Unexpectedly, both NirK and Nor were expressed aerobically. The inability to express Nor did not result in a decreased NO2-tolerance of the Nor-deficient strain. Based on these observations we conclude that, i) *N. europaea* has the ability to express NirK and Nor, ii) *N. europaea* has NO and N2O producing mechanisms that that do not involve NirK or Nor, iii) the denitrifying enzymes of *N. europaea* are involved in the protection of the cells against NO2- and NO produced during aerobic nitrification, iv) aerobic expression of NirK in response to NO2- involves a novel regulator, v) NirK and Nor are not functionally coupled as in 'true' denitrifiers.

E03

Direct analysis of mass isotopomers of most primary metabolites in *Saccharomyces cerevisiae* using LC-MS

W.A. van Winden, J.C. van Dam, C. Ras, W.M. van Gulik, J.J. Heijnen
Kluyver Laboratory for Biotechnology, Bioprocess Technology, Delft, the Netherlands

Introduction. Metabolic fluxes in cells are increasingly determined by measuring the ^{13}C-labelling of metabolites in cells grown on labelled medium. Ideally, one directly measures the labelling of primary metabolites, but so far this has been prohibited by their high turnover rates and low concentrations. Instead, the labelling of primary metabolites is deduced from GC-MS or ^{13}C-NMR measurements of ^{13}C-labelled accumulating compounds. As this indirect method has important disadvantages we developed a method for direct measurement of ^{13}C-labelling of primary metabolites.
Methods. *S. cerevisiae* was grown in a chemostat on a glucose/ethanol mixture. After taking a first sample of cells grown on unlabeled medium we switched to medium containing labelled glucose and ethanol and took two more samples after 40 and 60 minutes. The samples were quenched in cold methanol. Intracellular metabolites were extracted in boiling ethanol and separated by anion exchange chromatography. Their mass isotopomer distributions were measured with a mass spectrometer.
Results. LC-MS analysis yielded mass isotopomer distributions of 16 primary metabolites. We compared the

distributions of metabolites formed from unlabeled medium with the theoretically expected distributions and found that only 34% of the measured mass fractions significantly differed (95% confidence level). The mass isotopomer distributions of glycolysis and pentose phosphate pathway (PPP) intermediates were identical in the samples taken after 40 and 60 minutes of ^{13}C-labelled medium supply (2.5% differed). Those of the tricarboxylic acid (TCA) cycle were not (65% differed).
Conclusions. The good agreement between the measured and calculated mass isotopomer distributions of unlabeled metabolites demonstrates the accuracy of the measurements. The reproducibility of the distributions of the glycolytic and PPP intermediates in the samples taken after the onset of ^{13}C-labelled medium supply shows both that these are in isotopic steady state within 40 minutes and that the sampling and quenching of the metabolism is sufficiently fast.

E04
Biochemical and molecular characterisation of arginase from *Agaricus bisporus*

M.J.M. Wagemaker[1], C. van der Drift[1], L.J.L.D. van Griensven[2], M.S.M. Jetten[1], H.J.M. op den Camp[1]
[1]University of Nijmegen, Microbiology, Nijmegen, the Netherlands, [2]Plant Research International, Cell Cybernetics, Wageningen, the Netherlands

An extensive survey of higher fungi reveals that members of the family Agaricaceae, including *Agaricus bisporus*, accumulate substantial amounts of urea in their fruit bodies. Also during postharvest storage the urea concentration increases. Such a large increase in urea content may affect the quality of the stored mushroom, e.g. by the formation of ammonia from urea through urease action. Production of urea is not limited to fruit bodies, but also occurs in the mycelium. Despite the abundance of urea in the edible mushroom *A. bisporus* little is known about its physiological role, although it was proposed that in fruit bodies urea is an end product of catabolic pathways. Two major mechanisms for urea formation can be envisaged: the ornithine cycle and nucleic acid degradation. Arginase is the ornithine cycle enzyme that catalyses the hydrolysis of arginine to urea and ornithine. In the scope of the study on urea metabolism, the work on the mushroom arginase was initiated with the isolation of the arginase cDNA, gDNA and promoter region. Sequence analysis revealed that the cDNA encodes a 311 aa protein which is probably expressed in the cytosol. Expression of the cDNA in *E. coli* was established as a His-tagged protein. The purified recombinant protein was used to determine enzyme kinetics. The characterised gene and enzyme are used as molecular markers to study expression and regulation during sporophore formation and postharvest development.

E05
Hierarchy in the adaptation of *Bacillus subtilis* to nitrogen starvation

W.C. van Heeswijk, D.J. Kiviet, K.J. Hellingwerf
Swammerdam Institute for Life Sciences, Amsterdam, the Netherlands

In *Bacillus subtilis*, a Gram-positive spore forming bacterium, three proteins (TnrA, GlnR and CodY) control gene expression in response to the availability of given nitrogen sources. To characterise the regulon of TnrA we have performed DNA micro-array analysis. For this we have determined the differential expression of genes in a strain over-expressing *tnrA vs*. a strain containing a *tnrA* null-mutation grown in various media. The data of this study will be presented.
To study the hierarchy in the adaptation to a limitation for the optimal nitrogen source, we have searched for growth conditions in which wild type *B. subtilis*, containing an *amtB-lacZ* reporter, shows diauxic growth on the combination of two different N-sources. *AmtB-lacZ* is a well-known reporter for TnrA signalling in *Bacillus subtilis*. When grown on a combination of ammonia plus either glutamate, aspartate, or proline as nitrogen source, induction of *amtB-lacZ* correlated strongly with depletion of ammonia in the medium. Work is in progress to correlate the growth characteristics of the cultures with a global analysis of gene-expression upon diauxic growth.

E06
Compartmentalisation in *Candidatus* 'Brocadia anammoxidans'

L.A. van Niftrik[1], M. Strous[2], J.G. Kuenen[1], M.S.M. Jetten[2], J.A. Fuerst[3]
University of Technology Delft, Biotechnology, Delft, the Netherlands, [2]University of Nijmegen, Microbiology, Nijmegen, the Netherlands, [3]University of Queensland, Microbiology and Parasitology, Brisbane, Australia

Planctomycetes form a distinct phylum and order of the domain Bacteria. They have many interesting properties, one of which is the presence of intracytoplasmic membranes. Various types of cellular compartmentalisation occur. One of its members, *Candidatus* 'Brocadia anammoxidans', contains an inner ribosome-free compartment, called the anammoxosome, bounded by a single membrane. The anammoxosome membrane lipids contain unique linearly concatenated cyclobutane rings. The anammoxosome contains at least one enzyme (hydroxylamine oxidoreductase) involved in the anammox catabolism. Anammox (anaerobic ammonium oxidation) is the anaerobic conversion of ammonium with nitrite (as the electron acceptor) to dinitrogen gas.
This project addresses the functionality of the anammoxosome. The hypothesis is that the closed cyclic system of the anammox catabolism is connected to the anammoxosome membrane and could lead to the build-up of a proton motive force (pmf) across this membrane allowing ATP-synthesis. A separate membrane dedicated to anammox catabolism could be advantageous to the organism because: (1) the anammoxosome enables total control of the physical chemistry of the postulated pmf and thus

more efficient energy generation. (2) The ladderane lipids in the anammoxosome membrane give rise to an exceptionally dense membrane, a tight barrier against diffusion, leading to a lower loss of valuable catabolic intermediates. (3) Membrane specialisation gives more freedom to the organism. The anammoxosome membrane could be used to generate a pmf and to keep toxic intermediates of the anammox catabolism away from the rest of the cell. The outer membrane could be used for homeostasis and thus would have to be relatively permeable. By dividing these tasks, the cell can overcome the problem of needing a single membrane to be both impermeable and permeable.

E07

Identification and analysis of quorum sensing two-component regulatory systems in the human isolate *Lactobacillus plantarum* WCFS1

M.H.J. Sturme[1], E.E. Vaughan[1], M. Kleerebezem[2], A.D.L. Akkermans[1], W.M. de Vos[1]

[1]Wageningen University, Laboratory of Microbiology, Wageningen, the Netherlands, [2]Wageningen Centre for Food Sciences, Wageningen, the Netherlands

Intercellular communication systems in Gram-positive bacteria are based on signalling peptides and involved in cell-density-dependent gene expression (quorum sensing). The exported signalling peptides regulate quorum sensing via two-component regulatory systems (TCS), consisting of histidine kinase (HPK) and response regulator (RR) genes. We are interested in the role of TCS that are involved in auto-inducing peptide (AIP)-regulated quorum sensing in the human isolate *Lactobacillus plantarum* WCFS1, of which the genome has been sequenced and annotated. Potential quorum sensing TCS have been identified in *L. plantarum* WCFS1, and characterisation of these TCS is being done by using transcription profiling with a DNA micro-array of *L. plantarum* WCFS1, bio-informatics and promoter-reporter studies. Genome-analysis has identified 13 TCS distributed over the genome, of which five showed significant homologies to the Agr-like quorum sensing TCS as found in *Staphylococcus aureus*. One TCS showed clear identity to the regulatory system involved in plantaricin production, two others were preceded by putative AIP sequences, and a fourth was located directly upstream of a cell surface protein precursor. One TCS was selected in order to study its putative quorum sensing function as it showed a similar gene organisation as the *S. aureus agr*-cluster and *Enterococcus faecalis fsr*-cluster (both involved in quorum sensing-regulated virulence), and contained a putative AIP. For this TCS a RR-mutant was generated by gene replacement and transcription profiles of wild-type and RR-mutant at different growth-phases were compared by micro-array analysis. Clear differences in gene expression were observed, mainly during mid-exponential growth, supported by promoter-reporter assays that indicated highest activity of the TCS at mid-exponential growth. Cluster-analysis of the transcription profiles and promoter-motif analysis of clustered genes were performed. The use of micro-arrays and genomics will now enable us to elucidate the function of these uncharacterised TCS.

E08

The evolution of hydrogenosomes

B. Boxma[1], J. Tjaden[2], F. Voncken[1], J.H.P. Hackstein[1]

[1]University of Nijmegen, Evolutionary Microbiology, Nijmegen, the Netherlands, [2]University of Kaiserslautern, Plant physiology, Kaiserslautern, Germany

Hydrogenosomes are membrane-bounded organelles with a size of approximately 1-2 micrometer that compartmentalise the terminal reactions of the cellular energy metabolism in a number of anaerobic protists. These protists belong to phylogenetically rather unrelated groups, and it is likely that they evolved several times. To study the evolution of hydrogenosomes, we isolated genes, which encode proteins that are crucial for the hydrogenosomal metabolism in the parabasalid *Trichomonas vaginalis*, the anaerobic ciliate *Nyctotherus ovalis* and the anaerobic chytridiomycete fungi *Neocallimastix* sp. and *Piromyces* sp. We performed a functional and phylogenetic analysis of hydrogenosomal ADP/ATP carriers[1], a phylogenetic analysis of the hydrogenases[2], and an analysis of the functional differences between the various hydrogenosomes. We provide experimental evidence that hydrogenosomes are not the same: The hydrogenosomes of anaerobic ciliates are a kind of anaerobic mitochondria that produce hydrogen[3]. The hydrogenosomes of anaerobic chytridiomycete fungi evolved from the mitochondria of their fungal ancestors through massive structural and metabolic reorganisations[4,5]. The hydrogenosomes of the parabasalid *T. vaginalis,* on the other hand, evolved from a pre-mitochondrial organelle, which 'most likely' produced hydrogen and ATP[1], as predicted by the hydrogen hypothesis of Martin and Mueller.

References

1. Tjaden, et al. submitted
2. Voncken FGJ, Boxma B, Hoek AHAM van, Akhmanova AS, Vogels GD, Huynen M, Veenhuis M, Hackstein JHP. Gene 2002;284:103-12.
3. Akhmanova AS, Voncken FGJ, Alen TA van, Hoek AHAM van, Boxma B, Vogels GD, Veenhuis M, Hackstein JHP. Nature 1998;396:527-8.
4. Akhmanova AS, Voncken FGJ, Hosea KM, Harhangi H, Keltjens JT, Camp HJM op den, Vogels GD, Hackstein JHP. Mol Microbiol 1999;32:1103-14.
5. Voncken F, Boxma B, Tjaden J, Akhmanova A, Huynen M, Verbeek F, Tielens AGM, Haferkamp I, Neuhaus HE, Vogels G, Veenhuis M, Hackstein JHP. Mol Microbiol 2002;44:1441-54.

E09

Characterisation of the predominant *Bacillus* BACREX cluster species in soil by cultivation and 16S rDNA analyses

Y. Li[1], A. Felske[2], W.M. de Vos[3], E.E. Vaughan[3], A.D.L. Akkermans[3]

[1]Huazhong Agricultural University, Key Laboratory of Agricultural Microbiology, Wuhan, China, [2]German Research Centre for Biotechnology, Microbiology, Braunschweig, Germany, [3]Wageningen University, Laboratory of Microbiology, Wageningen, the Netherlands

Culture independent molecular tools were developed for 16S rRNA-targeted detection of *Bacillus* species from the BACREX cluster that was recently discovered to be abundant in soil samples. New primers were designed for BACREX-specific amplification of the V6-V8 16S rDNA region. These primers are suitable for use with Denaturing Gradient Gel Electrophoresis (DGGE), which allows for the rapid

evaluation of composition and activity of bacterial populations in response to changing environmental parameters. Convenient sources of information about the microbial succession over time were archived soil samples from the Wieringermeer polder, where the environment has changed from anaerobic to aerobic and from saline to fresh water during polder formation, since 1932. After each period of change the bacterial ecosystem remained in relative equilibrium over a long time, suggesting that the bacterial community had reached its climax. The influence of different crops on the bacterial community in the polder soils over time was also studied. DGGE results showed that the type of planted crop influenced the total microbial community composition in the samples, as well as the BACREX cluster. The molecular methods were complemented with cultivation experiments. Although some of the polder samples were older than 50 years, BACREX strains could be isolated. The comparison of the BACREX communities in the old polder soil samples with those in the fresh soils indicated that they were more abundant in the latter.
The development of new specific primers now allows for fast detection and screening of the BACREX community in the different soil samples. The present study contributes to our knowledge on the diversity and abundance of this interesting group of microbes in soils throughout the world.

E10

Reconstruction of the O2 uptake rate and CO2 evolution rate on a time scale of seconds

L. Wu[1], H.H.J. Bloemen[1], W.M. van Gulik[1], M.H.G. Verhaegen[2], J.J. Heijnen[1]
[1]Kluyver Laboratory for Biotechnology, Bioprocess technology, Delft, the Netherlands, [2]Delft University of Technology, Faculty of Information Technology and Systems, Delft, the Netherlands

This poster addresses the reconstruction of the dynamics of the oxygen uptake rate (OUR) and carbon dioxide evolution (CER) rate from off-gas concentration measurements and DO measurements during dynamic conditions, like during a pulse experiment. These exchange rates are essential in understanding and modelling the *in vivo* kinetics of micro-organisms. The algorithm fits a model for the mass transport to the measurement data, using so-called smoothing techniques. The model of the mass-transport, including sensor dynamics, is a grey-box model, of which the parameters of the 'white' part are derived from physical knowledge, and of which the parameters of the 'black' part are computed by fitting the model to an identification data set. It is further demonstrated that the net gas production or consumption can have a significant effect on the estimated OUR, and therefore is included into the model. Besides the reconstruction of the OUR and the CER, the monitoring of the mass transfer coefficient is incorporated into the algorithm.

E11

Development of methods for the *in vivo* kinetic modelling of the metabolism of *Saccharomyces cerevisiae*

W.M. van Gulik, L. Wu, M.R. Mashego, J.C. van Dam, C. Ras, A. Proell, J.L. Vinke, W.A. van Winden, J.J. Heijnen
Kluyverlaboratory for Biotechnology, Delft University of Technology, Delft, the Netherlands

The development of recombinant-DNA techniques has opened the possibility of precise modifications in microbial metabolism. The goals of such 'rational metabolic engineering' are de novo or improved production of desirable chemical compounds. However, often product biosynthesis pathways are interconnected at various points with the primary metabolic pathways. Due to the complexity of the resulting metabolic network, recombinant-DNA based improvement of product formation and/or introduction of novel pathways driven by intuition alone is not feasible. A detailed insight in the *in vivo* kinetics of the biosynthesis pathway(s) + interconnected primary routes, in the form of a mathematical model, is therefore indispensable.
Estimation of *in vivo* kinetic parameters of such models requires pulse-response measurements i.e. disturbance of a steady state situation followed by measurement of the concentration profiles of all relevant intracellular and extracellular metabolites within a short time window (typically 200 seconds). This is performed by fast sampling with time intervals of a few seconds followed by rapid quenching of metabolic activity. A new device has been developed (BioSCOPE) to carry out pulse response measurements outside the fermentor, thus preventing disturbance of the steady state and enabling to carry out several pulse response experiments shortly after one another. The bottleneck in this kind of pulse response experiments is the fast and accurate analysis of large numbers of samples. We developed a method for the analysis of the glycolytic intermediates using HPLC with tandem mass spectrometric detection and enzyme based assays carried out in microtiter plates for the analysis of nucleotides and TCA-cylce intermediates.
Comparison of metabolite concentration profiles from pulse response experiments carried out in the BioSCOPE with profiles obtained from fermentor pulse experiments show strikingly similar results. It is concluded that the BioSCOPE is a powerful new tool too carry out pulse reponse studies for *in vivo* estimation of kinetic parameters.

F02

The systemic mycosis array test (SMART)

F.B.J.M. Thunnissen[1], G.S. de Hoog[2], J.F.G.M. Meis[1], H.E. Viëtor[3]
[1]Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands, [2]Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, [3]Multigen Diagnostics, Abcoude, the Netherlands

Introduction. One of the main health problems in the hospitalised population of immunocompromised patients, now that bacterial infections are largely under control, may be systemic infections by fungi.
This may cause an increased morbidity and mortality in patients who might have overcome their primary disorder. Recognition of a fungal infection, as well as identification of the agent

requires specialised histopathological and time consuming culturing skills. It is our central hypothesis that (1) systemic fungal infections are much more common than currently established, and that (2) the diversity of the agents concerned differs significantly from the species-spectrum published in most handbooks. In order to gain insight into this problem, first-line fungal diagnostics should be simplified to such an extent that tests can routinely be applied. To this aim we will develop an 'easy-do' diagnostic micro-array using staining and microscopic techniques available in any clinical lab and which provides specific information within a single day.
Material and methods. The project aims to develop a DNA micro-array for the identification of all potential agents of systemic filamentous fungal infection in humans, either primary pathogens or opportunists, as recently listed in the 'Atlas of Clinical Fungi' (2000); a total of about 150 species will be covered. The array will initially be based on selected ribosomal and intron sequences, which have been proven to deviate sufficiently among the relevant species. The micro-array procedure for genotyping will be similar to the one used for human papilloma viruses in the Canisius Wilhelmina Hospital. The procedure after PCR can be applied in any microbiology or pathology laboratory as this follows the basics of routine immunohistochemistry for visualisation.
Results. The set-up of the HPV system will be presented. Over 36 different HPV types are present in triplicate on a microscope object slide. If a type is present in the PCR amplification this is easily visible in the light microscope.
Conclusion. An 'easy-do' diagnostic micro-array is developed using staining and microscopic techniques available in any clinical lab and for diagnosis and examination of pathogenicity of systemic fungi.

F03
Labelled peptide nucleic acids (PNA) as species-specific probes for fluorescence in situ hybridisation (FISH) enabling an easy-to-perform identification of fungi in blood cultures

G. Haase[1], H. Stender[2]
[1]*Institute of Medical Microbiology, University Hospital RWTH Aachen, Aachen, Germany,* [2]*AdvanDx, Inc, Concord, MA, USA*

In PNAs, the negatively charged phosphate linkages in DNA are replaced with peptomimetic neutral amide linkages. PNA-DNA/RNA complexes form more quickly and are tighter and more specific than analogous DNA-DNA/RNA complexes thereby allowing more robust hybridisation applications. Especially relative insensitivity to ionic strength and pH and resistance to nucleases and proteases during hybridisation provides a wider platform for specific DNA/RNA detection thereby simplifying diagnostic procedures, such as FISH. A novel FISH method is reported that uses PNA probes for identification of *Candida albicans* directly from positive-blood-culture bottles in which yeast was observed by Gram staining. The test is based on a fluorescein-labelled PNA probe that targets *C. albicans* 26S rRNA. The PNA probe is added to smears made directly from the contents of the blood culture bottle and hybridised for 90 min at 55°C. Unhybridised PNA probe is removed by washing of the mixture (30 min), and the smears are then examined by fluorescence microscopy. The specificity of the method was confirmed with 15 reference strains and 75 clinical isolates representing clinical
important yeast species including *C. albicans* (n=35), *C. dubliniensis* (n=30), *C. glabrata* (n=10), *C. krusei* (n=3), *C. parapsilosis* (n=5), and *C. tropicalis* (n=7). The performance of the *C. albicans* PNA FISH method as a diagnostic test was evaluated with 13 routine and 20 simulated yeast-positive blood culture bottles (BacAlert®) and showed 100% sensitivity and 100% specificity. It is concluded that this 2.5-h method for the definitive identification of *C. albicans* directly from yeast-positive blood culture bottles provides important information for optimal antifungal therapy and patient management. Application of the described method holds a great potential for identification of other fungi causing systemic infection, e.g. in case of biopsies containing fungal elements and is therefore well suited as a reliable, cost-effective and easy-to-perform procedure in the diagnostic laboratory.

F04-05
Cryptococcal glucuronoxylomannan (GXM) inhibits adhesion of polymorphonuclear leukocytes (PMN) to stimulated endothelium *in vitro* by affecting both PMN and endothelial cells in both static and dynamic adhesion models

P.M. Ellerbroek, A.M. Hoepelman, F. Wolbers, F.E.J. Coenjaerts
University Medical Centre, AGI, Utrecht, the Netherlands

Introduction. Cryptococcal infections are often characterised by a paucity of PMN in infected tissues. Previous research has shown that the capsular polysaccharide GXM inhibits PMN migration by interfering with chemotactic pathways. In this study we investigated whether GXM can affect the migration of PMN through the endothelium by interfering with adhesion. For this purpose, we employed both static and dynamic adhesion models.
Methods and results. In the static model, pre-treatment of PMN with GXM inhibited PMN adhesion to tumour necrosis factor-α (TNFα)-stimulated endothelium up to 44%. Treatment of TNFα -stimulated endothelium with GXM led to 27% decrease in PMN adhesion. GXM treatment of both PMN and endothelium did not have an additive inhibitory effect. We demonstrated that GXM-induced L-selectin shedding does not play an important role in the detected inhibition of adhesion. L-selectin was still present on PMN after GXM treatment, since it could be further inhibited by blocking antibodies. Furthermore, blocking of GXM-related L-selectin shedding did not abolish the GXM-related inhibition of adhesion. GXM most likely exerts its effect on PMN by interfering with E-selectin-mediated binding. The use of blocking monoclonal antibodies against E-selectin - shown to decrease adhesion in the absence of GXM - did not cause additive inhibition of PMN adhesion after GXM pre-treatment. By using blocking antibodies we demonstrated that the inhibiting effect found after GXM treatment of endothelium probably involves interference with both intercellular adhesion molecule-1 and E-selectin binding. These findings were confirmed in the 'dynamic' rolling model. Here, pre-treatment of PMN with GXM caused inhibition of PMN rolling both on endothelium and on E-selectin transfectants.

F06
Yeasts of the world, an interactive CD-ROM

T. Boekhout[1], V. Robert[1], J. Stalpers[1], G. Gijswit[2], C.P. Kurtzman[3], J.W. Fell[4], I. Roberts[5]
[1]*Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands,* [2]*Expertisecentre Taxonomic Indentification, University of Amsterdam, the Netherlands,* [3]*NRRL-USDA, Peoria, USA,* [4]*RSMAS, University of Miami, Key Biscayne, USA,* [5]*NCYC, Norwich, UK*

This CD-ROM presents a complete taxonomic data set of all currently accepted yeast species, including morphological and physiological data, and ribosomal DNA sequences. The interactive software contains modules for the comparison and integrated use of physiological, sequence and morphological information, facilitating the identification of yeasts using complementary data sets. Many species are illustrated by microscopic and macroscopic images. This product will be useful in a wide range of yeast studies throughout the agro-industrial and medical sciences.

F07
Repellents of the phytopathogenic fungus *Ustilago maydis*

H.J. Deelstra[1], R. Bohlmann[2], J. Dijksterhuis[3], R. Kahmann[4], H.A.B. Wösten[1]
[1]*University of Utrecht, Microbiology, Utrecht, the Netherlands,* [2]*Ludwig Maximiliaans University, Genetics, Munich, Germany,* [3]*Fungal Biodiversity Centre, Utrecht, the Netherlands,* [4]*Max Plank Institute for Terrestric Microbiology, Organismic Interactions, Marburg, Germany*

Repellents of *U. maydis* are polypeptides of 35-53 amino acids cleaved off the larger precursor Rep1 and located in the cell wall of the dikaryon. Crosses of knockout strains do no longer project dikaryotic filaments of the phytoparasitic stage into the air when grown *in vitro*. Repellents seem to be analogous to the hydrophobins of filamentous fungi in this respect. A hydrophobin gene (*Hum2*) has also been found to be expressed in the dikaryotic phase of *U. maydis*. The location and function of the hydrophobin and the functions of Rep1 are the subject of the described research.
Dikaryons of Ä*Hum2* and Ä*Rep1* strains were compared to the wild-type with respect to aerial hyphae formation, cell wall constitution, hydrophobicity and pathogenicity. A fusion of *Hum2* with a LA epitope (Low affinity Haemagglutin Epitope) was used to study the location of Hum2 in cell fractions by immunoblotting.
Immunoblotting showed the presence of Hum2-LA in the cell wall fraction of celextracts, but in such low quantities that it could not be visualised on a proteingel using standard hydrophobinextraction procedures. Scanning electron microscopy (SEM) showed that Ä*Hum2* and wild-type dikaryons produce the same amount of aerial hyphae. Surprisingly, SEM revealed that Ä*Rep1* strains do produce aerial hyphae under dry conditions, but up to 80% of these hyphae cluster together in bundles of 2-6 hyphae. Wetting of the Ä*Rep1* aerial hyphae results in their collapse, whereas wetting of Ä*Hum2* and wild-type aerial hyphae results in a waterdroplet with a high contact angle on the colony surface. Pathogenicity of Ä*Hum2* dikaryons and Ä*Rep1* dikaryons is comparable to the wild-type; about 50% of the exposed plants develop disease. Cell walls of Ä*Rep1* dikaryons contain more alkaline-soluble sugars than wild-type and Ä*Hum2* dikaryons.
These results indicate that repellents have an effect on cell wall architecture, analogous to the effect of the hydrophobin Sc3 on the cell walls of *Schizophyllum commune*. Repellents, although completely different in primary sequence, share more than one function with hydrophobins.

F08-09
Population genetics of the AIDS-associated fungus, *Penicillium marneffei* in South East Asia

M.C. Fisher
Institute of Zoology, London, UK

Globalisation of the HIV virus has resulted in a number of diseases emerging as humans become susceptible to environmental cycles of infection. The fungus *Penicillium marneffei* has appeared in Southeast Asia as the third most common opportunistic infection in AIDS patients. In endemic areas, epidemiological studies suggest that wild rodents may act as zoonotic reservoirs of infection, and that environmental cycles enhance infection rates. However, these hypotheses remain untested.
An MLST typing scheme has been established for *P. marneffei* to investigate these questions. Genetic diversity within 6 genes (both intronic and exonic sequence) has been used to establish phylogenetic relationships between spatially and ecologically separated populations of the fungus. These same isolates have then been genotyped using a panel of 25 di, tri and tetra nucleotide microsatellites. The relative utility of these two classes of genetic markers (i) for describing the population structure of *P. marneffei* and (ii) for use as epidemiological tools, will be shown.

G01-09
Food microbiology

Food microbiology is a branch of microbiology that is characterised by a diversity of disciplines. From an applied point of view it is crucial to be able to calculate chances of contamination, survival and outgrowth of unwanted micro-organisms. For that purpose mathematical models for inactivation, survival and growth are being generated and combined with stochastic (probability estimation) models of the occurrence of micro-organisms in the food chain. However, micro-organisms are not static systems but are able to adapt themselves to the environment in which they find themselves. Moreover, with respect to heterogeneity they are comparable to any biological population. Knowledge of the level of stress response and adaptation options of micro-organisms has always been the prerogative of the research area of microbial physiology. Finally, medical microbiology occupies itself with the study of infection and virulence of amongst others food-borne microbial pathogens. In view of the vast scientific and economical impact of food related infections food microbiology still is highly interdisciplinary and relevant as ever. The unrivalled and ever more fast developments in Molecular Biology have made it possible that we now know the sequence of complete genomes of pathogenic and spoilage micro-organisms such as

Escherichia coli O157:H7, *Salmonella enteritidis, Bacillus subtilis, Bacillus cereus, Campylobacter jejunii, Listeria monocytogenes, Saccharomyces cerevisiae, Aspergiullus niger, Candida albicans*. With the aid of molecular techniques covered in 'genomics' (genome knowledge, transcript analysis, proteome knowledge and a complete metabolic insight in cellular behaviour) we are now able to measure genome-wide the microbial response on its environment. That leads to large amounts of data (e.g. studying Bacillus between 4000 and 5500 datapoints per measurement in a time-resolved analysis of acid stress response). Without the equally astonishing developments in the area of bioinformatics these 'floods of data' would not be translated in substantial increase of knowledge of microbial behaviour in any environment, be that laboratory or e.g. a food related environment. Data-analysis of expression patterns using correlations of gene-expression to certain signal transduction routes in cells is now possible with the aid of advanced algorithms such as REDUCE of Bussemaker *et al*.
In this Food Microbiology session various aspects of food microbiology are covered in 4 seminars. Marcel Zwietering will kick-off the session as newly appointed professor of food microbiology in Wageningen with a review of what stochastic calculations and a knowledge of the probability of occurrence of micro-organisms mean for the control of food safety. Stanley Brul, professor of Molecular Biology in particular Microbial Food Safety, of the Swammerdam Institute for Life Sciences in Amsterdam will highlight new possibilities offered by the genomics techniques and bioinformatics for the study of the function of micro-organisms of relevance to food safety. Examples will include studies with fungi and spore-forming Bacilli. Tjakko Abee, senior scientist and project leader at the Wageningen Centre for Food Sciences (WCFS) will highlight specifically the added value of a thorough understanding of the stress reactions of pathogens in maintaining and enhancing food safety and controlling virulence of food pathogens. Emphasis will be here on *Listeria monocytogenes* and *Bacillus cereus*. Finally, Serve Notermans, senior scientist at TNO-Food Research in Zeist will put all these new developments in the context of the practice of ensuring the safety of food. What can and will industry and public agencies do with the new knowledge on the behaviour of unwanted food borne micro-organisms in the production of nutritious safe food of a high organoleptic quality. How can the governmental organisations use this knowledge in the monitoring of food safety and what does the consumer see of the discussed new developments in food microbiology?

H01

Recent surge in outbreaks of viral gastro-enteritis in Europe may be related to epidemic spread of a new Norovirus variant

M.P.G. Koopmans[1], B. Lopman[2], E. Kohli[3], E. Schreier[4], B. Bottiger[5], L. Svensson[6], C. von Bonsdorff[7], Y. Duijnhoven[8], E. van Strien[1], H. Vennema[1]
[1]RIVM, LIS, Bilthoven, the Netherlands, [2]PHLS, CDSC, London Colindale, UK, [3]University of Dijon, Virology, Dijon, France, [4]Robert Koch Institute, Virology, Berlin, Germany, [5]Statens Serum Institute, Diagnostic Virology, Copenhagen, Denmark, [6]Karolinska, Virology, Solna, Sweden, [7]University of Helsinki, Virology, Helsinki, Finland, [8]RIVM, Infectious Diseases Epidemiology, Bilthoven, the Netherlands

Recently, some highly publicised outbreaks of Norovirus-associated gastro-enteritis in hospitals in the UK and Ireland, and on cruise ships in the US, sparked discussions and speculations on Promed whether this reported activity was unusual. We analysed data collected through a collaborative research and surveillance network of viral gastroenteritis in 9 countries in Europe by compiling data on total number of outbreaks by month and by country, and by comparing genotypes of the viruses involved. The data were compared with historic data, available through a systematic retrospective review of existing surveillance systems in these countries, and in a central database of viral sequences. While there is no conclusive evidence for a true increase in the total numbers of outbreaks since sustained surveillance is not existent in most countries, the total numbers of reported outbreaks were highest for all reported years since 1995. Almost all outbreaks appear to be caused by a single genotype, designated Lordsdale-like, which had a consistent set of mutations in the polymerase gene sequence. This motif was not present in any of the viruses belonging to this genotype for which sequence information was available in the database prior to 2002 (n=450 from 14 countries from 1995 till 2001). Phylogenetic analysis of outbreak strains showed that viral sequences detected in January 2002 were tightly clustered, with an increasing diversity noted later in the year. This data shows that the recent outbreaks are due to an epidemic of a single variant, which emerged early in 2002. The unusually high numbers of outbreaks points at properties of the virus or its transmission, which allow its rapid spread. The poor understanding of epidemics due to enteric viruses, with a possible role of food in their transmission, is worrisome, and points at the need for sustained surveillance for these pathogens and the oral-faecal mode of transmission.

H02

Genotype differences determine *cytomegalovirus* dissemination in the mouse

I. Vliegen, S.B. Herngreen, G. Grauls, S. Stevens, C.A. Bruggeman, F.R.M. Stassen
University Hospital Maastricht, Medical Microbiology, Maastricht, the Netherlands

Introduction. ApoE-/- mice, bred on a C57Bl/6J background, are widely used to study the role of cytomegalovirus (CMV) in atherosclerosis. Unfortunately, the C57Bl/6J background strain has been described as being rather resistant to mouse

CMV (MCMV). In this study, we investigated whether knocking out the apoE gene renders C57Bl/6J mice more susceptible to MCMV, as previously shown for bacterial infections.

Material and methods. After infection with 5.10^4 PFU, viral dissemination was determined in salivary gland, spleen, liver, lung, bone marrow, aorta, blood and heart of ApoE-/- (KO) and ApoE+/+ (WT) mice at 1, 2, 4, 6, 14 and 28 days post infection (p.i.) using real time PCR (n=4/group). Plasma IFNγ and TNFα titres were determined by ELISA. Furthermore, spleen macrophages (mφ) were isolated from both KO and WT, infected with MCMV or mock and incubated for 24, 48 or 72 hrs. Production of various cytokines (IL10, IL12, IL18, IFNγ and TNFα) was determined at all time points.

Results. Virus dissemination was similar in KO and WT, with peak levels in spleen, liver and blood at 2 days p.i., while in bone marrow, aorta, heart and lung peak levels were observed 4 to 6 days p.i. At 14 days p.i., peak levels were seen in the salivary gland, while MCMV titres were very low or undetectable in all other organs at this time point. Surprisingly, the number of MCMV DNA copies was significantly higher in all organs from WT mice. No differences were observed in plasma IFNγ and TNFα levels. With respect to spleen mφ, IL-18 production was significantly increased in KO mφ, both under baseline conditions (mock) as well as p.i. Furthermore, IL-12 production following infection was significantly elevated in KO mφ. IL-10, TNFα and IFNγ production was not different between KO and WT mφ.

Conclusions. In contrast to our hypothesis, WT mice seem to be more sensitive to MCMV infection than KO mice. As IL-18 production by KO mφ is already increased at baseline, this suggests a higher activation status of the immune system in KO mice, which may allow the host to surmount an early attack on the virus, thus leading to inefficient viral dissemination.

H03

Hepatitis E in the north-eastern part of the Netherlands, a retrospective study

K. Waar[1], M.P.G. Koopmans[2], C.A. Benne[3]

[1]University Hospital Groningen, Department of Medical Microbiology, Groningen, the Netherlands, [2]National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands, [3]Regional Public Health Laboratory Groningen and Drenthe (SLGD), Groningen, the Netherlands

Hepatitis E virus (HEV) is the major aetiological agent of enterically transmitted non-A, non-B hepatitis in central Asia, the Middle East, north and West Africa and Mexico. In these regions, >50% of the acute sporadic hepatitis is caused by HEV. Evidence provided in recent years shows that hepatitis E is also prevalent in very low numbers in non-endemic countries and HEV is sporadically found as a causative agent of acute hepatitis in patients without a history of travel.

In the area covered by the Regional Public Health Laboratory Groningen and Drenthe (SLGD), we recently discovered HEV antibodies in a cohort of 3 patients with acute hepatitis but no history of travel. HEV genome sequence clustering with HEV from pigs in the Netherlands was detected in one of the patients. Since it is very important for treatment to differentiate between viral and auto-immune hepatitis, we decided to study whether HEV is present more frequently among patients with acute hepatitis in the SLGD area. The seroprevalence of hepatitis E was determined among 209 patients with signs of hepatitis between August 2000 and June 2002. All sera tested negative for hepatitis A to C and other causes of hepatocellular damage. Antibodies were detected with two different Enzyme Immune Assay's; Genelabs Diagnostics HEV ELISA for IgG and IgM and the Abbot HEV EIA for IgG.

By use of the Genelabs EIA we found 16 (7.7%) patients with IgG and 15 (7.2%) patients with IgM anti-HEV, with the Abbot EIA we found 15 (7.2%) patients with IgG anti-HEV. Eleven (5.3%) patients were positive in both the Genelabs and Abbot IgG test and 4 (1.9%) patients were positive in all three tests. The prevalence of anti-HEV IgG in this group was higher than the prevalence of 1.1% as reported by Zaaijer *et al.* (Nature 1993;341:826) among blood donors, suggesting there might be a role for HEV among patients with acute hepatitis in the SLGD area. Future work will focus on confirmation of results by immunoblot and PCR as the EIAs are not based on European strains. Also, studies will have to be conducted to determine the seroprevalence among healthy people in the area.

H04

Risk factors for adenovirus infection and death in paediatric stem cell transplant recipients

J. Schinkel[1], M.J.D. van Tol[2], A.C.M. Kroes[1], W. Dinkelaar[2], C.M. Jol-van der Zijde[2], J.M. Vossen[2]

Leiden University Medical Centre, [1]Medical Microbiology, [2]Department of Paediatrics, Leiden, the Netherlands

Introduction. Adenovirus (ADV) infection in stem cell transplant (SCT) recipients is increasingly recognised as an important pathogen causing significant morbidity and mortality. We studied the records of paediatric SCT patients between 1985 and 1998 to assess incidence and risk factors for the occurrence of ADV infection and ADV related death.

Methods. The study group consisted of 328 patients who received an allograft for malignant or non-malignant haematological disease. Incidence and risk factors for ADV infection as evidenced by positive faecal, throat or urine culture during the first 6 month after SCT were studied using Kaplan-Meier (KM) product limit methods and Cox-survival analysis. A separate survival analysis was carried out among the ADV positive patients to asses the risk to die from ADV and its predictive parameters.

Results. in 37 of 328 patients ADV infection occurred after a median of 35 days. KM estimated incidence of ADV infection was 12%. Incidence of ADV infection increased from 4% in the period 1985-1994 to 22% from 1995 onwards. Independent risk factors associated with the occurrence of ADV infection were: age <= 4 years (HR 2.4), rigorous T-cell depletion (TCD: HR 2.2), a non-related or non-identical donor (HR 3.5), transplant period >= 1995 (HR 4.0). Seven patients died as a result of ADV infection (KM incidence 22%) with rigorous TCD being the only significant predictor of death (HR 5.2).

Conclusion. The incidence of ADV infection is increasing in paediatric SCT patients. This cannot solely be explained by an increase in the occurrence of the identified risk factors (age, T-cell depletion and donor type). The occurrence of an

ADV infection has a significant risk of death and is associated with a rigorous T-cell depletion of the graft.

H05

Fitness and evolution of *Human Immunodeficiency Virus* during antiretroviral treatment

M. Nijhuis, R. Schuurman, D. de Jong, P. Schipper, C.A.B. Boucher

University Medical Centre, Virology, Utrecht, the Netherlands

Introduction. During the past decade potent antiretroviral drugs have been developed for treatment of HIV infection. However, virological failure occurs frequently and is usually associated with the emergence of drug resistant viruses. The development of drug resistance may not be completely hopeless, because selection of resistant viral variants may affect the fitness of the viral population and change the pathogenesis of the disease.

Methods. The effect of antiretroviral therapy on two viral factors contributing to the fitness of the viral population is under investigation: the replication capacity and the overall degree of diversity. The viral replication capacity is measured in viral competition experiments in donor peripheral blood mononuclear cells and the degree of viral diversity is determined by clonal sequencing of the variable V3 loop of the HIV envelope.

Results. We demonstrate in patients treated with either protease-inhibitor monotherapy or reverse transcriptase (RT)-inhibitor monotherapy that selection of the initial drug resistant viral variants is accompanied by a reduction in the variation in the viral population (population bottlenecking). In addition to a change in viral variation we also demonstrate that the viral variants that are initially selected during treatment display a severe reduction in viral replication capacity. Upon continuous treatment, novel viral protease variants with additional mutations are selected. These variants display no further increase in drug resistance but demonstrate an increase in viral replication capacity. Interestingly, some of these viral protease variants replicate even better than the original wild-type protease. To what extent restoration of the viral replication potential subsequently influences changes in viral diversity is currently being investigated, but we have demonstrated in a small patient group that the bottlenecking effect persists at least six months.

Conclusion. We have demonstrated that antiretroviral treatment results in a change in both the variation in the viral population and in the replication capacity of the viral population. This means that after treatment failure we have a completely different viral population, which may not be able to respond well to changes in the environment.

H06

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

A. Meijer[1], E.E.H.M. van de Kamp[1], G. Koch[2], T.G. Kimman[3]

National Institute of Public Health and the Environment, [1]Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, [3]Laboratory for Vaccine-Preventable Diseases, Bilthoven, the Netherlands, [2]Central Institute for Animal Disease Control, Section Notifiable and Exotic Viral Diseases, Lelystad, the Netherlands

Increased use of antiviral drugs to manage influenza requires, ideally, universal methods to determine the susceptibility of influenza virus strains for these drugs. For this purpose, a Cell-ELISA assay (cELISA) which can be used with the old (amantadine) and new (neuraminidase inhibitors oseltamivir and zanamivir) anti-influenza drugs was developed and characterised. The assay is based on detection of influenza virus nucleoprotein 18 hours post inoculation in MDCK cells. Influenza A viruses A/Chicken/Pennsylvania/21525/83 (H5N2), A/Chicken/Saoudi-Arabia/569017/00 (H9N2), and A/PR/8/34 (H1N1) were used. Because reproducible virus spreading is a prerequisite for a good performing cELISA, the heterogeneous MDCK cell line was cloned. Two clones, MDCK-T CB4 and MDCK-I BD5, were selected that showed the highest level of virus spreading. For amantadine, the IC50s were similar with both clones. The A/PR/8/34 virus was resistant for amantadine. The IC50s of the avian viruses for oseltamivir and zanamivir using clone MDCK-T CB4 paralleled those estimated using a fetuin-based biochemical neuraminidase inhibition (NI) assay. For A/PR/8/34 virus, the IC50s estimated using the cELISA were always up to 70-fold higher than in the NI-assay. Using MDCK-I BD5 cells, the IC50s for the neuraminidase inhibitors could not be estimated or only with a higher estimate and a wider confidence interval than with MDCK-T CB4 cells due to extensive neuraminidase-induced pile up of virus on the membranes of infected cells. In conclusion, since the cELISA performed well with three very distinct influenza viruses and three different antiviral drugs our results suggest that the cELISA using MDCK-T CB4 cells can be universally used. However, since adjustment of the cELISA appeared to be virus specific this approach is only recommended in a research setting and not for routine estimation of IC50s. Because results between the cELISA and the NI-assay may differ for particular strains, further work should indicate which method gives the best prediction of the *in vivo* situation.

J01

Actinomycetoma of the thumb caused by *Gordona terrae*

M.W.H. Wulf[1], X.R. Bakker[2], P.H.M. Spauwen[2], T. Schülin[1]

University Medical Centre St Radboud, [1]Medical Microbiology, [2]Plastic Surgery, Nijmegen, the Netherlands

In 2001 an 18-year-old patient from Sierra Leone presented himself with a painful/painless lesion on the left thumb (tenar region) that had been slowly progressive for almost

four years. There were multiple sinuses draining pus and granules. The patient had received no previous treatment and there were no other complaints. X-ray of the hand showed no bone involvement. Cultures of swabs and granules remained sterile.

Surgical debridement was performed. Examination of biopsy material showed sporadic leukocytes with sporadic gram-positive rods. Culture on sheep-blood agar yielded raised, rough, salmon-coloured colonies after 5 days of incubation at CO2. Gram stain of cultures showed non-branching Gram-positive rods. ZN/kinyoun stains were negative. Susceptibility testing was performed by disc diffusion on sheep blood agar.

Biochemical determination as *Rhodococcus* species was performed with biochemical tests (Api Coryne, Biomerieux) and *Gordona terrae* (formerly *Rhodococcus terrae*) was identified by means of 16S rRNA gene sequencing. This aerobic, Gram-positive, slightly acid-fast, non-motile short rod belongs to the family of the *Nocardiaceae, genus Gordona*. It does not form spores or capsules and does usually not produce aerial hyphen. *Gordona terrae* has been isolated from soil and sputum. Only a few cases of human infection are described. To our knowledge this is the first description of a mycetoma caused by *Gordona terrae*.

After surgery, the patient received two weeks of doxycycline 100 mg once daily with a starting dose of 200 mg. The lesion healed and no relapse has occurred.

J02

Pseudotumour of the right upper arm due to *Bartonella henselae* infection

F. Bosma[1], M.W.H. Wulf[1], C. Smeets[2], M. Pruszczynski[3], C.M.E. Weemaes[4], J.M.D. Galama[1]

University Medical Centre St Radboud, [1]Medical Microbiology, [2]Pediatrics, [3]Pathology, [4]Pediatrics, Nijmegen, the Netherlands

An 8-year-old girl was referred to a paediatric surgeon with fever and a painful swelling of the right elbow, which was initially considered as a bursitis due to a judo injury. Initial treatment with flucloxacilline had no effect. At physical examination we saw an apparently healthy girl with a temperature of 38°C and no lymphadenopathy or skin lesions. At the elbow a painful, solid tumour of 23 cm was palpable. MRI showed a soft tissue tumour near the distal side of the right humerus, closely related to vascular and nervous structures, appearing as a malignancy. Therefore, the patient was admitted to the pediatric oncology ward for a diagnostic biopsy, where histopathology showed a granulomatous inflammation with focal necrosis. Gram, Ziehl-Neelsen, Grocott and silver staining according to Steiner showed no micro-organisms. Mycobacterial cultures remained sterile. Differential diagnosis included *Bartonella* infection; the family kept several pets, including a cat. *Bartonella* ELISA results, however, were negative (National Institute of Public Health and Environment, RIVM, Bilthoven, the Netherlands). Polymerase chain reaction, targeting the 16S rRNA gene of *Bartonella. henselae*, performed on the biopsy tissue was positive (Department of Medical Microbiology, St. Elisabeth Hospital, Tilburg, the Netherlands).

B. henselae causes cat-scratch disease (CSD) and is classically described as self-limited regional lymphadenitis in young immunocompetent patients. Unusual manifestations of CSD include osteomyelitis, endocarditis, encephalopathy, transverse myelitis and oculoglandular syndrome. This case demonstrates that *Bartonella* infection should also be considered in the differential diagnosis of a soft tissue tumour. Molecular techniques used on biopsy tissue contribute to a quick and adequate diagnosis of *B. henselae* infection, especially when serology results are negative.

J03

First report on methicillin resistant staphylococci from animals in the Netherlands

E. van Duijkeren[1], A.T.A. Box[2], W. Wannet[3], J.A.H. Smit[1], A.C. Fluit[2]

[1]Veterinary Microbiological Diagnostic Centre, Utrecht University, Utrecht, the Netherlands, [2]Eijkman-Winkler Institute, University Medical Centre Utrecht, Utrecht, the Netherlands, [3]RIVM, LIS, Bilthoven, the Netherlands

In the Netherlands, methicillin resistant *Staphylococcus aureus* (MRSA) are cultured regularly from humans, but not from animals. We present the first isolation of MRSA from animal origin in the Netherlands. A coagulase positive staphylococcus was cultured from an infected wound in a Dutch dog, which had recently been operated in Italy. The staphylococcus was resistant to ampicillin, amoxycillin+ clavulanic acid, cephalexin, erythromycin, lincomycin, tetracycline, gentamicin, enrofloxacin and methicillin. It was identified as *S. aureus* by fermentation of mannitol and Martineau-PCR. The presence of *mecA* was confirmed by PCR. Further analysis of the staphylococcal cassette chromosome *mec* (SCC*mec*) showed that it was of type I, a type common in MRSA from human origin. Of ten multiresistant staphylococci from the strain collection of the Veterinary Microbiological Dianostic Centre 5 were *mecA* positive. These clinical isolates were from three dogs, a horse and a cat. Four were coagulase positive staphylococci and one was coagulase negative. Most of these isolates were unknown SCC*mec* types suggesting that these isolates are not from human origin. In conclusion, veterinarians should be aware of the risk of infections with methicillin resistant staphylococci, not only from patients returning from abroad, but also in animals without a recent history of travelling abroad.

J04

Borrelia burgdorferi-associated lymphocytoma cutis of the glans penis simulating a primary cutaneous B-cell lymphoma

F.G.C. Heilmann[1], H.M. Bruns[2], H. van Dessel[1], J. Kissing[1], J.F.P. Schellekens[3]

Gelre Hospitals, [1]Medical Microbiology and Infectious Diseases, [2]Dermatology, Apeldoorn, the Netherlands, [3]National Institute of Public Health, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, Bilthoven, the Netherlands

Introduction. The histological distinction between primary cutaneous B-cell lymphoma (PCBCL) and B-cell pseudo-lymphoma may be difficult, particularly in some cases of *Borrelia burgdorferi* (Bb)-associated lymphoid proliferations. In this case, while histology suggested PCBCL, positive *Bb*

Abstracts

serology led to the diagnosis of *Bb*-associated pseudolymphoma (lymphadenosis benigma cutis {LBC}).
Case history. A 61-year-old man presented with a 3 month-existing red plaque on the glans penis. A course of local corticosteroid was ineffective and a biopsy was performed. Since histology showed a very dense infiltrate of large lymphoid cells with enlarged nuclei, a PCBCL was suspected. The patient lived in an endemic region for *Bb* infections, therefore the possibility of a LBC was also considered. *Bb* serology revealed highly elevated IgG antibody titres. IgG-immunoblot (with human isolate A39, *Borrelia afzelii*) showed strong antibody-binding to 17kD, 22kD, 41kD, 58kD and 92kD; IgM-immunoblot showed strong antibody-binding to 18kD, 22kD and 41kD. Serological reations on *Treponema pallidum* were negative. Histological staining for *Bb*-like organisms were negative and no *Bb*-specific DNA sequences could be detected by PCR. The patient was treated with doxycycline 100 mg twice daily orally for 4 weeks. After 2 weeks the tumour had regressed considerably and after 8 weeks it had disappeared completely.
Discussion. The typical histological features of LBC include a dense infiltrate of lymphocytes and histiocytes characterised by follicles mostly with germinal centres. Because of the absence of germinal centres a provisional diagnosis of PCBCL was made. A *Bb* induced LBC was considered based on high endemicity of *Bb* infections in the region and the presence of *Bb*-specific IgM and IgG antibodies. The diagnosis was confirmed by prompt and complete cure by antibiotic therapy.
Conclusion. This case suggest that in *Bb*-endemic regions, patients with PCBCL of LBC should be evaluated for *Bb* infection. As far as we are aware this is the first description of *Bb* pseudolymphoma localised at the glans penis.

J05
Brucellosis in a game butcher: need for reliable subspeciation to trace the infection

J.A. Wagenaar[1], C. Appels[2], A.H.W. Schoormans[1], E.A.P.M. Thewessen[3], T. Koster[2]
[1]Veterinary Microbiological Diagnostic Centre, Utrecht, the Netherlands, Groene Hart Hospital, Departments of [2]Internal Medicine and [3]Clinical Microbiology and Infection Prevention, Gouda, the Netherlands

A male patient was hospitalised with ongoing peaks of high fever. The patient reported health problems that started 3 weeks earlier and were characterised by fever (peaking with intervals of 24 hours), headache, progressive pain in muscles of neck, general malaise and an 8 kg weight loss. Treatment with cefuroxim was started. Bacteriological cultures performed on 5 blood samples taken on two separate days showed aerobic growth in 2-3 days and subsequent subculturing showed a Gram-negative rod on blood agar within 24 hours. The bacterial isolates were biochemically typed as *Brucella spp*. After this finding the patient was treated with doxycyclin and rifampicin whereafter his clinical situation improved rapidly. Three months later the patient was still suffering from muscle pain but after half a year the patient had fully recovered. One of the Brucella isolates was single colony cloned several times and submitted to three different (reference) laboratories in and outside the country for determination of species and serovar. This resulted in three different definitive typing results: *Brucella suis* serovar 1, *B. suis* serovar 4, and *Brucella melitensis*. The patient was a butcher processing game meat from the Netherlands and from other countries all over the world. The three Brucella species that were identified by the laboratories are related to different animal hosts, which makes tracing and intervention rather difficult. This case study underlines the difficulties in pheno- and genotyping Brucella due to the clonality of the genus.

J06
Clonal identity of *Staphylococcus aureus* isolates in repeat bacteraemia, demonstrated by pulsed-field gel electrophoresis

J.A. Jacobs[1], P.L.J.M. Leroy[2], A.W.D. Gavilanes[3], N. London[1], C. Driessen[1], C. Vink[1]
University Hospital Maastricht, [1]Medical Microbiology, [2]Pediatrics, [3]Neonatology, Maastricht, the Netherlands

Background. *Staphylococcus aureus* belongs to the most frequently recovered organisms in bacteraemia. Bacteraemia with *S. aureus* may be difficult to eradicate. In repeat bacteraemia, reinfections must be distinguished from relapsing infections that originate from a persistent infectious focus. Recently, we observed two infants with in each patient consecutive episodes of *S. aureus* bacteraemia occurring more than two months apart.
Patients and methods. Patient 1 was a premature girl delivered by Caesarean section at a gestational age of 30 weeks. After an episode of necrotising enterocolitis for which hemicolectomy was performed, bacteraemia with *S. aureus* occurred at day 24, 25, 26 and 105 after birth, respectively. A right atrial thrombus was considered as the possible focus of persistent infection. Patient 2 was a newborn girl with oesophageal atresia and severe tracheomalacia for which surgical correction was performed. Bacteraemia with *S. aureus* occurred at day 19 and 95 after birth. Also this patient suffered from an intravascular thrombus (inferior caval vein). Genetic relatedness between the consecutive *S. au*reus isolates in both patients was assessed by pulsed-field gel electrophoresis (PFGE) analysis of *Sma*I restricted chromosomal DNA.

K01-10
Work group epidemicity markers and bacterial typing (WET)

The Netherlands working group of epidemicity markers and bacterial typing (Werkgroep Epidemiologische Typering, WET) is an ad hoc group of microbiologists involved in microbial typing. The group organises workshops and symposia, and has a website (www.typeringen.nl) with information on typing activities in the Netherlands.
In the present session, invited speakers will discuss several topics.
The first lecture deals with the use of a combination of typing methods (polyphasic characterisation) to characterise bacteria of the *Burkholderia cepacia* complex.
A second talk is on the generation and electronic exchange of microbial fingerprints using the Internet.
Next, a book on microbial typing methods will be presented.

This book has been written by members of the WET and expert colleagues, and is published with support of the NVVM.
Further talks will deal with the epidemiological typing of enteric viruses, which is a new field, and by the presentation of 'screening flows' to assess the diversity of cultures in biotechnology by the use of genotypic methods.

L10

Primary toxoplasmosis in a renal transplant patient

M.W.H. Wulf[1], R. van Crevel[2], A. van der Ven[2], J.M.D. Galama[1]
University Medical Centre St Radboud, [1]Medical Microbiology, [2]General Internal Medicine/section Infectious Diseases, Nijmegen, the Netherlands

A 58-year-old woman with end stage renal failure due to polycystic kidney disease received a renal transplant from a related living donor. Five months later she was admitted to our hospital because of fever, headache and severe dyspnoea. The chest X-ray showed an interstitial pneumonia. She was treated empirically with ciprofloxacin and co-trimoxazole. Despite extensive cultures of broncho-alveolar lavage fluid no diagnosis was made. She recovered but because of weakness attributed to critical illness neuropathy she was transferred to a rehabilitation centre. Four weeks later she collapsed, was lethargic, dysartric and complained of headaches. A subsequent CT scan showed multiple hypodense areas. Empirical therapy with ceftriaxone, voriconazole and co-trimoxazole was started. Cerebrospinal fluid analysis showed elevated protein level, normal glucose and no leukocytosis. Bacterial and fungal cultures remained negative as were cryptococcal antigen and aspergillus galactomannan testing. A brain biopsy showed inflammation. Funduscopy showed chorioretinitis. She was seropositive for *Toxoplasma gondii* (*T. gondii*) IgG but PCR on CF a brain biopsy were negative. Retrospective analysis of sera showed a seroconversion for *T. gondii* IgG during the episode of the pneumonia. PCR for *T. gondii* on blood and bronchoalveolar lavage fluid from this episode was positive. The patient was treated successfully with pyrimethamine, sulfadiazine and folinic acid.
Our patient had a primary *T. gondii* infection starting with pneumonitis and progressing into chorioretinitis and encephalitis. Diagnosis was made by serology and PCR on blood and broncho-alveloar lavage fluid. It is unclear how this primary infection was acquired; the donor had high IgG levels on time of transplantation but no IgM or IgA antibody. The interval between transplantation and start of this unusual presentation of *T. gondii* infection is longer than in other transplant-acquired cases (5 versus 1 month).

L11

West Nile virus encephalitis in an elderly Dutch patient

S. Kuipers[1], J.J.W. Prick[2], H.D.R. Kuipers[3], J.H.R. Vliegen[2], G.J.J. van Doornum[4]
[1]Regional Public Health Laboratory, Microbiology, Enschede, the Netherlands, Medical Spectrum Twente, [2]Neurology, [3]Internal Medicine, Oldenzaal, the Netherlands, [4]Erasmus Medical Centre, Virology, Rotterdam, the Netherlands

Shortly after returning to the Netherlands from a journey to those parts of Canada where West Nile virus is prevalent, a 69-year-old man developed fever, and signs and symptoms of an encephalitis. Electroencephalogram results and cerebrospinal fluid examination were compatible with the diagnosis of encephalitis of viral origin. MRI of the brain did not contribute to the diagnosis. Two consecutive sera showed positive IgM antibody as well as rising IgG antibody titres against West Nile virus, thereby confirming the diagnosis of West Nile virus encephalitis. Antibodies or viral RNA could not be detected in cerebrospinal fluid. The patient fully recovered, and the EEG normalised too. This report describes the first imported Dutch case of West Nile virus infection contracted on the North American continent.

L12

Response to the West Nile virus threat in the Netherlands

M.P.G. Koopmans[1], K. van den Wijngaard[2], W. van Pelt[2], A. Bosman[2]
RIVM, [1]Diangostic lab for Infectious Diseases, [2]Infectious diseases Epidemiology Centre (CIE), Bilthoven, the Netherlands

Following reports on the introduction of West Nile virus (WNV) in the USA, an initial risk assessment was done to determine the likelihood of introduction of WNV in the Netherlands, and no further action was taken. However, the recent reports on flavivirus in non-migratory birds in the UK and Austria suggested a changing epidemiology for flaviruses in Europe as well. Therefore, in October 2002, a project group was launched to study trends in neurological illness of possible viral aetiology in the Netherlands. The aim of this project is to make an inventory of data sources that may provide information on emerging illness due to endemic flavivirus transmission, and design a cost effective prospective surveillance system for encephalitis. Neurological illness of possible viral aetiology other than acute flaccid paralysis is not notifiable in the Netherlands. We reviewed data provided by the administration of hospital discharge records, and data from submissions of cerebrospinal fluids for testing for neurotropic viruses, which were extracted from a routine laboratory surveillance network (coverage 2.6 million; 16% of the population). In addition, we initiated a retrospective study of CSF samples that tested negative for known pathogens. The hospital discharge records indicate that approximately 400 cases of encephalitis e.c.i. occur each year. The laboratory submission data showed 3879 CSF analyses for the past 3 years, most of which (80%) were done by 2 laboratories. The vast majority of tests (>96%) were negative. The submissions, including those for persons between 50 and 70 years of age showed a slight increase during summer in 2001 and 2002, whereas for submissions of CSF from elderly a peak in winter of 2001/2002 was noted. The comparison of lab results shows that diagnostic evaluation of CNS disease is poorly harmonised, that the majority of cases goes without a diagnosis, and that only a small proportion is tested for WNV in the national reference laboratory for imported viral diseases handles. We conclude that low level WNV activity might go undetected in the Netherlands.

L13

Febrile seizures and viral infections: a case presentation

J.H. van Zeijl[1], B. Wilbrink[2], J.M.D. Galama[3]

[1]Laboratorium voor de Volksgezondheid in Friesland, Medische Microbiologie, Leeuwarden, the Netherlands, [2]Rijksinstituut voor Volksgezondheid en Milieu, Laboratorium voor Infectieziektendiagnostiek en Screening (LIS), Bilthoven, the Netherlands, [3]Institute of Medical Microbiology and Nijmegen University Centre for Infectious, Virology Section, Nijmegen, the Netherlands

Background. Although fever is commonly held responsible for the occurrence of febrile seizures (FS) alternative explanations have been proposed, with emphasis on a more direct pathogenic role of particular infectious agents. In this context viruses are far more frequently involved than bacteria, and certain viruses more often than others. Enteroviruses have been isolated from CSF in patients with FS, and may be responsible for meningo-encephalitis. Other viruses that have been isolated from several clinical specimens are influenza virus, parainfluenzavirus, respiratory syncytial virus (RSV), and adenovirus. However, publications on this topic show considerable variation, and cerebrospinal fluid seldom showed positive findings in viral culture. The introduction of molecular techniques elaborated the rapid diagnosis of infections with herpes simplex virus as well as other herpesviruses (e.g. HHV-6/7).

Methods. During the 2001/2002 winter period a 6 months old boy suffered from three episodes of FS. A nasopharyngeal swab for viral culture was obtained on all three events. Samples were inoculated on HEL, Hep2, $LLcMK_2$, and RD-cells, respectively.

Results. Three FS events were documented, with progression of severity of the convulsion in time. In these three episodes, virus culture revealed parainfluenzavirus, RSV-B, and RSV-A, respectively.

Conclusion. By this case presentation we attempt to underline the impact of viral infections as a trigger in FS. Some viral agents may be candidate for preventive measures. Complications like recurrent FS as well as the development of non-febrile convulsions may be related to specific viral agents.

L14

A patient with pain in the back. Pain everywhere

A. van Griethuysen[1], A. Rolink[2], C. Richter[2], L. Verschoor[2], C. Swanink[1]

Hospital Rijnstate, [1]Medical Microbiology, [2]Internal Medicine, Arnhem, the Netherlands

A 61-year-old lady of Turkish origin presented at the outpatient clinic of internal medicine with generalised pain, mainly located in the lower back and muscular pain in the shoulders and lower limb girdle. Three weeks before the general practitioner started with prednison 20 mg/day under the presumptive diagnosis of polymyalgia rheumatica. At evaluation a high Erythrocyte Sedimentation Rate (ESR) of 67 mm/h, a C-reactive protein level (CRP) of 24 mg/l, a haemoglobulin concentration (Hb) of 6.0 mmol/l and a normal leukocyte count (8.8 x 10 E9/l) was found. An X-ray of the spine showed degenerative changes with narrowing of the disk space and spondylarthrosis. Six weeks later she was hospitalised for further analysis. Anamnestic and at physical examination she had increased pain at both knees, shoulders and back. At physical examination there were no signs of arthritis. Laboratory investigation showed: ESR 91 mm, CRP 115 mg/l, Hb 5.6 mmol/l and LDH 560 U/l. After admission a MRI-scan was performed that showed discitis in Th8, Th9, L2 and L3 vertebrae. A punction under CT guidance was send to the laboratory for culture but showed no growth. Blood cultures however became positive with Gram-negative coccobacilli, which were later identified as *Brucella spp*. Serum samples drawn before and at admission were positive for antibodies against Brucella, titers >1:1280. The patient was treated with doxycycline and rifampin. She probably acquired Brucellosis eating unpasteurised cheese at a stay in Turkey. At our laboratory several technicians had sniffed the culture before they suspected a *Brucella spp*. These technicians were offered prophylaxis with doxycycline for 3 weeks. One can discuss if brucellosis can be acquired by merely sniffing a culture and whether prophylaxis can prevent this. This case illustrates the importance to warn the laboratory when Brucella is suspected and the importance for the technician to handle an isolate that grows after 48 h with special safety precautions.

L15

A HIV patient with neurological deterioration and discrepant viral load between plasma and CSF

B.U. Ridwan, C.A.B. Boucher, R. Schuurman, M. Schneider

University Medical Centre, Utrecht, the Netherlands

Case presentation. A 40-year-old HIV patient was admitted because of neurological deterioration. The patient received antiretroviral therapy (AZT, 3TC and nelfinavir/saquinavir) since 1996. The patient had been clinically stable until six months before admission, when progressive confusion and memory loss developed. At that time his CD4 count was 908/mm^3 and peripheral HIV viral load was 83 copies/ml. The MRI showed general white matter lesions. Serology for Lues, Borrelia and Toxoplasmosis was negative. CSF PCR for CMV, EBV, JC/BK was also negative. However the HIV viral load in the CSF was 6000 copies/ml.

The discrepancy between the low peripheral viral load and a significantly higher viral load in the CSF, suggests selective replication of HIV at a 'sanctuary site', i.e. the brain, which might be reflected by the presence of specific (resistance) mutations in the virus detected in the CSF. To confirm this, HIV resistance genotyping was performed on both the CSF and plasma samples, as well as on a plasma sample obtained during a single peak in viral load several years earlier. In all samples exactly the same profile of mutations conferring resistance to NRTIs was observed in the RT genes. In contrast, no protease inhibitor resistance associated mutations were observed in any of the samples, including the CSF sample. Apparently, replication of HIV-1 in the peripheral blood remained suppressed by the protease inhibitors in the treatment regimen.

Follow-up. The therapy was switched to efavirenz, kaletra and amprenavir. Four months later he showed a marked clinical improvement and both peripheral and CSF viral load were lower then 50 copies/ml.

Conclusions. The discrepancy in viral load between the plasma and the CSF could not be explained by the presence of a distinct (resistant) virus population in the brain. The results suggest that preferential replication of HIV in the brain might be due to poor penetration of the protease inhibitors into the brain. HIV replication in the CSF may be ongoing in the presence of a low peripheral blood viral load.

L16
Borrelia burgdorferi-associated acute disseminated encephalomyelitis

F. Bosma[1,6], S. van Assen[2,6], L.M.E. Staals[3], W.J.G. Melchers[1,6], B.J. Kullberg[2,6], M. Lammens[4,5], P. Vos[5], B.G. Fikkers[3] , J.M.D. Galama[1,6]
University Medical Centre St Radboud, Departments of [1]Clinical Microbiology, [2]General Internal Medicine, [3]Intensive Care Medicine, [4]Pathology and [5]Neurology, [6]Nijmegen Centre for Infectious Diseases, Nijmegen, the Netherlands

Acute disseminated encephalomyelitis (ADEM) is an uncommon inflammatory demyelinating disease of the central nervous system, typically occurring one to three weeks after a non-specific febrile illness, infection, or immunisation.
We describe a 45-year-old man who developed acute blindness, meningism, fever and transverse myelitis, rapidly followed by flaccid tetraparesis, locked in syndrome, autonomic disregulation and eventually death. MRI and postmortal pathology findings were compatible with ADEM. An extensive workup for infectious diseases associated with ADEM and encephalomyelitis was performed, using serology, culture and molecular techniques. Initially, *Borrelia* serology was negative, using in house ELISA and immunofluorescence techniques. Western blotting (National Institute of Public Health and Environment, Bilthoven, the Netherlands) showed no specific IgG response, but an IgM response was found in paired serum samples: a *B. burgdorferi* specific 22 kD band was seen, and a weaker, possibly aspecific band was seen at 34 kD; the *Borrelia* specific 41kD band showed no reactivity. PCR targetted to the *B. burgdorferi* OspB gene (Biozym, Landgraaf, the Netherlands) performed on two different cerebrospinal fluid samples and brain biopsy tissue demonstrated the presence of *B. burgdorferi* DNA in our patient's central nervous system. CSF cultures for *Borrelia* spp on Barbour-Stoenner-Kelly medium remained sterile. To our knowledge, this is the first case of ADEM associated with *B. burgdorferi* infection.

M01-11
Education

Further to the 2002 session, which gave a general overview on microbiology education, the present session has the topic Computer Assisted Education.
Several examples of ICT at different universities are shown. Another session addresses the rapidly emerging field of Bioinformatics. The session ends with the challenging game 'Genomics, who wins the Nobel prize Biology?' This game is a collaborate initiative of NIBI, Senter, IOP, Praktisch Onderwijs in de Natuurwetenschappen, and the Nationaal Regie-Orgaan Genomics (www.praktijk.nu).

N01
Rodlins are involved in but not sufficient for assembly of the streptomycete rodlet layer

D. Claessen[1], D. Jager[2], C. Leeflang[3], F. Goedegebuur[3], H.J. Deelstra[1], N.A. Penninga[1], C. Bormann[4], J. Salas[5], L. Dijkhuizen[1], H.A.B. Wösten[6]
[1]University of Groningen, Microbiology, Haren, the Netherlands, [2]BioMaDe, Groningen, the Netherlands, [3]Genencor International, Research and Development, Leiden, the Netherlands, [4]University Tübingen, Microbiology/ Biotechnology, Tübingen, Germany, [5]University Oviedo, Departament Biology Funcional, University Institute Oncology, Oviedo, Spain, [6]University of Utrecht, Microbiology, Utrecht, the Netherlands

Streptomycetes are Gram-positive soil bacteria with a complex life cycle. After a submerged feeding mycelium is established hyphae grow into the air. These aerial hyphae septate into spores that are dispersed to give rise to a new substrate mycelium. Aerial hyphae and spores of streptomycetes are hydrophobic. The surface layer that confers hydrophobicity is called the rodlet layer and has an ultrastructure of a mosaic of parallel rods. Two homologous proteins, RdlA and RdlB, are involved in formation of this layer in *Streptomyces coelicolor*. In a strain in which both *rdlA* and *rdlB* were deleted (Ä*rdlAB* strain) no rodlet layer was observed. We here show that *Streptomyces tendae* and *Streptomyces griseus* also contain two rodlin genes that are highly homologous to those of *S. coelicolor* and that are able to complement the Ä*rdlAB* strain. Interestingly, the *rdlA* and *rdlB* genes are not redundant but both essential as was shown by deleting either *rdl* gene. On the other hand, recombinant rodlins did not form a rodlet layer *in vitro*. This indicates that formation of this surface layer *in vivo* is not simply the result of self-assembly of a rodlin.

N02
Long-term aerobic glucose-limited chemostat cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D

M.R. Mashego, M.L.A. Jansen, W.M. van Gulik, J.T. Pronk, J.J. Heijnen
University of Technology Delft, Bioprocestechnology, Delft, the Netherlands

The study of the *in vivo* kinetic properties of microbial metabolism requires accurate measurement of the dynamics of intra- and extracellular metabolite levels as response to a certain stimulus, e.g a step change of the extracellular substrate concentration in a steady state chemostat. So far possible changes in the properties of the metabolic network, as a result of the selective pressure which takes place under chemostat conditions, has been neglected.
To study this, intracellular and extracellular metabolites were followed during prolonged aerobic glucose-limited chemostat cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D at a specific growth rate of 0.05 1/h. It was observed that during 1200 hours of cultivation (corresponding to 90 generations) the concentrations of residual glucose, glycolytic intermediates, some TCA-cycle intermediates, NAD and most of the specific activities of the enzymes in the lower part of glycolysis decreased significantly. This phenomenon has a large impact on the study of *in vivo*

dynamic behaviour of *S. cerevisiae* metabolism since it indicates that the physiology of this organism is continuously changing under these conditions. From these observations it can be concluded that pulse response experiments carried out to elucidate the *in vivo* kinetic properties of metabolic pathways should be carefully designed in order to eliminate ageing effects.

N03

The *ramA* gene is not involved in multidrug-resistant *Salmonella typhimurium*

R.J.H.M. van der Straaten[1], C.M. Janssen[1], D.T. Mevius[2], J.T. van Dissel[1]
[1]University Medical Centre, Infectious Diseases, Leiden, the Netherlands, [2]Cidc-Lelystad, Infectious Diseases, Lelystad, the Netherlands

The *Salmonella typhimurium* gene *ramA*, also described as *rma*, is a gene involved in resistance against oxidative stress. However, when overexpressed in *E. coli* it confers resistance not only to superoxide generating agents but also to multiple unrelated antibiotics. The *ramA* gene is present in *S. typhimurium*, other *Salmonella* serotypes and *Klebsiella pneumoniae* but is lacking in *E. coli*. multidrug resistance against various unrelated antibiotics is assumed to be mediated by the *mar* operon which, upon activation, allows transcription of genes necessary for a protective response. Gene products regulated by *mar* are involved in decreasing the permeability of bacteria for antibiotics or increasing the efflux of antibiotics (e.g. the effluxpump AcrAB). MarA activates these genes by binding to a consensus sequence in their promoter region, resulting in transcription. RamA is a novel transcriptional activator that was previously shown to also bind to this specific concensus sequence, suggesting that RamA and MarA can activate the same subset of genes resulting in multidrug resistance (MDR). This hypothesis was confirmed in *E. coli* strains lacking *marA*: overexpression of RamA indeed confers MDR. To study the physiological role of RamA in *Salmonella*, i.e. when expressed at normal levels, we inactivated *ramA* in wild-type *Salmonella* and in a range of multidrug resistant clinical isolates of *S. typhimurium*. Inactivation of RamA did not affect resistance against multiple unrelated antibiotics in wild-type *Salmonella* or any of the clinical MDR isolates, except for tetracycline resistance, which was somewhat altered in several MDR strains. In conclusion, when overexpressed, RamA can activate MarA regulated genes resulting in MDR, but inactivation of RamA in *Salmonella* has no effect on the MDR phenotype, suggesting that under physiological conditions RamA does not directly activate *mar*-regulated genes.

N04

Cytochromes *c* of *Candidatus* 'Kuenenia stuttgartiensis'

I. Cirpus[1], M.C. Schmid[1], I. Schmidt[2], H.J.M. op den Camp[2], D. Lepaslier[3], J. Weissenbach[3], M. Wagner[4], J.G. Kuenen[1], M. Jetten[2], M. Strous[2]
[1]University of Technology, Delft, the Netherlands, [2]Ku Nijmegen, the Netherlands, [3]Genoscope, [4]University Vienna, Austria

The planctomycete *Candidatus* 'Kuenenia stuttgartiensis' anaerobically transforms ammonium (NH_4+) and nitrite (NO_2-) to dinitrogen (N_2). A pathway for the anaerobic ammonium oxidation (anammox) has been proposed: Nitrite is reduced by a nitrite-reducing enzyme, NR, to hydroxylamine (NH_2OH). Hydroxylamine and ammonium are condensed to hydrazine (N_2H_4) by a hydrazine hydrolase, HH. Hydrazine is then converted to dinitrogen by hydroylamine oxidoreductase, HAO. Released electrons are recycled to reduce nitrite to hydroxylamine.
Candidatus 'K. stuttgartiensis' exhibits an unusually high content of cytochromes *c*, which can constitute as much as 15% of the total proteins present. The anammox enzymes HAO and NR are known to be *c*-type cytochromes. Interestingly, HAO with its 24 heme groups already contributes 10% of the total protein content.
Genome analysis of *Candidatus* 'K. stuttgartiensis' preliminary sequence data revealed many ORFs with the classical heme C-binding motif, CXXCH. Two of the *c*-type cytochromes deduced from the genome analysis correspond to cytochromes previously isolated: the high molecular-mass cytochrome HAO, and a small cytochrome *c* with unknown function. Some of the membrane and soluble *c*-type cytochromes are most likely involved in electron transfer in the anaerobic ammonium oxidation.

N05

Assessment and evaluation of the effects of probiotics on faecal microbiota of patients with inflammatory bowel disease

K. Ben-Amor, I.G.A. Heikamp-de Jong, S. Verhargh, A.D.L. Akkermans, W.M. de Vos, E.E. Vaughan
Wageningen University, Agrotechnology and Food Sciences, Laboratory of Microbiology, Wageningen, the Netherlands

Probiotics have the potential to improve human health in intestinal disorders: lactose intolerance, acute gastro-enteritis, and inflammatory bowel disease (IBD). However, very little data is available on the role of probiotics in IBD due to either the inadequacy of the methods used or the complexity of the intestinal ecosystem. In this study, part of the EU-PROGID project, we evaluated the efficiency of two probiotic strains (*Lactobacillus salivarius* subsp. salivarius UCC118 and *Bifidobacterium infantis* 35624) on IBD patients during two distinct long-term, large-scale, multicentre, randomised, double blind, placebo-controlled feeding trials on maintenance of remission. The influences exerted by the probiotics on colonic microbiota was assessed by: (i) monitoring the faecal microbial changes using DGGE, (ii) enumerating the major intestinal bacteria in faecal samples using Fluorescent in Situ Hybridisation (FISH) and Flow Cytometry (FCM). Since the large number of faecal samples

required a rapid DNA extraction method, four different protocols were compared: 1) in-house (WU) method based on phenol extraction and bead beating, 2) BIO101 FastDNA kit, 3) QIAmp stool kit and, 4) combination of the 2 kits. Different primer-sets were used to amplify the 16S rDNA of total bacteria, lactobacilli and bifidobacteria. The DGGE profiles showed that for total bacteria, method 1 and 4 gave better results in terms of total bacterial diversity. For Lactobacillus and Bifidobacterium, method 3 and 4 performed best. Using method 4, PCR-DGGE fingerprinting of the faecal microbiota from patients at different time points of the trial was compared. For a more quantitative and as a high throughput approach, FISH was used in combination with FCM to enumerate faecal bacteria from IBD patients. Group specific probes Bif164, Lab158, Bac303, Erec482, Ecoli1531 and Ato291 were used to count *Bifidobacterium spp.*, Lactobacillus/Enterococcus group, Bacteroides/Prevotella cluster, Eubacterium rectale/Clostridium coccoides group, Enterobacteriaceae and Atopobium group respectively. The FISH probes, originally used for fluorescent microscopy and image analysis, were revalidated for FCM application and the signal to noise ratio was optimised.

N06
Development and use of a *pspA*-based reporter system for screening the mode of action of natural preservatives

S. Brul, K.J. Hellingwerf, W. Crielaard
University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, the Netherlands

The phage-shock-protein (*psp*) operon improves the survival of *Escherichia coli* in late stationary phase at alkaline pH, and protects the cell against dissipation of its proton-motive force upon various challenges. The *psp* operon is strongly induced *e.g.* by the addition of filamentous phage pIV, by ethanol stress, and by the synthesis of a mutant-form of an outer membrane protein, that does not translocate properly through the cytoplasmic membrane. The *psp* operon consists of the poly-cistronic *pspA-E* operon and the divergently transcribed *pspF*.
We will present the construction of reporter strains that express lacZ or GFP under control of the *pspA* promotor. Also the differential effect of (natural) preservatives (ethanol, NaCl, sorbic acid, CCCP) on growth and the expression of the pspA operon will be shown. The strains are currently being used to test our hypothesis that reporter enzyme/protein expression in such strains is exclusively induced by decreasing the size of the proton motive force across the cytoplasmic membrane. The application of our strains in screening the effect of large numbers of naturally occurring preservatives in *Escherichia coli* will be discussed.

N07
Novel archaeal and bacterial divisions from mediterranean deep hypersaline anoxic basins

P.W.J.J. van der Wielen, J.K. Brons, H. Bolhuis
University of Groningen, Laboratory of Microbial Ecology, Haren, the Netherlands

Recent survey of the Eastern Mediterranean seafloor revealed the presence of several (brine lakes). These brines are most likely developed by the dissolution of 5 to 8 million year old Messinian evaporites. They are well described geochemically, but little is known about the microflora present and their ecological role in these environments. As part of the EC project 'BIO technologies from the DEEP' (BIODEEP) the microflora in the brine and the brine/seawater interface of four different brine lakes (L'Atalante, Bannock, Discovery and Urania) was studied by sequencing of 16s rRNA genes. Clone libraries of bacterial and archaeal 16S rDNA were constructed and 96 clones from the brine/seawater interface were sequenced.
Analysis of the sequence data revealed a relatively high bacterial diversity in the interfaces (~30 operational taxonomic units, OTU, >97% sequence homology) and brines (~40 OTUs). In addition, each brine lake had its own specific bacterial community with approximately 50% of the OTUs that were not found in any of the other brine lakes. Only one sequence was found that was present in all four brines. The archaeal diversity in the brine and brine/seawater interface was much lower (~14 OTUs) and most sequences could be found in all of the four brines although different sequences dominated the different brines. A phylogenetic survey with closest known relatives revealed sulfate reducing bacteria, sulfur-oxidising bacteria and methanogenic archaea suggesting that these prokaryotes play an important role in these extreme environments, contributing to sulfur speciation as well as methane production. However, the most exciting finding is the discovery of large number of unique and yet unknown bacterial and archaeal sequences that show very low homology (<90% sequence similarity) with known sequences from the GenBank database. This finding opens possibilities for the discovery of novel biotechnological and medical applications the major aim of the BIODEEP programme.

N08
Gene expression profiles of acid tolerant *Salmonella typhimurium* DT104 isolates

A.P.H.M. Hermans[1,2], T. Abee[2], H.J.M. Aarts[1]
[1]*RIKILT institute of food safety, Department Microbiology, Wageningen, the Netherlands,* [2]*Wageningen University, Food Hygiene and Microbiology Group, Wageningen, the Netherlands*

During the last decades, the incidence of foodborne diseases has increased in many parts of the world and new foodborne pathogens, like *Salmonella typhimurium* DT104, have been identified. These pathogens have the potential to adapt to a wide variety of food preservation related stress conditions, i.e. starvation, temperature extremes and (weak) acids, which can result in an increased survival in the GI-tract.
The objective of our research is to analyse gene expression profiles of acid tolerant *S. typhimurium* DT104 isolates to gain

detailed insight in the molecular pathways involved in low pH survival.
Acid tolerant and acid sensitive variances were selected from a number of food *S. typhimurium* DT104 isolates, by exposure to pH=2.5 after adaptation at pH=5. A thematic micro-array was constructed containing oligo's homologous to *S. typhimurium* LT2 stress response and virulence related genes. Cells of both isolates were harvested in the exponential, transitionary and stationary growth phase at pH=7 and pH=5 from the high and low responder *S. typhimurium* DT104 isolates and the obtained RNA samples were hybridised to the micro-array.
The micro-array data analysis resulted in many differential expressed genes between growth phases of both pHs. Furthermore a comparison of the acid tolerant and acid sensitive isolates resulted in a limited number of differential expressed genes. Nevertheless some interesting acid survival related genes were up regulated in the acid tolerant isolate.
The thematic micro-array provides indeed a tool to gain detailed insight in the molecular pathways involved in low pH survival.

N09

Population dynamics, toxin induction and early detection of toxic cyanobacteria (DYNATOX)

I. Janse[1], M. Meima[1], M.P. Kamst-van Agterveld[1], E. Kardinaal[2], G. Zwart[1]
[1]Centre for Limnology, Dutch Institute of Ecology (NIOO-CL), Nieuwersluis, the Netherlands, [2]IBED/Aquatic Microbiology, University of Amsterdam, Amsterdam, the Netherlands

Blooms of toxic cyanobacteria occur worldwide and constitute a health hazard of growing concern. To reveal which conditions may lead to toxic blooms, we study the population dynamics and toxin production of cyanobacteria in Dutch lakes. In addition, we intend to develop and validate a sensitive method based on DNA probe techniques for early detection and monitoring of toxic cyanobacteria to aid research and water management.
Five lakes were sampled at regular intervals during two subsequent years to correlate the composition of the cyanobacterial community to toxin concentrations and environmental parameters. A number of potentially toxic cyanobacteria, mostly from the genera *Microcystis*, *Planktothrix*, *Aphanizomenon*, and *Anabaena*, were isolated and cultivated. Toxin production and sequences of marker genes were characterised in these cultures. As an additional approach to correlate genotypes to toxin production, we determined DNA sequences and the presence of toxic peptides (by MALDI-TOF) in single *Microcystis* colonies isolated from field samples.
Composition and dynamics of cyanobacteria was studied mainly using molecular techniques such as denaturant gradient gel electrophoresis (DGGE) and DNA sequencing. Since toxin production may vary between closely related cyanobacteria, even below species level, a method for high-resolution analysis of cyanobacterial populations was required. For this purpose, primers were designed which enable specific amplification of variable regions of the cyanobacterial genome and yield PCR products suitable for DGGE analysis. The primers were targeted at the ribosomal RNA internal spacer (rRNA-ITS), and at the phycocyanin intergenic spacer (PC-IGS). Comparison of a number of isolated *Microcystis* strains showed that the resolution of this method is sufficient to distinguish closely related organisms, even strains within the same species.
Cyanobacterial community dynamics varied significantly between the lakes under investigation, ranging from one lake with a stable community composition throughout the sampling period, to lakes exhibiting successions of different cyanobacterial species and strains. Community dynamics is correlated to limnological parameters. Comparison of toxin concentrations and the occurrence of particular organisms in environmental samples, supported by the data on toxin production and genotypes of isolated cyanobacteria, enables identification of some major toxic cyanobacteria. Once identified, DNA sequences will be used to design specific probes targeted at toxic and non-toxic cyanobacteria. These probes will be applied in a reversed line blot assay for detection and monitoring of toxic cyanobacteria.

N10

Extreme thermoactive amylomaltase from *Pyrobaculum aerophilum* IM2

T. Kaper[1,2], M. Habets[1,2], J. van Munster[1,2], T. Ettema[4], M.J.E.C. van der Maarel[1,3], L. Dijkhuizen[1,2]
[1]Centre for Carbohydrate Bioengineering TNO-RUG, Groningen, the Netherlands, [2]Microbial Physiology Research group, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Haren, the Netherlands, [3]Innovative Ingredients and Products Department, TNO-Nutrition and Food Research, Groningen, the Netherlands [4]Laboratory of Micriobiology, Wageningen University, Wageningen, the Netherlands

Amylomaltases (EC 2.4.1.25) transfer a segment of a α-1,4-glucan onto glucose or another α-1,4-glucan. Amylomaltases are intracellular enzymes involved in maltose metabolism and energy storage. They belong to family 77 of glycoside hydrolases and the α-amylase superfamily together with family 13 and 70. Acting upon starch, amylomaltases can produce cycloamylose or a thermoreversible starch gel, which are both of commercial interest. Due to the low solubility of starch at ambient temperatures, our research is focussed on amylomaltases from hyperthermophilic organisms, which can be used at high temperature. Here we present the results of the characterisation of amylomaltase from the hyperthermophilic archaeon *Pyrobaculum aerophilum* IM2. Gene PAE1209 was cloned and expressed in *E. coli* and purified by heat incubation and Ni-affinity chromatography. The enzyme was active up to 100°C, with an optimal activity at pH7.0. Kinetic parameters have been determined for glucose release from malto-oligosaccharides. The disproportionating activity of the enzyme was determined by HPLC and the preferred binding mode of the malto-oligosaccharides in the active site has been deduced. By homology modelling, a 3D model was constructed and analysed for active site and thermostability features.

N11

Characterisation of a glucansucrase gene from *Lactobacillus reuteri* strain 121

S. Kralj, G.H. van Geel-Schutten, M.J.E.C. van der Maarel, L. Dijkhuizen

Centre for Carbohydrate Bioengineering, TNO-RUG, Haren, the Netherlands

Glucansucrases or glucosyltransferases (GTFs, EC 2.4.1.5) of lactic acid bacteria are large extracellular enzymes responsible for the synthesis of glucan from sucrose. Much research is performed on glucansucrases from oral streptococci and *Leuconostoc* species, whereas little is known about the glucansucrases of lactobacilli. Glucansucrases are large enzymes with a common structure consisting of: (i) a signal peptide of 32-34 amino acids; (ii) a highly variable region of 123-129 amino acids; (iii) a conserved catalytic domain of approximately 1,000 amino acids; and (iv) the glucan binding domain with a number of repeats at the C-terminus. It has been reported that *Lactobacillus reuteri* strain 121 is capable of producing a highly branched glucan with an unique structure containing α-(1$\rightarrow$4) and α-(1$\rightarrow$6) linkages (Van Geel-Schutten *et al.* 1999).

A DNA fragment containing the putative glucosyltransferase gene (*gtfA*), was isolated from the chromosomal DNA of *Lactobacillus reuteri* strain 121. The size of the *gtfA* ORF was 5343 bp, encoding a protein of 1781 amino acids with a deduced molecular weight of 198,637 Da and a pI of 5.04. The deduced amino acid sequence of GTFA revealed clear similarities with other glucosyltransferases. GTFA has a relatively large variable N-terminal domain (702 amino acids), containing five unique distinct repeats and a relatively short glucan-binding domain (267 amino acids), containing eleven 'YG-repeats'.

The *gtfA* gene was expressed in *Escherichia coli*, yielding an active GTFA enzyme. NMR, HPSEC-MALLS, and methylation analysis of the glucans produced by this *E. coli* transformant and of glucans isolated from supernatants of *Lb. reuteri* grown on sucrose revealed that both glucans were virtually identical. The N-terminal amino acid sequence of the glucosyltransferase of *Lb. reuteri* strain 121, purified from culture supernatants using 2D-PAGE (i.e. iso electric focussing followed by SDS-PAGE), was the same as the deduced N-terminal amino acid sequence of the *gtfA* gene. SDS-PAGE and iso electric focussing of the purified *L. reuteri* enzyme confirmed the predicted molecular weight and iso electric point of GTFA. Therefore it is concluded that the *gtfA* gene encodes the active glucosyltransferases of *Lb. reuteri* 121.

N12

Analysis of the extracellular proteome of *Agaricus bisporus*

R. ten Have[1], G. Straatsma[2], P.J. Schaap[1]

[1]*Microbiology, section Fungal Genomics, Wageningen, the Netherlands,* [2]*Proefstation voor de champignoncultuur, Horst, the Netherlands*

The edible mushroom *Agaricus bisporus* is by far the most popular mushroom crop in the world. Mushrooms are cultivated on a fermented mixture of wheat straw, chicken and horse manure and gypsum. Cultivation of the mushroom crop on an industrial scale can be separated into two major phases. In the spawning phase the compost is fully colonised. Mushroom development occurs in the second phase and is initiated by applying a casing layer on top of the colonised compost. Usually there are three consecutive rounds of mushroom development and harvesting. During compost colonisation the fungus utilises the compost as a carbon source by secreting a large array of extracellular enzymes mostly (hemi)cellulases, which must have a synergistic interaction with lignin degrading enzymes for efficient utilisation of the lignocellulose complex. So far, information on the full extracellular proteome is limited. Eleven individual extracellular enzymes have been subject to a biochemical characterisation, but nothing is known about the synergistic effect of their combined mode of action.

As a first step of a full understanding of compost degradation and utilisation by this fungus we used 2-D protein gels to analyse the complete extracellular proteome of *A. bisporus* starting from compost colonisation up to and including the first round of full mushroom development. Proteins that showed a differential pattern of expression were excised from the gels and subjected to QTOF-MS mass analysis for identification. The sequence information from the mass analysis already resulted in the putative identification of a number of enzymes that are novel for this species.

N13

Molecular characterisation of the general stress response of *Bacillus subtilis*

A. ter Beek[1], K.J. Hellingwerf[2], S. Brul[1]

Laboratory for [1]*Molecular Biology & Microbial Food Safety and* [2]*Microbiology, Swammerdam Institute for Life Science, Biocentrum, University of Amsterdam, Amsterdam, the Netherlands*

Bacillus subtilis cells respond to a variety of stresses by the induction of the general stress regulon, which is controlled by the transcription factor sigma-B. Activation of this sigma factor by energy- or environmental stresses, such as carbon limitation, or ethanol-, acid- or salt shock, induces the expression of 200 or more different stress proteins. In this study, a beginning is made, by way of comparison to the induction of sporulation, with the characterisation in well defined MOPS buffered media and under defined stress conditions, of the general stress response system. The cells are grown in different media, in which the energy -source and/or -availability is varied and are followed both during their logarithmic growth and during the transition into and maintenance of stationary phase. The general stress response is monitored both through the use of a *lacZ* and a fluorescence reporter gene. The latter is being used to investigate heterogeneity within the culture with respect to the time of induction of the sigma-B system and in combination with image analysis the extent of induction. These experiments are extended with the determination of cfu's and an assessment of the amount of spores present to come to an overall picture of the stress responses chosen. Results until now show that a low ratio of carbon to nitrogen favours the onset of the general stress response measured as a ctc-LacZ fusion protein. This process precedes sporulation.

N14
Typical freshwater bacteria

G. Zwart[1], M.P. Kamst-van Agterveld[1], E. van Hannen[1], K. van der Gucht[2], E.S. Lindström[3], T. Lauridsen[4], S. Declerck[5]

[1]Centre for Limnology, NIOO-KNAW, Nieuwersluis, the Netherlands, [2]Department of Biology, Laboratory of Protistology and Aquatic Ecology, Gent, Belgium, [3]Department of Limnology, Evolutionary Biology Centre, Uppsala University, Sweden, [4]Department of Lake and Estuarine Ecology, The National Environmental Research Institute, Denmark, [5]Laboratory of Aquatic Ecology, University of Leuven, Leuven, Belgium

Introduction. Systematic analysis of bacterial 16S rRNA gene clone libraries identified specific clusters of sequences that occur frequently in freshwater ecosystems. The identification of these phylogenetically narrow clusters has provided a basis for probe design. Using these probes we have investigated distribution and ecology of dominant freshwater bacteria.

Methods. Water samples from 81 diverse lakes from Belgium, the Netherlands, Denmark, Sweden and Norway (Spitsbergen) were tested in reverse line blot for reactivity against 15 oligonucleotide probes which were designed specifically for these freshwater clusters. In addition to these molecular community data, information was obtained on biomasses of phytoplankton groups, zooplankton groups, macrophytes and fish.

Results. Six probes reacted with the large majority of tested lake samples. Probes against two actinobacterial clusters reacted to more than 90% of the samples. From this we can point out actinobacterial, verrucomicrobial, proteobacterial and cyanobacterial groups that are represented in the large majority of North-West European lakes. Multivariate analysis showed that in Denmark, Belgium and the Netherlands, biomasses of several phytoplankton groups were the best explanatory variables of bacterial community structure.

Conclusions. 1. Bacterial communities in freshwater are dominated by a few narrow groups of bacteria. Some of these groups can be found in almost every lake in Northwest Europe. Clone library results indicate that the distribution of most of the groups is globally. 2. Phytoplankton is a major determinant of bacterial community composition in Northwest European freshwater ecosystems.

N15-17
The need of a polyphasic approach in microbial ecology

G. Muyzer

Environmental Microbiology, Kluyver Laboratory for Biotechnology, Delft, the Netherlands

Our knowledge of microbial diversity has drastically increased over the last decade. This is mainly due to the use of small subunit ribosomal RNA genes as molecular markers for phylogenetic inferences. Comparative sequence analysis of 16S rRNA genes amplified from environmental samples showed that microbial diversity is much greater than anticipated, and that traditional culture-based approaches are insufficient in retrieving this enormous reservoir of diversity. However, although we can now determine microbial diversity in fine detail, we only know little about the driving forces that generate and maintain microbial diversity, and about the value of this diversity for ecosystem functioning. Examples of questions that are still unanswered are: Why are there so many closely related coexisting populations with a similar or identical metabolism in one and the same environment? What is their role? Are they all active at the same time, or do they become active under different environmental conditions, or upon the availability of different substrates? What does diversity mean for community stability? To answer these questions, we have to use a polyphasic approach, combining concepts and methodology from different disciplines, such as ecology, microbiology, molecular biology, and genomics. In this seminar I will give examples of the use of molecular methods to study the diversity and activity of microbial communities, and will discuss the need for a renewed effort to isolate ecologically important micro-organisms.

Q04-06
De secundaire laboratoriumdiagnostiek van tuberculose

D. van Soolingen

Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, Nederland

Er zijn in Nederland 43 perifere laboratoria die zich bezighouden met de primaire laboratoriumdiagnostiek van tuberculose. Hierbij worden diverse kleurtechnieken gebruikt om mycobacteriën in klinisch materiaal aan te tonen. Daarnaast wordt gebruik gemaakt van verschillende kweektechnieken op vaste- en vloeibare voedingsbodems. Moleculaire technieken spelen ook een steeds grotere rol om mycobacteriële infecties sneller te diagnosticeren. Een deel van de laboratoria voert zelf een (gedeeltelijke) identificatie- en soms een beperkte gevoeligheidsbepaling uit. Er vindt op dit moment een inventarisatie plaats van de gebruikte technieken en er wordt gewerkt aan het opstellen van richtlijnen voor de primaire laboratoriumdiagnostiek van tuberculose.

In het kader van de nationale surveillance van tuberculose ontvangt het RIVM alle ongeveer 1100 *Mycobacterium tuberculosis* complex isolaten en tevens zo'n 400 atypische mycobacteriële stammen die jaarlijks geïsoleerd worden in Nederland. De identificatie vindt plaats met Accuprobetesten en andere moleculaire technieken zoals DNA fingerprinting en DNA sequentie analyse. Er vindt op dit moment onderzoek plaats om snellere identificatie met reversed-line-blot methoden mogelijk te maken. Voor de gevoeligheidsbepaling wordt gebruikt gemaakt van een Minimal Inhibition Concentration methode op 7H10 agar. Naast eerstelijnscontroles, vinden tweedelijnscontroles plaats via uitwisseling van monsters met een ander laboratorium. Er wordt ook meegedaan aan proficiency studies van de WHO. Voor epidemiologische typeringen worden restrictie fragment lengte polymorfisme (RFLP) typeringen uitgevoerd. Deze typeringen worden gebruikt om het contactonderzoek te ondersteunen, maar daarnaast ook om bredere analyses van transmissie van tuberculose in Nederland mogelijk te maken. Verder blijkt de laatstgenoemde methode ook belangrijk om laboratorium kruis-contaminaties op te sporen.

R01

Effects of clarithromycin on oropharyngeal and nasal flora: a double-blind placebo-controlled study

H.F. Berg[1], J.H.T. Tjhie[2], E.E. Stobberingh[3], M.F. Peeters[1], P.H.J. van Keulen[4], J.A.J.W. Kluytmans[4]
[1]*St Elisabeth Hospital, Medical Microbiology, Tilburg, the Netherlands,* [2]*PAMM laboratory, Medical Microbiology, Veldhoven, the Netherlands,* [3]*University Hospital Maastricht, Medical Microbiology, Maastricht, the Netherlands,* [4]*Amphia Hospital, Medical Microbiology, Breda, the Netherlands*

Background. The use of antimicrobial agents is associated with the emergence of resistance to these drugs in almost all human pathogens. Whether this also accounts for clarithromycin, as a relatively new macrolide antibioticum, has not yet been evaluated. Therefore, a prospective, placebo-controlled, randomised, double blind study was performed to investigate the effect of clarithromycin slow-release on colonisation and resistance in oropharyngeal and nasal flora.
Patients and methods. 296 patients with documented coronary artery disease were randomised to receive a daily dose of clarithromycin 500 mg slow release (CL) or placebo tablets (PB) until the day of surgery, during the preoperative period. Nose- and throat swabs were taken before initiating therapy and after. Minimal Inhibitory Concentration (MIC) was determined for oral *Haemophilus* species and nasal *Staphylococcus aureus*. Throat swabs were also cultured for genotypic macrolides, lincosamides, and streptogramins b (MLS) cross-resistance detection in indigenous flora.
Results. Oropharyngeal carriage of *Haemophilus* species was not influenced in either of the treatment groups (79.1% before treatment in both groups, and 70.3% after CL versus 74.3% after PB). Nasal carriage of *S. aureus* however, decreased significantly from 34.7% before treatment, to 4.9% after CL treatment (compared to PB: 32.4% before and 30.1% after; RR=13.2). The resistance for clarithromycin was significantly increased in *Haemophilus* species (p=0.0002; RR=1.9; 95% Confidence Interval: 1.4-2.7). The MLS resistant bacteria accounted for 37% of the total indigenous flora. Directly after therapy and 8 weeks after therapy, the percentage of MLS resistant bacteria increased significantly in patients who received clarithromycin (77% in CL group versus 30% in PL group directly after, and 53% (CL) versus 27% (PL) 8 weeks after).
Conclusion. These results show a rapid effect of clarithromycin slow-release on resistance, and suggest the capability of transfer of MLS resistance genes from indigenous flora to pathogenic flora.

R02

Antimicrobial peptides derived from bovine milk proteins

M.I.A. van der Kraan, R. de Bruijn, J. Groenink, J.G.M. Bolscher, E.C.I. Veerman, A.V. Nieuw Amerongen
ACTA, Dental Basic Sciences-Oral biochemistry, Amsterdam, the Netherlands

Many reports refer to the therapeutic use of milk for the benefit of oral health. We selected five potential antimicrobial peptides from two bovine milk proteins, i.e. lactoferrin and casein. We search for new antimicrobial peptides in bovine milk, which is abundantly available, that might be used for the benefit of oral health, for example to prevent oral candidosis. Lactoferrin (Lf) is an iron-binding glycoprotein that has been found in a large number of secretions of mammals, including saliva, tears and milk. Upon peptic digestion a strong antimicrobial peptide, referred to as lactoferricin (amino acid residues 17-41), is released from the native protein. Two peptides within the lactoferricin sequence were manufactured, referred to as bLf 17-30 and bLf 19-37. Four different caseins are present in bovine milk, namely $á_{S1}$-, $á_{S2}$-, â- and ê-casein. In three types of casein, peptide domains with bactericidal activity have been identified. We manufactured three peptides from $á_{S2}$-casein, referred to as CBPá2 154-173, CBPá2 158-173 and CBPá2 162-173. *Candida albicans* was used as a model micro-organism to establish the killing activity of the five selected peptides. Killing of *C. albicans* was established within 5 minutes by the lactoferrin peptides (LC_{50} values of 0.9-1.6 μM) and within 15 minutes by the $á_{S2}$-casein peptides (LC_{50} values of 1.5-6.4 μM). Internalisation of the peptides, labelled with FITC, was found using confocal and fluorescence microscopy. None of the peptides were associated with the cytoplasmic outer membrane. The peptides showed different distribution patterns in the cell. Fluorescence of FITC-labelled peptide bLf 17-30 was found intracellular in the area of the cell where budding takes places. CBPá2 158-173 seemed to be concentrated in one organelle. The other peptides showed a more diffuse distribution in the cell, suggesting localisation in the cytoplasm. Our data indicate that the potential antimicrobial peptides have fungicidal activity and with distinct cellular targets.
This research was supported by DMV-International, Dutch Dairy Foundation and by The Dutch Technology Foundation STW, Grant VTH5180.

R03

Low susceptibility to antibiotics of the causing agents of bacterial conjunctivitis in primary care

R.P. Rietveld[1], J.H. Sloos[2], H.C.P.M. van Weert[1], P.J.E. Bindels[1]
[1]*Academic Medical Centre, Department of Primary Care,* [2]*Laboratory for Clinical Microbiology, Amsterdam, the Netherlands*

Objectives. The aim of the study was to identify the bacterial cause and their susceptibility to antibiotics of conjunctivitis in general practice
Methods. In 176 patients from 10 general practices with clinically suspected bacterial conjunctivitis using strict criteria, cultures were taken from the conjunctiva of both eyes by the general practitioner. Cultures were send to the laboratory for microbiology and performed according to the American Society for Microbiology. MICs from several topical antibiotics were determined using Etest.
Results. Of the most affected eye, 58/176 cultures were positive with pathogenic bacteria, including *Streptococcus pneumoniae* (n=26), *Staphylococcus aureus* (n=11), and *Haemophilus influenzae* (n=11). Of *S. pneumoniae*, 4 isolates were resistant against fusidin acid (MIC 3-16 mg/l), 4 against trimethoprim (MIC 8-32 mg/l), 3 against gentamicin (MIC 6-8 mg/l), and 1 against chloramphenicol (MIC 12 mg/l). Of *H. influenzae*, 4 isolates were resistant against fusidin acid (MIC 3-48 mg/l), 2 against trimethoprim (MIC 6-256 mg/l), and 1 against

gentamicin (MIC 8 mg/l). Of *S. aureus*, 6 isolates were resistant against fusidin acid (MIC 12-256 mg/l), 3 against trimethoprim (MIC 8-32 mg/l), and 1 against gentamicin (MIC 8 mg/l). None of the isolates tested were resistant to ciprofloxacin.
Conclusions. 1. The sensitivity of strict clinical criteria for bacterial conjunctivitis in general practice is low, when the culture is used as the golden standard. 2. From a bacteriological point of view, only one quarter of cases of clinically suspected bacterial conjunctivitis can be empirically treated effectively using local application of antibiotics. 3. In case of culture confirmed bacterial conjunctivitis, resistance against common topical antibiotics is relatively high. 4. General practitioners should be withholding in prescribing antibiotics for the empirical treatment of clinically suspected bacterial conjunctivitis

R04
Evaluation and optimisation of antibiotic treatment in a Dutch teaching hospital

I.H. Bartelink[1], G.J. van Asselt[2], J.W.P.M. Overdiek[1], P.A.M.M. Boermans[1], P.M. Oostvogel[2], H.J. ter Horst[1], R.J.M. Brüggemann[1]
Medisch Centrum Haaglanden, [1]Hospital pharmacy, [2]Medical Microbiology, the Hague, the Netherlands

Introduction. Although an antibiotic formulary is established in many hospitals, the implementation of its guidelines has not been studied extensively. The goal of this study was to optimise the use of the antibiotic formulary in the hospital, starting in the surgical department. Results of interventions in other departments are in progress.
Methods. A pharmacist, specifically assigned for this study, performed a two-month evaluation of antibiotic therapy, using the formulary as 'golden standard'. Criteria to judge the quality of therapy were: 1) correctness of dose, 2) route of administration, 3) length of therapy, 4) choice of antibiotics and 5) justification of the use of antibiotics. A treatment was defined as optimal when all these criteria were met.
In order to obtain adherence to formulary guidelines interventions followed. Physicians and nurses were instructed monthly, guidelines for an early switch from intravenous to oral antibiotics were introduced and interventions took place by giving advice in individual cases.
Results. In the evaluation period 42 surgical patients were reviewed. On average, the pharmacist reconsidered the choice of antibiotics three times per patient. Only 42% of infections were treated according to all criteria. The most common deviations were 1) choice of antibiotics (28%) and 2) route of administration (14%). In a three-month intervention period the percentage of optimal therapies in 77 patients was 64%. A deviation from the formulary was the reluctance to use gentamicin, which did not improve much during the interventions. Excluding these indications, 75% of therapies were optimal. All evaluated parameters improved.
Conclusion. This study shows a need to control and change the antibiotic therapy extensively during treatment. In the course of the evaluation only 42% of therapies in the surgical department were optimal. A large improvement to 75% was therefore possible. The results show a positive effect of interventions on antibiotic policy. However, some systematic deviations tempered this effect. Consensus meetings will lead to improvement.

R05
Effects of 16S rRNA gene mutations on tetracycline resistance in *Helicobacter pylori*

M.M. Gerrits, M. Berning, A.H.M. van Vliet, E.J. Kuipers, J.G. Kusters
Erasmus Medical Centre, Department of Gastroenterology and Hepatology, Rotterdam, the Netherlands

Objective. Tetracycline is frequently used in anti-*Helicobacter pylori* therapy. Most *H. pylori* strains are susceptible to tetracycline (MIC <2 μg/ml), but the incidence of tetracycline resistance has increased over the past two years. Strikingly, the *H. pylori* strains with the highest tetracycline resistance levels contain the same triple basepair substitution $AGA_{926\text{-}928}$® TTC within both the 16S rRNA genes. In this study we determined whether single, double, or triple basepair substitutions of the TTC mutation are required for high-level tetracycline resistance in *H. pylori*.
Methods. Mutants containing single, double or triple basepair substitutions at position 926-928 were constructed in the 16S rRNA genes of the tetracycline-sensitive *H. pylori* reference strain 26695. Subsequently the effects of the different 16S rRNA gene mutations were assessed on growth rate, stability, and level of tetracycline resistance.
Results. The mutant that contained the naturally occurring TTC substitution had the highest growth rate and MIC value (MIC = 8 μg/ml). Although mutants containing one or two basepairs substitutions were able to grow on low concentrations of tetracycline, these mutants displayed reduced growth rates and their MIC values were all ≤2 μg/ml. Despite these reduced growth rates, all mutations and MIC values were stable during repeated subculturing both in the presence and absence of tetracycline.
Discussion. The substitution of one or two basepairs at position 926-928 in the 16S rRNA genes confers low-level tetracycline resistance to *H. pylori*. Only the naturally occurring triple basepair substitution TTC results in resistance levels with clinical relevance. Together with the higher growth rates of the TTC mutant this explains the preference for TTC mutation in the naturally occurring high-level tetracycline resistant isolates. Based on these results, a molecular screening approach for tetracycline resistance *H. pylori* isolates focusing solely on the TTC mutation seems feasible.

S01
Chronic persistence and reactivation of *Salmonella typhimurium* infection in mice

A. van Diepen, J.S. van de Gevel, H. Beekhuizen, R. Janssen, J.T. van Dissel
Leiden University Medical Centre, Infectious Diseases, Leiden, the Netherlands

Following an acute infection with *Salmonella*, bacterial growth is suppressed but the pathogen is often not completely eliminated. *Salmonella* can persist for a long time without causing manifest disease, but may exacerbate ('reactivate') at a later timepoint. A better insight in the interaction between *Salmonella* and its host may lead to new methods of immune intervention and preventive screening of reactivating infections. To this end, we studied the reactivation of an *S. typhimurium* infection in C3H/HeN (Ity^r)

mice that suffer a systemic infection when injected subcutaneously in the flanks, reaching a peak in liver and spleen between day 5 and 19. Eventually, the infection is cleared and the mice recover: they gain weight, show no signs of discomfort, and bacteria can no longer be detected microbiologically. By exposing mice thus recovered to total body irradiation or by selective *in vivo* depletion of $CD4^{+}$ T cells, we were able to reactivate the *S. typhimurium* infection that runs a systemic course similar to that in the primary, acute infection. We are currently further analysing this reactivation model to determine the specific contribution of various immune cells in suppression of *Salmonella* infection during microbial dormancy.

S02
Trans-cell envelope signalling in *Pseudomonas aeruginosa*

M. Llamas, W. Bitter
Free University Medical Centre, Medical Microbiology and Infection prevention, Amsterdam, the Netherlands

Pseudomonas aeruginosa is a remarkably versatile pathogen which grows in soil, fresh and salt water habitats, and is not only a major opportunistic pathogen for humans, but is also able to infect a large range of other species, including plants, insects and nematodes. This does not only mean that *P. aeruginosa* must possess a wide range of virulence factors, but also a complex regulatory network to control these factors. Analysis of the *P. aeruginosa* genome sequence has recently shown that a high proportion (9%) of the 5,570 predicted open reading frames is involved in gene regulation. The two-component system and the quorum sensing system are the predominant signal transfer systems in Gram-negative bacteria. In addition, another signal transfer system has been identified recently, the trans-cell envelope regulatory system (TCR). The TCR pathway involves three proteins, an outer membrane receptor, an inner membrane protein and a cytoplasmic alternative sigma-factor. The receptor is sensing the extracellular presence of an specific signal and transduces the signal to the inner membrane protein. This protein functions as an anti-sigma factor, which only effectively inhibits the activity of a specific alternative sigma factor (σ-70) in the absence of the extracellular signal. Upon induction, the σ-70 is activated and directs the RNA polymerase to the promoter region of the genes under the control of the TCR system. The analysis of the *P. aeruginosa* genome sequence resulted in the identification of 14 potential TCR systems. To study the role of the *P. aeruginosa* TCR systems, the regulatory operons has been amplified and cloned, and the genes encoding the sigma factors have been cloned in a broad-host range plasmids and introduced in *P. aeruginosa*. (Over)expression of these alternative sigma factors usually results in the expression of the sigma-dependent genes in the absence of the inducing signal. Differential expression patterns of *P. aeruginosa* cells (over)expressing different sigma factors are being established by (2D) SDS-PAGE analysis and by micro-array analysis. The preliminary results indicate that one of the new TCR systems regulates the swarming behaviour of *P. aeruginosa*.

S03
The functional status of the putative *Helicobacter pylori* adhesin *sabB* as a marker for clinical outcome

R. de Jonge[1], R.G.J. Pot[1], R.J.L.F. Loffeld[2],
A.H.M. van Vliet[1], E.J. Kuipers[1], J.G. Kusters[1]
[1]*Erasmus Medical Centre, Gastroenterology and Hepatology, Rotterdam, the Netherlands,* [2]*de Heel Zaans Medical Centre, Internal Medicine, Zaandam, the Netherlands*

Background. *Helicobacter pylori* factors that contribute to disease outcome are largely unknown, but intimate contact to host cells as mediated by outer membrane proteins (OMPs), is thought to play an important role. Expression of the OMPs OipA, HopZ, SabA, and SabB depend on CT-repeats in the coding region of the respective genes. The correlation between the expression status of these four genes and the clinical outcome of colonisation with *H. pylori* was determined in a Dutch population.
Patients and methods. *H. pylori* strains isolated from 95 Dutch patients (28 with gastritis, 28 with duodenal ulcer, 21 with gastric ulcer, 9 with gastric carcinoma, and 9 with lymphoma) were analysed for the presence of the *cag* PAI and the on/off expression status of the *H. pylori* genes *oipA*, *hopZ*, *sabA*, and *sabB* genes by DNA sequence analysis.
Results. The off-status of *sabB* and the presence of the *cag* pathogenicity island were significantly associated with duodenal ulcer (P=0.03 and P=0.04 respectively). The expression status of *oipA*, *hopZ*, and *sabA* did not correlate with clinical outcome. Furthermore, lymphoma strains appear to express a significant smaller amount of putative adhesins when compared to gastritis, gastric ulcer, duodenal ulcer and gastric carcinoma strains (P=0.01 for all groups tested).
Conclusions. The presence of the *cag* PAI and the off status of *sabB* in *H. pylori* strains are associated with duodenal ulcer disease. SabB may be involved in intimate attachment to host cells and *H. pylori* strain harboring a functional *sabB* gene may therefore be more susceptible to phagocytosis.

S04
The mycobacterial surface glycolipid lipoarabinomannan (LAM) binds to dendritic cells through its mannose caps and downregulates DC via the DC-SIGN

B.J. Appelmelk[1], T.L. Lowary[2], T. Geijtenbeek[3],
C.H. Hokke[4], Y. van Kooyk[3], R.R. Gadikota[2], W. Bitter[1],
C.M.J.E. Vandenbroucke-Grauls[1]
Free University Medical Centre, [1]*Medical Microbiology,* [3]*Mol Cell Biol, Amsterdam, the Netherlands,* [2]*Ohio State University, Chemistry, Columbus, USA,* [4]*Leiden University Medical Centre, Parasitology, Leiden, the Netherlands*

Introduction. The mycobacterial surface glycolipid lipo-arabinomannan (LAM) plays an important role in the interaction of *Mycobacterium tubercu*losis and the host. LAM consists of a glycosylphosphatidyl anchor, a mannan core and an arabinan domain terminally substituted with 1 to 3 mannose residues. We aim to identify the parts of LAM that binds to host cells, to identify host receptors, and study the biological effect their interaction
Methods. Synthetic oligosaccharides representing LAM termini were synthesised and binding to dendritic cells analysed by FACS. Lectin-blocking Mabs were included to

identify relevant DC-lectin (-s). Binding of recombinant lectins to LAM termini was investigated by Elisa. The effect LAM-lectin interaction was studied by measuring cytokine production. Mycobacterial mannosyltransferases were identified in silico, cloned and overexpressed in *M. smegmatis*.
Results. We synthesised LAM termini containing arabinose (ara) and/or mannose (man): ara, (ara)5, man-ara, (man)2-ara and (man)3ara . The oligosaccharides were linked to serum albumen (25 glycan chains/mol) and biotinylated.FACS analysis showed that (man)1-3ara bound well to DC while ara and (ara)5 did not bind. Mabs to DC-SIGN blocked binding of LAM to DC, which proves that DC-SIGN is a major LAM receptor In Elisa, rDC-SIGN-Fc bound to (man)ara <<(man)2ara <(man)3ara, but no binding to ara and (ara)5 was found. Addition of LAM to activated DC led to secretion of immunosuppressive IL-10. Eight mycobacterial mannosyltransferase genes were cloned and overexpressed in *M. smegmatis*; the interaction of these complementants with DC are under study.
Summary. Synthetic LAM termini allow us to to elucidate the interaction between mycobacteria and DC on the molecular level. The LAM-induced secretion of IL-10 provides a model how mycobacteria may persist in phagocytic cells. Our data also suggest the possibility for novel therapies (MT-blocking agents) or synthetic carbohydrate vaccines for prevention of mycobacterial disease.

S05

Zebrafish embryos as a model for the real time analysis of *Salmonella typhimurium* disease development

A.M. van der Sar[1], R.J.P. Musters[2], F. van Eeden[3], C.M.J.E. Vandenbroucke-Grauls[1], W. Bitter[1]
Free University Medical Centre, [1]Medical Microbiology, [2]Physiology, Amsterdam, the Netherlands, [3]Hubrecht laboratorium, Utrecht, the Netherlands

Bacterial virulence is best studied in animal models. However the lack of possibilities for real time analysis and the need for laborious and invasive sample analysis limit the use of experimental animals. Fish species belonging to the Teleost family, such as the zebrafish (*Danio rerio*), have a well-developed immune system, both innate and adaptive, which is quite similar to the mammalian immune system. In the zebrafish embryo, macrophage-like cells appear at about 24 hours post fertilisation (hpf). These cells are able to phagocytose intravenously injected bacteria and upon sensing the presence of bacteria migrate into infected areas to eradicate the microbial invaders. Furthermore, zebrafish embryos develop externally and are optically transparent during their development. These characteristics render the zebrafish a useful model for the study of vertebrate development, and as such zebrafish has become the preferred model for developmental biologists. In the present study we wanted to test the possibility to use zebrafish embryos as a model to analyse *in vivo* disease development. 24-hour-old zebrafish embryos were infected with DsRed-labelled cells of *Salmonella typhimurium*. With wide-field deconvolution microscopy we were able to determine the exact location and fate of these bacterial pathogens in a living vertebrate host for three days. A low dose of wild-type *S. typhimurium* resulted in a lethal infection with bacteria residing and multiplying both in macrophage-like cells and at the epithelium of small blood vessels. LPS mutants of *S. typhimurium* proved to be non-pathogenic in the zebrafish embryos and were partially lysed in the bloodstream or degraded in macrophage-like cells. However, injection of LPS mutants in the yolksac of the embryo resulted in uncontrolled bacterial proliferation. Heat-killed wild-type bacteria were completely lysed within minutes after injection. In conclusion, the zebrafish embryo model allows for rapid, non-invasive and real-time, in situ analysis of bacterial infections in a vertebrate host.

T03-04

Toepassing van beslissingsondersteuning in de medische microbiologie: ontwikkeling van een aanvraagmodule

P.A. de Clercq[1,2], C.H.E. Boel[4], H.H.M Korsten[1,3]
[1]Technische Universiteit Eindhoven, Eindhoven, Nederland, [2]Medecs BV, Eindhoven, Nederland, [3]Catharina Ziekenhuis, Intensive Care, Eindhoven, Nederland, [4]Stichting PAMM, Veldhoven, Nederland

Dankzij standaarden en richtlijnen, technologische oplossingen, de 'human-factor' en het beschouwen van het systeem waarin medische professionals moeten werken is de kwaliteit van de medische zorg de afgelopen dertig jaar sterk gestegen. Aan de andere kant wordt er in een recent editorial in het New England Journal of Medicine gesteld dat deze kwaliteit nog steeds sterk verbeterd worden. Een voorbeeld hiervan is een recent onderzoek in de Verenigde Staten, uitgevoerd door het Institute of Medicine. Hieruit bleek dat ten minste 48.000 tot 98.000 doden per jaar zijn te betreuren door medische fouten in Amerikaanse ziekenhuizen. Het toepassen van kennistechnologie wordt als een belangrijk hulpmiddel gezien om de noodzakelijke kwaliteitsverbeteringen en de verhoging van de veiligheid in ziekenhuizen door te voeren.
Er zijn in Nederland verscheidene projecten gestart die zich richten op het toepassen van kennistechnologie in de medische wereld, met name op het gebied van de beslissingsondersteuning. Hieruit bleek dat, afhankelijk van het specialisme, er verschillende soorten beslissingsondersteuning mogelijk zijn, zoals ondersteuning bij de consultatie en ondersteuning bij de rapportage. Een voorbeeld van ondersteuning bij de consultatie is het presenteren van 'intelligente' invoerformulieren die op basis van de al eerder ingevoerde of bekende informatie getoond worden. Als gevolg hiervan wordt dus alleen die informatie aan een zorgverlener gevraagd die op dat moment relevant is. Een andere vorm van ondersteuning is ondersteuning bij de rapportage achteraf op basis van de ingegeven informatie. Een voorbeeld hiervan is het genereren van advies, waarschuwingen en rapporten op basis van de aanwezige informatie (bijvoorbeeld laboratoriumuitslagen) en kennis (bijvoorbeeld richtlijnen of protocollen).
Recent is een project gestart waarin de toepasbaarheid van bovenstaande vormen van ondersteuning in het specialisme van de Microbiologie onderzocht en getoetst wordt. Hiertoe wordt met behulp van de ontwikkelomgeving GASTON (waarmee beslissingsondersteunende systemen snel en eenvoudig door de specialisten zélf kunnen worden ontwikkeld), een consultmodule ontwikkeld, waarmee elektronische ge-

gevens kunnen worden ingegeven en worden geanalyseerd. Door gebruik te maken van de al bekende informatie, zoals de gegevens uit een laboratoriuminformatiesysteem (LIMS) of een Elektronisch Patiënten Dossier (EPD), worden zogenaamde contextgevoelige invoersystemen ontwikkeld worden: systemen waarin zowel het bedieningsgemak als de invoertijd verbeterd wordt door alleen die gegevens te vragen die op dat moment relevant zijn. Tevens wordt er gebruik gemaakt van een rapportagemodule die op basis van informatie (uit verschillende informatiesystemen zoals het LIMS of EPD) resultaten kan combineren, analyseren en presenteren. Het systeem is zo opgezet dat het kan communiceren met een willekeurige LIMS of EPD.
De tijd is rijp om kennistechnologie in te zetten in verschillende specialismen, zo ook binnen de Medische Microbiologie. Gegevens worden steeds vaker elektronisch opgeslagen, gegevensuitwisseling tussen diverse informatiesystemen begint van de grond te komen, User Interfaces waarmee ook leken kunnen werken zijn inmiddels dankzij Windows en het World Wide Web gemeengoed, en de maatschappelijke acceptatie van deze technieken is groeiende. Ook de kennistechnologie wordt volwassen genoeg om te kunnen leiden tot betere zorgkwaliteit, grotere effectiviteit, betere toegankelijkheid en transparantie en dit alles tegen lagere kosten. In de voordracht worden enkele aspecten van deze nieuwe ontwikkelingen gedemonstreerd.

V01

Increased prevalence of multiresistant *Enterobacteriaceae* during an *Enterobacter cloacae* outbreak: coincidence or transfer of resistance genes?

N. Al Naiemi[1], B. Duim[1], J.E.M. de Bruijn[1], P.H.M. Savelkoul[3], L. Spanjaard[1], E. de Jonge[2], J. Dankert[1], A. Bart[1], M.D. de Jong[1]
[1]Academic Medical Centre, Medical Microbiology, [2]Intensive Care, Amsterdam, the Netherlands, [3]Free University Medical Centre, Medical Microbiology and Infection Contro, Amsterdam, the Netherlands

Introduction. During an outbreak in an Intensive Care Unit (ICU) of an aminoglycoside-resistant, extended spectrum â-lactamase (ESBL)-positive *Enterobacter cloacae*, an increased prevalence of several other *Enterobacteriaceae* with similar resistance patterns was observed. We investigated whether this could be ascribed to interspecies transfer of resistance genes.
Methods. Relatedness between E. cloacae was determined with amplified fragment length polymorphism (AFLP) analysis. Plasmids were isolated from 10 outbreak strains and 42 multidrug-resistant Enterobacteriaceae isolated from ICU patients during the outbreak. For restriction fragment length polymorphism (RFLP) analysis, plasmids were digested with EcoRI. Transfer of resistance was investigated by conjugation experiments. PCR and sequence analysis, using generic primers for the TEM and SHV â-lactamase genes was performed.
Results. AFLP analysis identified that the E. cloacae outbreak was caused by a single clone. Aminoglycoside resistance and ESBL phenotype could be transferred separately via conjugation. Analysis of the transconjugants showed that these antibiotic resistance determinants were located on different plasmids. The specific RFLP patterns of both plasmids were observed in the epidemic clone as well as in several other Enterobacteriaceae, indicating interspecies plasmid transfer. PCR and sequence analysis revealed the presence of an SHV-12 ESBL gene in the epidemic E. cloacae and other Enterobacteriaceae. The precise extent of transfer of the SHV-12 ESBL- and aminoglycoside-resistance genes to other strains is currently investigated.
Conclusion. Our findings indicate the simultaneous occurrence of spread of resistance plasmids via interspecies transfer and a clonal outbreak of E. cloacae carrying the same plasmids. Beside relatedness between strains, characterisation of transferable resistance genes deserves attention in hospital epidemiology.

V02

Nosocomial infection (NI) in a neonatal intensive care unit (NICU): results from a surveillance study with definitions for infection specifically designed for neonates

W.C. van der Zwet[1], A.M. Kaiser[1], R.M. van Elburg[2], W.P.F. Fetter[2], C.M.J.E. Vandenbroucke-Grauls[1]
Free University Medical Centre, Departments of [1]Medical Microbiology and Infection Control and [2]Neonatology, Amsterdam, the Netherlands

Introduction. The incidence of NI in the NICU is high compared to other wards. No specific definitions for NI are available for neonates. The aim of our study was to measure the incidence of NI and to identify risk factors for NI in the NICU of the VU University Medical Centre.
Methods. A prospective surveillance was performed from 1998-2000. We designed definitions for NI specific for neonates by adjusting current definitions of the Centres for Disease Control (CDC) for children <1 year. Analysis for risk factors was performed by multivariate analysis. Neonates were stratified in 4 birth weight categories: <1000 g (I), 1001-1500 g (II), 1501-2500 g (III), >2500 g (IV).
Results. Of 742 included neonates, 191 neonates developed 269 infections. Blood stream infection (BSI) (n=129, 15/1000 patient days) and pneumonia (n=73, 8/1000 patient days) comprised the main part of infections. Of BSI 58% was caused by coagulase negative staphylococci; 22% remained culture negative. Of pneumonias 36% was caused by Enterobacteriaceae; 27% of cultures remained negative. Compared to surveillance data from the Nosocomial Infection Surveillance System of the CDC, our device associated utilisation ratios were high (ventilator days II-IV and central line days IV: >90% percentile) and device-associated NI rates were high (central line associated blood stream infection rate and ventilator associated pneumonia rate I-II: >90% percentile). The main risk factors for BSI were low birth weight (Odds Ratio (OR) II vs IV = 5.22, I vs IV = 5.64), and the presence of an umbilical vein and artery catheters (presence up to 4 days: OR = 3.56 and 2.44 respectively). The main risk factors for pneumonia were low birth weight (OR II vs IV = 5.41, I vs IV = 7.2) and intubation (1-4, 5-8 and >8 days: OR = 3.35, 6.64 and 3.02 respectively. The Clinical Risk Index for Babies was not predictive for infection.
Conclusions. Our local NI rates are relatively high. This can be partially explained by (1) the use of our definitions for NI, which are more suitable for this patient category, and (2) the relative high device associated utilisation ratios.

V03

The effect of timing of antibiotic prophylaxis on the incidence of surgical site infections after total hip replacements

J. Manniën[1], M.E.E. van Kasteren[2], I.C. Gyssens[3], B.J. Kullberg[2], J.C. Wille[4], A.S. de Boer[1]

[1]National Institute of Public Health and the Environment, Centre for Infectious Diseases Epidemiology, Bilthoven, the Netherlands, [2]University Medical Centre St Radboud, Department of Internal Medicine, Nijmegen, the Netherlands, [3]Erasmus University Medical Centre, Department of Medical Microbiology and Infectious Diseases, Rotterdam, the Netherlands, [4]Dutch Institute for Healthcare Improvement CBO, Utrecht, the Netherlands

Introduction. Perioperative antibiotic prophylaxis is used to prevent surgical site infections (SSIs). Its timing is considered to be crucial in preventing SSIs. We examined the relation between the timing of prophylaxis and the SSI incidence in total hip replacements of the CHIPS study, a multicentre intervention study which investigated the quality of perioperative prophylaxis in Dutch hospitals.

Methods. Eleven hospitals collected data on both SSIs and prophylaxis for elective total hip replacements, excluding surgery in dirty or infected tissue. Only data of surveillance during admission were included. Timing of the administration of prophylaxis was stratified as follows: very early (>120 minutes before incision), early (31-120 minutes before incision), optimal (1-30 minutes before incision), and late (after incision). Relative risks (RR) for a SSI of suboptimal timing compared to optimal timing were stratified for the risk factors gender, age, ASA score, and duration of surgery.

Results. Data were collected on 851 total hip replacements. The SSI incidence was 0.9 (95% conficence interval (CI): 0.3-2.2) when timing of prophylaxis was optimal, 0.0 (95% CI: 0.0-43.4) when timing was very early, 4.1 (95% CI: 2.5-6.8) when timing was early, and 3.9 (95% CI: 1.1-13.2) when timing was late. The crude relative risk for an SSI of suboptimal versus optimal timing was 4.6 (95% CI: 1.6-13.8). RRs for a SSI of suboptimal compared to optimal timing, stratified for gender, age, ASA score and duration of surgery, did not differ significantly from the crude RR.

Conclusions. These data highlight the importance of a correct timing of antibiotic prophylaxis, as the incidence of SSI was higher when timing was suboptimal. This study suggests that the common recommendation to administer antibiotic prophylaxis within two hours before incision may be narrowed down to 30 minutes before incision.

V04

Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn centre

A.M.D. Kooistra[1], S.R. van Dijk[2], G.I.J.M. Beerthuizen[2], W.H.M. Vogels[3], A.A. van Zwet[1], H.A. Verbrugh[4]

Regional Public Health Laboratory, [1]Research & Development, [3]Medical Microbiology, Groningen, the Netherlands, [2]Martini Hospital, Burn Centre, Groningen, the Netherlands, [4]Erasmus Medical Centre, Medical Microbiology and Infectious Diseases, Rotterdam, the Netherlands

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

The aim of this study was to evaluate the effect of eradication of nasal *S. aureus* with mupirocin in patients with regard to *S. aureus* colonisation of their burn wounds.

Methods. From September 2000 to March 2001, 42 patients admitted to our 10-bed burn centre were screened for *S. aureus* in nose and burn wounds. Isolates were genotyped with PFGE. All patients received nasal mupirocin at the time of admission to the burn centre. Fifty-five patients from the same unit who were followed from September 1997 till May 1998 and had not received mupirocin prophylaxis served as a historical control group.

Results. At admission 29/42 (69%) patients in the mupirocin trial had no *S. aureus* colonisation in their burn wounds. Of this group 25 patients were non-carriers and 4 patients were nasal carriers of *S. aureus*. Seven (28%) non-carriers and 2 (50%) nasal carriers acquired burn wound colonisation with *S. aureus* during their stay at the centre. Of the historical control group, 39 (71%) patients had no *S. aureus* colonisation of their burn wounds at the time of admission. Of this group 31 patients were non-carriers and 8 patients were nasal carriers; 18 (58%) non-carriers and 7 (88%) nasal carriers acquired *S. aureus* wound colonisation during their stay. The overall probability of wound colonisation in the patients treated with mupirocin was significantly lower than in the historical non-treated group (p=0.01, chi-square test with Yates correction).

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* colonisation 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.

V05

Risk factors for carriage of an epidemic vancomycin-resistant *Enterococcus faecium* strain

E.M. Mascini[1], K.P. Jalink[1], T.E.M. Kamp-Hopmans[1], H.E.M. Blok[1], J. Verhoef[1], M.J.M. Bonten[2], A. Troelstra[1]
University Medical Centre Utrecht, [1]Eijkman Winkler Centre for Microbiology/Infectious Diseases and Inflammation, [2]Division of Internal Medicine and Dermatology, Utrecht, the Netherlands

Introduction. In May 2000, the first outbreak of vancomycin-resistant *Enterococcus faecium* (VREF) was detected in the University Medical Centre Utrecht at the nephrology ward. It is not known what determines why some VREF strains spread among hospitalised patients, whereas other strains do not.
Methods. 30 patients who were found colonised with VREF between May and November 2000 were included in the study. Molecular typing and subsequent comparison of strains with an international databank confirmed that 19 of them carried an identical epidemic strain which harboured the *esp*-gene, while 11 were colonised by non-epidemic strains which were all *esp*-negative. Among these patients we analysed risk factors for acquisition of the outbreak strain. In addition, to study duration of colonisation, prospective surveillance of VREF carriage for a 6-months period starting from the date of first isolation of VREF was realised for 25 patients.
Results. Acquisition of the outbreak strain was significantly associated with diabetes mellitus, renal transplantation, and receipt of 2 or more antibiotics, especially cephalosporins, in the 2-months period before the date of first isolation of VREF. After 6 months, VRE was still detectable in faecal samples from 60% of carriers of the outbreak strain versus 20% of carriers of non-epidemic strains ($P<.05$). Diabetes mellitus was identified as a significant risk factor for prolonged colonisation of the outbreak strain.
Conclusion. A particular outbreak strain preferentially colonised patients who had been subjected to extensive antibiotic exposure and certain patient categories with underlying conditions such as diabetes mellitus and renal transplantation. The fact that this strain was recovered from faecal samples of the host for a significantly longer period of time than non-epidemic strains, may facilitate dissemination of the strain. We suggest, that next to a careful restrictive antibiotic policy at wards at risk for spread of VREF isolation, precautions may be focused on patients who are colonised with outbreak strains that harbour the *esp*-gene.

V06

Determination of epidemiological relationships between pneumococci: pulsed field gelelectrophoresis versus amplified fragment length polymorphism

C.H.W. Klaassen[1], J.A. de Valk[1], C. Neeleman[2], J.W. Mouton[1]
[1]Canisius Wilhelmina Hospital, Medical Microbiology and Infectious Diseases, Nijmegen, the Netherlands, [2]University Medical Centre St Radboud, Nijmegen, the Netherlands

Introduction. Pulsed field gelelectrophoresis (PFGE) is still considered to be the gold standard for determination of epidemiological relationships between pneumococcal isolates (and many other micro-organisms as well). However, it is a laborious and time-inefficient method. For obvious reasons polymerase chain reaction (PCR) based protocols like amplified fragment length polymorphism (AFLP) are much more favoured over the classical PFGE protocol. We explored the use of AFLP for epidemiological fingerprinting of *Streptococcus pneumoniae* isolates, especially focussing on the comparison with PFGE.
Methods. 89 erythromycin resistant *S. pneumoniae* isolates were collected from laboratories nationwide as part of another study. PFGE and AFLP were performed using established protocols. A *LytA* PCR was performed on all isolates to verify the identification as *S. pneumoniae*. All non-pneumococci were identified by 16S rRNA sequencing.
Results. Clusters of epidemiologically-related *S. pneumoniae* isolates were more easily recognised by using AFLP analysis than by using PFGE. These relationships were confirmed by PFGE, which proved to have a higher discriminatory power on a strain level. Furthermore, AFLP analysis also showed the formation of clusters on a species level allowing a simultaneous differentiation of species. All non-pneumococci were not recognised as such by PFGE. A total of 31 isolates (35%) were not grouped into the pneumococci cluster by AFLP. All pneumococci (according to the AFLP clusters) were *LytA* positive, all non-pneumococci were *LytA* negative. Of these 31 isolates, 17 (19%) were *Streptococcus mitis*, 9 (10%) were other streptococci and 5 isolates (6%) were non-streptococci. Epidemiological analysis of all isolates was much more easily established by using AFLP than by using PFGE.
Conclusion. AFLP analysis should be the preferred method for epidemiological fingerprinting of pneumococci.

W01

Development and application of a multiplex real-time PCR for diagnosis of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis*

K.E. Templeton[1], S.A. Scheltinga[1], H. Goossens[2], E.C.J. Claas[1]
[1]Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands, [2]UIA, Medical Microbiology, Antwerp, Belgium

Objectives. To develop a multiplex real-time PCR for clinical diagnosis of *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP) and *Bordetella pertussis* (BP) with an internal control.
Methods. Monoplex real-time PCR assays using molecular beacons as probes were developed for each pathogen. The molecular beacons were labelled with FAM (MP), HEX (CP), Texas RED (BP) so that discrimination in a single tube could be determined in one multiplex. All assays were first optimised in the monoplex assays and thereafter into one 4 ?target-multiplex reaction. The multiplex included an internal control reaction (phocine herpes virus) which was also detected by a labelled probe (Cy5). Standards of the bacteria were obtained by achieving a titre by colour changing units (CCU)/ml, inclusion forming units (IFU)/ml and Mcfarland turbimetric standards for MP, CP and BP respectively. The assay was evaluated using samples from 93 patients with respiratory symptoms requesting investigation for at least one of the pathogens. All samples were tested by

both the conventional diagnostic methods (serology or culture) and by means of the multiplex reaction. Results. The same limit of sensitivity was seen in the monoplex reactions and multiplex reaction, in that 50 CCU/ml, 0.049 IFU/ml, 100 CFU/ml was detected for MP, CP and BP respectively. The specificity of the monoplex and multiplex was also the same. No other respiratory pathogens were detected by the assays except that BP assay also detected *B. holmesii* in both monoplex and multiplex formats. In the clinical testing 9/93, 0/93 and 6/93 were detected by conventional methods and 12/93, 0/93 and 15/93 were detected by the multiplex PCR for MP CP and BP respectively. Of these 2 extra positives were obtained which were not suspected by the clinician.
Conclusion. The Multiplex real-time PCR for MP, CP and BP provides sensitive and specific diagnosis of these respiratory pathogens, which are difficult to culture. The multiplex assay is suitable for diagnostic use and may improve patient diagnosis and management.

W02
Development and assessment of a real-time PCR for *Giardia lamblia*

L. Schinkel, J.J. Verweij, D. Laeijendecker, A.M. Polderman
Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands

Introduction. Microscopy is considered the gold standard in diagnosis of a *Giardia lamblia* (GL) infection, however, this method is time-consuming and appears to be less sensitive than methods based on antigen detection. We developed a real-time PCR for the detection of GL and assessed its performance in comparison with the Alexon antigen test and microscopy.
Methods. The 18 SSU ribosomal gene was chosen as a target. To select conserved yet specific GL sequences, alignments were made of 31 Genbank sequences that included GL and 15 sequences of other parasitic and bacterial (non-)pathogens. Primers and probe were chosen that correspond to position 80 - 99 and 127 - 142 for the forward and reverse primers respectively and to 106 - 125 for the probe. The probe was labelled with FAM. Before column based DNA extraction, the faeces suspension containing 2% polyvinylpyrolydone was incubated at 100 C for 10 minutes to reduce inhibition. PCR was performed using a commercial mix that includes Hotstar Taq polymerase (Qiagen).
Patients. 96 stool samples of different patients were selected that were microscopy positive (n=86) and/or antigen positive (n=94). Eighteen additional samples were included that were microscopy positive but on which no antigen test was performed. Additionally, 24 stool samples were selected from patients with a negative antigen test and microscopy. Specificity of the assay was also tested on DNA obtained from 12 different bacterial cultures.
Results. PCR was positive in 102/104 (98.1%) of microscopy positive samples. The 2 PCR negative samples were both antigen-positive. In addition, PCR was positive in 10 samples that were microscopy negative but antigen positive. The only 2 out of 96 samples that were antigen negative were both PCR and microscopy positive.
PCR was negative in all 24 microscopy and antigen negative samples, as well on DNA from bacterial cultures.
Conclusion. Our in-house real-time PCR for the detection of *Giardia lamblia* is as sensitive and specific as the Alexon antigen test and is more sensitive than microscopy.

W03
Real-time detection of *Salmonella* DNA in faeces without culture enrichment

T. Schuurman, M.C. Scholts, A.M.D. Kooistra, A.A. van Zwet
Regional Public Health Laboratory for Groningen and Drenthe, Research and Development, Groningen, the Netherlands

Background. The diagnosis of salmonellosis is based on detection of *Salmonella spp*. in faeces, which consists of enrichment, selective culture and determination. This procedure may take several days. Recently molecular methods have become available for the detection of *Salmonella spp*. in faeces. However these methods still require a culture enrichment step resulting in a delay of at least 16 hours. In order to diminish 'turn-around time' as much as possible we embarked on a study investigating the possibilities of detecting *Salmonella* DNA directly in faeces.
Methods. During a consecutive period of 10 months 76 culture positive faecal samples were collected and stored at -20°C directly after the initiation of bacterial culture. Another 74 culture negative faecal samples were randomly selected. All faecal samples were suspended in lysis buffer at 33-50% (wt/v) and DNA was extracted from these suspensions. Real-time amplification was carried out on the invasion A gene, which is considered to be specific for *Salmonella spp*. The overall sensitivity of the assay was determined at 400-800 CFU/gram of faeces.
Results. During sample preparation 6 faecal samples, all with the same gluey structure, could not be homogenised. These samples were regarded as a separate group (P). All these 6 samples were culture positive. The remaining 70 culture positive samples all yielded positive PCR results. In the culture negative samples, one sample yielded a positive PCR result (sensitivity 100%, specificity 99% as compared to culture). In group P, PCR was negative when the standard extraction method was performed. However when a more rigorous homogenisation procedure was applied 3 out of 6 samples became PCR positive after all.
Conclusion. Our study shows that it is possible to detect *Salmonella* DNA directly in faeces without enrichment. A small group of physically distinct faecal samples is not suitable for this procedure. This problem needs further study before this molecular method can be introduced in our routine laboratory as a means of rapid detection of *Salmonella spp*. in faeces.

W04

16S rDNA PCR and sequencing of cerebrospinal fluid in the diagnosis of bacterial meningitis: results of a multicenter study

T. Schuurman, R.F. de Boer, A.M.D. Kooistra, A.A. van Zwet

Regional Public Health Laboratory, Department of Research and Development, Groningen and Drenthe, the Netherlands

Background. The diagnosis of bacterial meningitis is based on Gram staining and bacterial culture of cerebrospinal fluid (CSF). However, in meningitis caused by fastidious or slow growing bacteria, or when antimicrobial treatment is started before CSF is obtained, culture sometimes remains negative. This study describes a broad-range bacterial PCR with subsequent DNA sequencing directly performed on CSF in comparison with conventional bacterial culture.

Methods. Laboratories from 10 regional hospitals in the northern part of the Netherlands participated in this study. During 11 months 138 CSF samples from 135 patients with suspected meningitis were collected. Both routine culture and molecular analysis were performed independently on the CSF samples. DNA was extracted from the sample by a bead-beating/silica/guanidinium thiocyanate procedure. Amplification was performed with universal bacterial primers and positive samples were subsequently sequenced for identification.

Results. Fifteen PCR positive and 115 PCR negative samples were in complete accordance with the results of culture. Culture yielded 5 additional positive results, 4 of those 5 positive samples contained coagulase-negative staphylococci (CNS), which were regarded and reported as laboratory contaminations. The PCR detected 3 additional clinical significant positive samples. Compared to culture and corrected for the CNS culture results, sensitivity was 94%, specificity 97%, positive predictive value 83% and negative predictive value 99%.

Conclusion. In general our assay for direct CSF examination with a broad-range PCR and subsequent DNA sequencing seems to be comparable with culture in detecting bacterial meningitis. However the fact that the molecular method was capable of detecting 3 additional clinical significant pathogens missed by culture, makes the molecular approach an important supplementary test in daily clinical practice.

W05

Multiplex real-time PCR detection of respiratory viral targets

K.E. Templeton, S.A. Scheltinga, M.F.C. Beersma, A.C.M. Kroes, E.C.J. Claas

Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands

Objectives. To develop two multiplex real-time PCR reactions for diagnosis of influenza virus A (FA), influenza virus B (FB), respiratory synctial virus (RSV), parainfluenza viruses 1 (PIV1), 2 (PIV2) and 3 (PIV3).

Methods. Monoplex real-time PCR assays using molecular beacons as probes were developed for each virus. The molecular beacons were labelled with FAM (FA/PIV3), HEX (PIV1), Texas RED (FB/PIV2) and RSV (Cy5) so that discrimination in a single tube could be determined in two multiplexes. Sensitivity for the assays was obtained by comparison with viral culture by 10-fold serial dilutions of the six different viruses to give a $TCID_{50}$. The clinical utility of the assays was assessed by performing viral culture on 220 respiratory samples and comparing to PCR. Culture was performed in shell vials of HEL, LLCK2 and HEp2. Immunoflouresence (IF) was performed at 3 days and to confirm any cytopathic effect (Dako, Imagen). The nucleic acids were extracted from stored samples using the Magnapure (Roche) and run in the two multiplex reactions.

Results. The same limit of sensitivity was seen in the monoplex reactions and multiplex reactions, 0.01 $TCID_{50}$ for FA, 0.001 $TCID_{50}$ for FB, 0.01 $TCID_{50}$ for RSV, 0.01 $TCID_{50}$ for PIV1, 0.001 $TCID_{50}$ for PIV2 and 0.1 $TCID_{50}$ for PIV3. The specificity of both the monoplex and multiplex assays were also the same and no other viral respiratory pathogens were detected. In the clinical testing 15 (7%)(2 FA, 1FB, 9 RSV, 1PIV1, 2PIV3) were positive by cell culture for one of these 6 pathogens. By multiplex PCR 29 (13%) (2FA, 1FB, 15 RSV, 5PIV1, 6 PIV3) were found positive. Nine of the samples positive only by multiplex PCR were from patients with another sample culture positive. Using this real-time PCR methodology results for 30 samples could be obtained in 5 hours, including extraction.

Conclusion. The two multiplex real-time PCR for FA, FB, RSV, PIV1, PIV2, PIV3 provides sensitive and specific diagnosis for these respiratory viral pathogens. This assay provides a sensitive and rapid method and is suitable for diagnostic use and may improve patient management and diagnosis.

W06

Evaluation of eight commercially available EIA kits and two immunoblots for the serodiagnosis of Lyme disease

A.H. Brandenburg[1], B.C. Meijer[2], E. Steendam[1], A.M. van Elsacker-Niele[1]

[1]*Public Health Laboratory Friesland, Department of Medical Microbiology, Leeuwarden, the Netherlands,* [2]*Regional Public Health Laboratory, Department of Microbiology, Groningen, the Netherlands*

Sero-diagnosis of Lyme disease can be troublesome. Infection with *Borrelia burgdorferi* can present with a wide variety of clinical symptoms and even confirmatory tests from reference labs often give conflicting results. Therefore it is difficult for routine labs to determine which test or combination of tests is most suitable for diagnostic use. To investigate which serological test would be most suitable in our situation we evaluated eight commercially available Lyme-EIA kits from DAKO, Biotest (both native and recombinant antigen EIA), Clindia, Dade Behring (Enzygnost Borreliosis), Microgen (recomWell), Novum diagnostica and Genzyme/Virotech and two Lyme-immunoblot assays (recomBlot (Microgen) and Biotest Western blot) in a panel of 104 sera. In this panel mainly sera from patients with chronic skin disease (ACA), arthritis and neurological symptoms were included. As possibly cross-reacting sera also EBV-IgM and TPPA positive samples were included. Because no gold standard is available the tests were compared (1.) in cluster analysis and (2.) to earlier serological results (DAKO-EIA followed by RIVM-blot), combined with

clinical symptoms and results from follow up sera and/or PCR results from skin biopsies.
In sensitivity Dade Behring, Microgen and Virotech performed best missing only two, one and one early neuroborreliosis samples respectively. In all other EIAs more early neuroborreliosis and also one or more ACA samples gave dubious or negative results.
All IgM EIAs generated several false positives, mainly in the EBV-IgM positive sera and in sera from patients with arthritis previously found negative by immunoblot. The IgM EIAs from Microgen and Novum were found to be the most specific. In IgG tests DAKO, Dade Behring and Novum were the most specific.
Of the immunoblots the Microgen blot was the most sensitive and specific. The recombinant antigen used in this blot gives sharp bands without background bands. This makes reading of the blots straightforward and reproducible and will be an advantage in routine diagnostics.

POSTERS

P01
Mycoplasma pneumoniae and *Chlamydia pneumoniae* as causes of pneumonia in the intensive care setting

C.F.M. Linssen[1], J.A. Jacobs[1], J.S.A.G. Schouten[2], S. van der Geest[3], W.N.K.A. van Mook[3], M. Drent[4], G. Ramsay[3]
University Hospital Maastricht, [1]Medical Microbiology, [2]Epidemiology, [3]Intensive Care Medicine, [4]Pulmonology, Maastricht, the Netherlands

Introduction. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are intracellular pathogens that occasionally cause severe community-acquired pneumonia (CAP) requiring admission to the intensive care unit (ICU). It is uncertain whether these pathogens may be the cause of hospital-acquired pneumonia. In this study we looked for the presence of both pathogens in bronchoalveolar lavage (BAL) fluid of patients hospitalised at the ICU and suspected of pneumonia.
Study design. Over a 48-month period (January 1998-December 2001), BAL fluid samples obtained from patients admitted with a CAP or suspected of a ventilator associated pneumonia (VAP) when hospitalised at the ICU were included. BAL fluid samples were centrifuged and stored in MEM-medium at ?80°C. A total of 276 BAL fluid samples were included. These BAL fluid samples were obtained in ventilated patients at days 1 (n=12), day 2 (n=13), day 3 (n=13) or later (n=238) during ICU stay. Out of the 276 BAL fluid samples, 121 confirmed the suspected diagnosis pneumonia (culture ≥10^4 cfu/ml; CAP: 11/121, early onset VAP: 9/121, late onset VAP: 101/121). *M. pneumoniae* and *C. pneumoniae* were diagnosed by an in-house polymerase chain reaction.
Results. *M. pneumoniae* and *C. pneumoniae* were diagnosed each in 1 out of 276 samples at days one and two respectively. In both samples the cut-off value (culture) for microbiologically confirmed pneumonia was not reached for other micro-organisms. Both BAL fluid samples had a normal total cell count and showed 59.2% and 74.2% polymorphonuclear neutrophils respectively. They were obtained in two out of 13 patients with CAP admitted to the ICU.
Conclusions. 1. In the ICU setting *M. pneumoniae* and *C. pneumoniae* are potential pathogens in patients admitted for CAP. 2. In this series neither *M. pneumoniae* nor *C. pneumoniae* were part of the offending flora in patients with VAP.

P02
Molecular detection of rifampicin resistance and spoligotyping of *Mycobacterium tuberculosis* directly from Ziehl-Neelsen stained slides

A.G.M. van der Zanden[1], E.M. te Koppele-Vije[1], F.G.C. Heilmann[1], D. van Soolingen[2], L.M. Schouls[3]
[1]Gelre Hospitals, Medical Microbiology and Infectious Diseases, Apeldoorn, the Netherlands, National Institute of Public Health, Departments of [2]Mycobacteria Reference and [3]Bacteriology, Laboratory for Infectious Diseases, Bilthoven, the Netherlands

Introduction. Multidrug resistance among new cases of tuberculosis is increasingly becoming a significant problem in countries with a high prevalence and with inadequate therapy of tuberculosis. Rifampicin resistance is widely used as a marker for multidrug-resistant tuberculosis (MDR-TB) and a new approach is introduced to measure rifampicin resistance retrospectively without the need of a viable culture. In many developing countries culture is unavailable and diagnosis relies on clinical manifestations and Ziehl-Neelsen staining of sputum smears.
Methods. We determined rifampicin resistance directly in DNA extracts obtained from 36 Ziehl-Neelsen slides by identifying of mutations in the *rpoB* gene using reverse line blot hybridisation and DNA sequencing.
Results. Analysis of the *rpoB* gene revealed that samples containing rifampicin resistant *Mycobacterium tuberculosis* carried altered codons representing amino acid positions 516, 526 and 531 of the RNA polymerase.
Conclusion. (1) Although the sensitivities of both methods were equal (84%), the sequencing results were much easier to interpret than the results obtained by the reverse line blotting. Furthermore, due to the strict hybridisation conditions reverse line blotting is technically more demanding than sequencing.
(2) Sequence analysis of the *rpoB* gene in extracts from Ziehl-Neelsen stained slides may be used to quantify more precisely the nature mutation leading MDR-TB and (3) more importantly provide information on the magnitude and trends of development of resistance on a global scale. (4) The nature of rifampicin resistance and the genotype can be determined by analysis of Ziehl-Neelsen stained slides in a laboratory equipped for sequencing and spoligotyping. As the method uses killed organism on stained slides, samples do not have to be shipped as bio-hazardous materials.

P03

Bronchoalveolar lavage fluid cytology in the prediction of early- and late onset ventilator-associated pneumonia

C.F.M. Linssen[1], J.A. Jacobs[1], J.S.A.G. Schouten[2], S. van der Geest[3], W.N.K.A. van Mook[3], M. Drent[4], G. Ramsay[3]
University Hospital Maastricht, Departments of [1]Microbiology, [2]Epidemiology, [3]Intensive Care Medicine and [4]Pulmonology, Maastricht, the Netherlands

Introduction. Ventilator-associated pneumonia (VAP) is a common nosocomial infection in intensive care patients. VAP may be distinguished in early-onset VAP (≤4 days of ventilation) and late-onset VAP (≥5 days of ventilation). In this study, we assessed the predictive value of bronchoalveolar lavage (BAL) fluid cytology for the diagnosis of early-onset and late-onset VAP.
Study design. Over a 30-month period (January 1999-June 2002), BAL fluid samples obtained from patients suspected of VAP were collected. VAP was defined as BAL fluid cultures $\geq 10^4$ colony forming units/ml. BAL fluid cytology included the total cell count/ml, the differential cell count on 500 nucleated cells including the % of cells containing phagocytised organisms (intracellular organisms, ICOs). Receiver operating characteristic (ROC) curves were plotted for various cytological parameters and their combinations. Predictive values were calculated.
Results. A total of 142 BAL fluid samples were collected in 127 patients, and included in the study. In 53 (37%) of BAL fluid samples VAP was confirmed by culture. In rank of decreasing frequency, organisms most frequently recovered in early-onset VAP were: *Streptococcus pneumoniae* (n=3) and *Haemophilus influenzae* (n=2). In late-onset VAP they included: *Pseudomonas aeruginosa* (n=13) and *Staphylococcus aureus* (n=13). For the prediction of early- and late-onset VAP combined, the area under the curve (AUC) of the ROC curves of various parameters was as follows: total cell count: 0.566, % Polymorphonuclear neutrophils: 0.660, and % ICOs: 0.905. The combination of % ICOs with any other parameter did not increase the AUC. At a cut-off value of 2% for ICOs early-onset VAP was diagnosed with an 88.9% and 88.9% positive (PPV) and negative predictive value (NPV). For the diagnosis of late-onset VAP, the PPV and NPV were 84.6% and 88.1% respectively.
Conclusions. 1. The % of ICOs proved to be the single and most reliable parameter. 2. The PPV and NPV of early- and late-onset VAP were nearly identical: 88.9% and 88.9% versus 84.6% and 88.1% respectively.

P04

Rapid detection of human metapneumovirus (hMPV) by real-time PCR in patients with respiratory tract infections

S.A. Scheltinga, K.E. Templeton, M.F.C. Beersma, A.C.M. Kroes, E.C.J. Claas
Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands

The aim of this study was to develop a real-time PCR assay for detection of human metapneumoviruses (hMPV).
The real-time PCR assay was designed from the conventional L-gene PCR developed by the Erasmus Medical Centre, Rotterdam (prof. A. Osterhaus). A consensus probe sequence was selected from aligned sequences of the different hMPV variants and used to design a molecular beacon, labelled with the fluorophore HEX and quencher Dabcyl. The assay was optimised and the specificity and sensitivity were determined. Two hundred respiratory samples (throat swab, nose wash and sputum) were used to evaluate the hMPV assay. Nucleic acids were isolated using the Total Nucleic Acid Kit of the Magnapure (Roche). Amplification was performed using the one-step RT-PCR kit (Qiagen) and the iCycler IQ real-time detection system (Biorad).
The real-time PCR assay for detecting hMPV is specific and has a sensitivity of 0.1 TCID50. Five patients (2.5%) were found positive for hMPV. Sequence analysis showed that four patients were infected with subtype A and one patient with subtype B hMPV. Of the four patients infected with type A hMPV, samples were taken in December and in January. Only two patients infected with type A hMPV (aged 1 and 35 years) showed respiratory illness like coughing and fever. The patient infected with subtype B hMPV, of whom the sample was taken in June (aged 11 years), was an immunocompromised patient undergoing a bonemarrow transplantation. None of the five patients were infected with other respiratory viruses.
1) Real-time PCR is a rapid method to detect the hMPV within 6 hours. 2) hMPV is a cause of respiratory disease in children and adults.

P05

Rapid diagnosis of *Bartonella* infections using automated DNA extraction and real time PCR

B. Duim, E. van der Maas, R. Boom, D. van der Riet, B. de Wever, M.D. de Jong
Academic Medical Centre, Mecical Microbiology, Amsterdam, the Netherlands

Introduction. Several *Bartonella* species are important human pathogens that cause a variety of clinical syndromes. Diagnosis of infections by *Bartonella* species is seriously hampered by their fastidious growth characteristics. We have developed a real-time PCR assay for rapid diagnosis of clinically relevant *Bartonella* species.
Methods. The MagNaPure LC for automated DNA isolation in combination with LightCycler PCR and DNA sequence analysis was used. DNA was extracted from *B. henselae, B. quintana* and *B. elizabethae* cultures. Before using the MagNa Pure Bacterial DNA isolation kit III were cells lysed with Proteinase K. PCR primers targeting the 16S-23S rRNA intergenic region of the genus Bartonella were used for PCR amplification. Amplicons were detected with meltingcurve analysis and biprobe hybridisation. A bartonella generic biprobe, labelled with the fluorophore Cy5 was used. When this probe binds to complementary sequences the Cy5 is excited by the energy transfer from Sybr Green I. After a positive probe result the amplicon is sequenced for determination of the Bartonella species.
Results. With serial dilutions of bacteria the sensitivity of real-time detection using Sybr Green I, was determined at 1,7 CFU of *B. henselae*, 0,7 CFU of *B. quintana* and 0,2 CFU of *B. elizabethae*. Meltingcurve analysis enabled differentiation of the three species. The probe assay was developed as the primers were found to amplify human DNA from clinical

samples. The sensitivity of the probes was identical to the Sybr Green I detection. The probe assay detected *B. henselae* in a lymph node from a patient with cat scratch disease. The MagnaPure isolation in combination with the probe assay is now being evaluated in a large number of clinical specimens. Conclusions. Automated DNA isolation and biprobe LightCycler PCR allowed rapid and sensitive detection of *Bartonella* species and, in combination with DNA sequencing is a promising and accurate method for identification of *Bartonella* infections.

P06
Improved diagnosis of acute respiratory tract infections with multiplex PCR as additional test, experience in three different paediatric wards

P. Gruteke[1], M. Dierdorp[1], W.B. Vreede[2], J.W. Pilon[3], S. Bruisten[1]
[1]*Municipal Health Service, Public Health Laboratory, Amsterdam, the Netherlands,* [2]*Flevohospital, Pediatrics, Almere, the Netherlands,* [3]*IJsselmeerhospitals, Pediatrics, Lelystad, the Netherlands*

Objectives. Current molecular methods allow improved diagnosis of Acute Respiratory Infections (ARI) in paediatrics. The aim of this study is to document the number of diagnoses obtainable with Multiplex-PCR, used in addition to conventional laboratory methods, as well as the turnaround time required.
Methods. Nasopharyngeal aspirate (NPA), per-nasal and throat swabs were accepted as samples. NPA samples were tested for RSV antigen on working days. M-PCR was performed twice weekly either on NPA samples if no RSV antigen was detected, or on per-nasal and throat swab samples, if NPA was not included in the sample set. M-PCR consisted of a DNA-set (*C. pneumoniae, M. pneumoniae* and adenovirus) and a RNA-set (Influenza A and B, Parainfluenza 1 and 3, Rhinovirus, Enterovirus and RSV). Paediatricians completed questionnaires on presentation, and on discharge if the patient was admitted.
Results. A total of 178 episodes, in 172 patients, was sampled. In 94 sample sets an NPA was included, and in 53 (56%) of these, RSV-antigen was detected. The number of sample sets tested by M-PCR was 125, with 76 (60%) yielding one or two possible pathogens. Rhinovirus was the most common finding; in 26 samples as a single pathogen, and in 19 samples with another possible pathogen. Bronchiolitis was the most commonly reported clinical symptom in these cases. Rhinovirus patients had a shorter duration of illness prior to presentation than RSV patients, possibly indicating a more severe disease. Influenza A was found in 10 samples as a single, and in 3 samples with another pathogen. Median turnaround time was 4 days for M-PCR. Wards differed considerably with respect to admission and sampling policy.
Conclusion. M-PCR is a useful additional test in paediatric practice. Rhinovirus patients presented to paediatricians may have more severe illness than RSV patients. M-PCR for ARI has now been established as a standard test for RSV-antigen negative NPA samples in our laboratory.

P07
Quantitative real-time PCR targeting the 23S-5S spacer for direct detection and differentiation of *Legionella spp.* and *Legionella pneumophila*

B.L. Herpers[1], B.M. de Jongh[1], K. van der Zwaluw[2], E.J. van Hannen[1]
[1]*St. Antonius Hospital, Department of Medical Microbiology and Immunology, Nieuwegein, the Netherlands,* [2]*National Institute of Public Health and the Environment (RIVM), Diagnostic Laboratory for Infectious Diseases And Perinatal Screening (LIS), Bilthoven, the Netherlands*

Legionellae can cause severe atypical pneumonia. Prognosis depends partially on rapid and accurate diagnosis, while conventional diagnostic techniques like culture on selective media, seroconversion assays, direct fluorescent antibody assays and urinary antigen tests either lack speed or sensitivity. Therefore, direct detection of *Legionella* DNA in clinical samples is a challenging alternative. Here we describe a dual colour, real-time PCR assay targeting the 23S-5S spacer region to detect and quantitate *Legionellae* in clinical samples. Our results show that the described dual probe assay is able to correctly distinguish *L. pneumophila* from other *Legionellae* in real-time. In comparison to previously described real-time 16S PCR assays, the more diverse 23S-5S spacer region allows this without post-PCR analysis. In a clinical context, this implies a maximum of 90 minutes assay time or less after DNA isolation before results are known. Such rapid diagnostic testing and differentiation of clinical specimens, here described for the ABI Prism 7000 system, provides a valuable tool in the evaluation of severe atypical pneumonia.

P08
Detection of *H. pylori* antigen in stool with a novel monoclonal EIA (HpSTAR)

A. van der Ende[1], S. Koletzko[2], J. Raymond[3], D.K. Bosman[4]
Academic Medical Centre, [1]*Medical Microbiology,* [4]*Pediatrics Amsterdam, the Netherlands,* [2]*Dr. v. Hauersches Kinderspital, Pediatrics, Munich, Germany,* [3]*St Vincent de Paul, Pediatrcs, Paris, France*

The aim of this study was to evaluate a novel EIA stool test based on monoclonal antibodies to assess Helicobacter pylori infection in children. Introduction: *H. pylori* (*H.p.*) infection is almost always acquired in early childhood and usually persists throughout life unless a specific treatment is applied. To investigate transmission, incidence, spontaneous clearance, and preventive measures of H.p. infection non-invasive tests are required which must be reliable in all age groups including toddlers, when the incidence of new infections is highest. Aim: Objective of this study was to evaluate the HpSTAR faecestest to diagnose a H.p. infection in children. Methods: Stool samples of 302 symptomatic children (148 girls, 154 boys, age 0.5-18.5 years) were frozen at -20°C until analysis. Only children with defined H.p. status based on biopsies were included. A child was considered positive if culture and/or al least two other methods (histology, RUT, 13C-Urea breath test) were positive, and H.p. negative if all test gave concordant negative results. None of the children received antimicrobial or acid suppressive therapy drugs during 4 weeks prior testing. The EIA was

performed using two different production lots according to the manufacturer's instruction at the three participating centres where the children were recruited. Results were read at the 450/630nm by spectrophotometry, optical density (OD) <0.150 was defined as negative, OD>0.150 as positive.
Results: Ninety-two children were H.p. positive and 210 were H.p. Negative; 116 were aged <6 years (18 H.p. pos., 98 H.p. neg.), 106 were aged 6-12 years (42 pos., 64 neg.) and 80 children were older then 12years (32 pos., 48 neg.). The OD values clearly separated the 92 H.p. positive children, median OD 2.729 (5th-95th percentile 0.232->4.000) and the 210 H.p. negative children, 0.021 (0.0009-0.076). Only 4 children were misclassified (2 false-positive and 2 false-negative). Sensitivity was 98%, specificity 99%, PPV 98%, NPV 99% and accuracy 98%. The OD were not related to age, neither in the group of H.p. infected nor in the non-infected children.
Conclusions. The HpSTAR is an accurate test for the diagnosis of H. pylori infection in children.

P09

Risk factors for carriage of an epidemic vancomycin-resistant *Enterococcus faecium* strain

E.M. Mascini, K.P. Jalink, T.E.M. Kamp-Hopmans, H.E.M. Blok, J. Verhoef, M.J.M. Bonten, A. Troelstra
University Medical Centre Utrecht, Eijkman-Winkler Institute, Utrecht, the Netherlands

Introduction. In May 2000, the first outbreak of vancomycin-resistant *Enterococcus faecium* (VREF) was detected in the University Medical Centre Utrecht at the nephrology ward. It is not known what determines why some VREF strains spread among hospitalised patients, whereas other strains do not.
Methods. 30 patients who were found colonised with VREF between May and November 2000 were included in the study. Molecular typing and subsequent comparison of strains with an international databank confirmed that 19 of them carried an identical epidemic strain which harboured the *esp*-gene, while 11 were colonised by non-epidemic strains which were all *esp*-negative. Among these patients we analysed risk factors for acquisition of the outbreak strain. In addition, to study duration of colonisation, prospective surveillance of VREF carriage for a 6-months period starting from the date of first isolation of VREF was realised for 25 patients.
Results. Acquisition of the outbreak strain was significantly associated with diabetes mellitus, renal transplantation, and receipt of 2 or more antibiotics, especially cephalosporins, in the 2-months period before the date of first isolation of VREF. After 6 months, VRE was still detectable in faecal samples from 60% of carriers of the outbreak strain versus 20% of carriers of non-epidemic strains ($P<.05$). Diabetes mellitus was identified as a significant risk factor for prolonged colonisation of the outbreak strain.
Conclusion. A particular outbreak strain preferentially colonised patients who had been subjected to extensive antibiotic exposure and certain patient categories with underlying conditions such as diabetes mellitus and renal transplantation. The fact that this strain was recovered from faecal samples of the host for a significantly longer period of time than non-epidemic strains, may facilitate dissemination of the strain. We suggest, that next to a careful restrictive antibiotic policy at wards at risk for spread of VREF isolation precautions may be focused on patients who are colonised with outbreak strains that harbour the *esp*-gene.

P10

Effect of hospitalisation on the antibiotic resistance of faecal Enterococci after discharge

S. Nys[1], N. Bruinsma[1], P.M.G. Filius[2], J. Degener[3], A.E.J.M. van den Bogaard[1], E.E. Stobberingh[1]
[1]*University Hospital Maastricht, Medical Microbiology, Maastricht, the Netherlands,* [2]*University Medical Centre Rotterdam, Medical Microbiology and Infectious Diseases, Rotterdam, the Netherlands,* [3]*University Hospital Groningen, Medical Microbiology, Groningen, the Netherlands*

Background. Hospitalisation is considered an important risk factor for the acquisition and spread of antibiotic resistance. This does not only apply for resistant pathogens, but also for bacteria of the commensal flora of hospitalised patients. These bacteria form a reservoir of resistance genes for (potentially) pathogenic bacteria. The, during their hospital stay, acquired resistance in the faecal flora, might be disseminated in the community. When the high antibiotic exposure and the selective pressure of hospitalisation disappear, one might expect that in time the antibiotic resistance of the patients will return to the level on admission.
Methods. The prevalence of antibiotic resistant enterococci in faecal samples of patients admitted to the surgical wards of the University Hospital Maastricht (azM, n=90), Rotterdam (azR, n=91) and Groningen (azG, n=82) was determined on admission, at discharge and 1 and 6 thereafter. This was done by comparing growth on antibiotic containing KF-agar plates with growth on antibiotic free agar plates as described previously {London, 1994 #196}.
Results. On admission significant differences in the prevalence of erythromycin and oxytetracycline resistance between azM and the two other hospitals were observed ($p<0.05$). This was most likely caused by a higher antibiotic use in the community in Maastricht. However, samples taken at the other points of time showed no significant differences in the prevalence of resistance against any of the antibiotics tested. In azR there was a significant increase of resistance for erythromycin ($p<0.03$) and oxytetracycline ($p<0.01$) at discharge. However this resistance was lost within six months after discharge. In Maastricht the same trend could be found for both antibiotics. Erythromycin resistance in Groningen had the same pattern over time, but oxytetracycline resistance continued to increase (not significantly) 6 months after discharge.
Conclusion. The results show that antibiotic use in surgical wards did cause a temporally increase in resistance which disappeared within 6 months after discharge.

P11

Isolation and AFLP typing of Brucella from harbour seals

J.A. Wagenaar[1,2], A.H.W. Schoormans[1],
M.A.P. van Bergen[2], B. Bakker[3], A.D.M.E. Osterhaus[4],
T. Kuiken[5]
[1]Veterinary Microbiological Diagnostic Centre, Utrecht, the Netherlands, [2]Institute for Animal Science and health, Lelystad, the Netherlands, [3]Central Institute for Animal Disease Control, Lelystad, the Netherlands, [4]Erasmus Medical Centre, Rotterdam, the Netherlands, [5]Seal Research and Rehabilitation Centre, Pieterburen, the Netherlands

This study was performed to assess whether (i) harbour seals (Phoca vitulina) can be carrier of Brucella in internal organs and to what extent, (ii) the presence of Brucella relates to gross lesions and (iii) the incidence and the load of Brucella in the harbour seal population changed during the Phocine Distemper Virus outbreak. The Brucella isolates were genotyped by AFLP (amplified fragment length polymorphism) to identify the genetic relationship of these isolates with other Brucella species. Animals were collected at the coasts in the Netherlands and the UK from 1999-2002 (before the PDV outbreak) and from June 2002-November 2002 (during the PDV outbreak). Samples from seven organs (lung, liver, spleen, kidney, tracheobronchial and external iliacial lymph nodes, and uterus or testis) from harbour seals of different ages (fetus, juvenile, or adult) were collected and cultured. Brucella was isolated from 12/39 animals prior to the PDV outbreak and from 13/132 animals during the PDV outbreak. Bacterial load in organs was similar prior and during PDV outbreak. Most Brucella were isolated from lung or tracheo-bronchial lymph node. Seals with bronchopneumonia associated with lungworm infection appeared to be predisposed to the isolation of Brucella. During the PDV outbreak which occured out of the lungworm season, the incidence of Brucella was lower compared to the pre PDV period. AFLP typing showed that the Brucella isolates from the seals are genetically different from other Brucella species. However, AFLP clearly showed the overall clonal character of the Brucella genus.

P12

Prevalence and high degree of antibiotic resistance in faecal samples of dogs and dog owners in the region Weert/Roermond, the Netherlands

S. Seeldrayers, A.E.J.M. van de Bogaard, M. Hazen,
E.E. Stobberingh
University Maastricht, Medical Microbiology, Maastricht, the Netherlands

Introduction. For many years antibiotics have been used by doctors and veterinarians for the treatment and control of bacterial diseases without restriction. Resistant bacteria from animals - zoonotic bacteria or intestinal flora - can infect or reach the human population by direct contact and also by food products of animal origin. These resistant bacteria can colonise humans and / or transfer their resistance genes to other bacteria belonging to the endogenous flora of humans. Most investigations in veterinary species are concentrated on production animals due to the extensive use of antimicrobials in agriculture and the well-documented transfer of resistant bacteria from animals to human beings by food. In contrast there is very little information about the extent to which antimicrobial resistance is present in the flora of domestic pets. Cats and dogs occupy a unique position among the domestic species because they are kept inside the house. Does mans best friend posses a risk for his owner as far as transmission of resistant genes is concerned? In this study, we try to find an answer to this question.
Material and methods. *Escherichia coli* and faecal enterococci from 55 fresh faecal samples of dog owners and their dogs were examined for the presence of resistance. Resistance of these indicator bacteria was measured by quantitative counts on selective media with and without antibiotics.
Results. After statistical analysis (Chi square test) no differences were observed with *E. coli*. With faecal enterococci there was a significant higher prevalence of resistance for oxytetracyclin, avilamycin and gentamicine in dogs. No differences were observed in the high degree.
Conclusion. The data suggest that having domestic pets does not appear to present a higher risk for transmission of antibiotic resistance.
Addendum. Prevalence (Prev. %) and high degree (HD %) of resistant *Escherichia coli* in dogs and dog owners in the Netherlands

Antimicrobial agents	Owner	Owner	Dog	Dog
	n=56	n=56	n=56	n=56
	Prev.	HD	PREV	HD
Ampicillin				

P13

Intra- and interspecies transfer of plasmids causing an unrecognised ESBL outbreak

B.J.M. Vlaminckx, E.J. van Hannen,
M.G. Nannini-Bergman, G.P. Voorn, B.M. de Jongh
Antonius Hospital Nieuwegein, Medical Microbiology and Immunology, Nieuwegein, the Netherlands

Introduction. In August to October 2002 the ICU of our hospital was confronted with an unprecedented increase in the incidence of multiresistant enterobacteriaceae. Typing of the involved isolates yielded Extended Spectrum Beta Lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella oxytoca*. In order to address whether inter-species transfer of ESBL encoding plasmids had occurred the nature of the genes encoding the ESBLs produced by the different species was evaluated.
Methods. ESBL positive isolates were tested for the presence or absence of TEM and SHV genes. TEM-PCR amplification product was sequenced using a 3100 Avant-automated sequencer (Applied Biosystems).
To determine whether intra-species transfer of plasmids had taken place, isolates of the same species were subjected to amplified fragment length polymorphism (AFLP) analysis.
Results. In 10 patients, admitted to the ICU from August 2002-October 2002, an ESBL positive isolate was obtained. From 5 of these 10 patients isolates were available for this study. It involved 2 *E.coli* and 3 *K. oxytoca*. Four of these 5 isolates proved to harbour a TEM-116 gene, whereas 1 isolate appeared to have only TEM-1. Upon revision, this isolate did not meet the criteria for phenotypically identifying ESBLs.

Both *E.coli* as well as both *Klebsiella* isolates had non-identical genotypes as determined by AFLP.
Conclusion. Though a diversity of species involved was initially taken as an indicator of non-relatedness, only one TEM-genotype (TEM 116) could be identified in all ESBL positive isolates. The dissemination of this TEM 116 gene in genetically dissimilar *E. coli* as well as *K. oxytoca* indicates that transfer of an ESBL encoding plasmid has taken place both within as well as between species. Therefore, a diversity in multiresistant species should not be taken as an indicator of non-relatedness or successful containment.

P14

A statistical model that identifies protease inhibitor treatment associated mutations in therapy naïve patients infected with the human immunodeficiency virus (HIV)

D.A.M.C. van de Vijver, R. Schuurman, C.A.B. Boucher
University Medical Centre, Eijkman-Winkler Centre, Department of Virology, Utrecht, the Netherlands

Introduction. Several reports have been published on the transmission of antiretroviral drug resistance in treatment naïve HIV patients. These reports used different consensus guidelines to define drug resistance. The guidelines, however, included some mutations for which there is not sufficient proof that they are associated with drug resistance. Furthermore, the guidelines do not include the same mutations. The aim of this study is to develop a statistical model that determines which mutations in the protease sequence of HIV are associated with protease inhibitor (PI) treatment.
Methods. Protease HIV subtype B sequences from treated and untreated individuals were obtained from the Stanford HIV reverse transcriptase and PI database. Two thirds of the sequences were randomised in the model development sample. The other sequences were used to validate the model. Using chi-square statistics, we assessed if mutations were associated with PI treatment (Bonferroni P=0.00038). Logistic regression was performed in mutations that were found in treated and untreated individuals, submitting all mutations that showed statistical significance in the chi-square test. The model consisted of a combination of the results of both statistical tests.
Results. Included were 1967 protease sequences (1167 treated with PI), of whom 1310 sequences (781) were allocated to sample for model development. The following mutations were only found in treated sequences: L24I, D30N, K45R, V32I, L33F, M46I/L, G48V, I54V, Q58E, G73C, T74S, V82A/T, I85V, and N88D. Logistic regression analysis identified A71T/V, L90M, any mutation at codon 20, N37S (negative association), and L63P combined with any mutation at codon 10. In both samples the model had a sensitivity of 78%, and a specificity of 87%.
Conclusion. We developed a logistic regression model that can be used for predicting transmission of PI resistant HIV. Further research has to be done to determine whether the model can also be used in non-B subtypes.

P15

Effects of different vaccination policies on the *B. pertussis* population in Europe

S.C.M. van Amersfoorth[1], H.G.J. van der Heide[1], L.M. Schouls[1], H.O. Hallander[2], A. Advani[2], K. Bondeson[3], C.H.W. von Koenig[4], M. Riffelman[4], C. Vahrenholz[4], N. Guiso[5], V. Caro[5], E. Njamkepo[5], Q. He[6], J. Mertsola[6], F.R. Mooi[1]
[1]RIVM, Laboratory for Vaccine Preventable Diseases, Bilthoven, the Netherlands, [2]Swedish Institute for Infectious Disease Control (SMI), Solna, Sweden, [3]Uppsala University, Department of Medical Sciences, Uppsala, Sweden, [4]Institut fur Hygiene und Laboratoriummedizin, Klinikum Krefeld, Germany, [5]Institut Pasteur, Unite de Bordetella, Department of Bacteriology and Mycology, Paris, France, [6]National Health Institute, The Pertussis Reference Laboratory, Turku, Finland

Introduction. Despite the widespread use of pertussis vaccines for a number of decades pertussis has remained an endemic disease with frequent epidemic outbreaks. Currently two types of vaccines are used, whole cell vaccines (WCV) and recently developed acellular vaccines (ACV). The long term aim of our study is to assess the effect of different vaccines on the population structure of *Bordetella pertussis* and ultimately on the disease burden.
Methods. 20 isolates from Finland, Sweden, Germany, the Netherlands and France from the period 1997-2000 were selected; ten of which were from unvaccinated hosts and ten from vaccinated hosts. Strains were characterised by sequencing of polymorphic genes for surface proteins (the pertussis toxin S1 and S3 subunits (PtxS1 and PtxS3), pertactin (Prn) and tracheal colonisation factor (TcfA)) and by typing based on variable number of tandem repeats (VNTR).
Results. Essentially no differences were observed in the frequencies of *ptxS1*. However, gene frequencies of *ptxS3* and *prn* were distinct in different countries. In general, strains with vaccine type protein variants were isolated in higher frequencies from non-vaccinated individuals compared to vaccinated individuals. VNTR analysis revealed little polymorphism in the Netherlands (5 VNTR types) whereas Finland showed the most polymorphism (10 VNTR types). This corresponded with the calculated genetypic diversity: 0.579 in the Netherlands and 0.832 in Finland. The low genotypic diversity found in the Netherlands suggests recent clonal expansion, possibly of strains adapted to a highly vaccinated population.
Conclusions. Though differences were seen between toxin and pertactin types in circulating strains of vaccinated and unvaccinated populations we could not find differences between populations vaccinated with WCV (Finland, the Netherlands and France) and ACV (Sweden, Denmark and Germany), respectively. This could be explained by the fact that ACV have only recently been introduced. Our results provide a base line which can be used to deduce changes in the *B. pertussis* in the coming years.

P16

Total genome polymorphism in the *uspA1* and *uspA2* genes of *Moraxella catarrhalis* in otitis prone and non-prone children up to 2 years of age

J. Hays[1], C. van der Schee[1], A. Loogman[1], K. Eadie[1], C. Verduin[1], H.A. Faden[2], H.A. Verbrugh[1], A. van Belkum[1]
[1]*Erasmus Medical Centre, Medical Microbiology and Infections Diseases, Rotterdam, the Netherlands,* [2]*Children's Hospital of Buffalo, Division of Infectious Diseases, New York, USA*

Intra-genomic variation in the *uspA1* and *uspA2* genes of *Moraxella catarrhalis* was studied using pulsed field gel electrophoresis (PFGE) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. From a set of 91 *M. catarrhalis* isolates taken from otitis prone and non-prone children up to 2 years of age, 19 pairs of PFGE identical isolates were found. 5 pairs originated from otitis non-prone children, 11 pairs from otitis prone children and for 3 pairs, one of the pair originated from an otitis prone and the other from an otitis non-prone child. No particular *M. catarrhalis* isolate or cluster of isolates was associated with either the otitis prone or non-prone groups. Sequence data obtained from these variants showed that PCR-RFLP pattern differences reflected actual changes in predicted amino-acid composition and that minor amino-acid changes in a 23 base pair 'NINNIY' repeat region (a conserved UspA1 and UspA2 protein binding site for the neutralising antibody mAb17C7) occurred. Variation in the *uspA2* 5? non-coding 'AGAT' repeat region was also observed as well as intra-genomic variation in the *uspA1* and *uspA2* genes. These results may have implications with regard to the suitability of the UspA1 or UspA2 proteins as components in a future *M. catarrhalis* vaccine.

P17

Clostridium difficile ribotyping analysis: fluorescent capillary electrophoresis versus agarose gelelectrophoresis

C.H.W. Klaassen, J.A. de Valk, A.M. Horrevorts
Canisius Wilhelmina Hospital, Medical Microbiology and Infectious Diseases, Nijmegen, the Netherlands

Introduction. At present, ribotyping is the method of choice for the analysis of the epidemiological relatedness of *Clostridium difficile* isolates. In ribotyping, the 16S-23S internal transcribed spacer (ITS) region from the ribosomal RNA gene is amplified by using the polymerase chain reaction (PCR). Since the rRNA gene is present in up to 14 copies per genome, and since the ITS region is highly polymorphic in *C. difficile*, up to 14 different amplicons can be obtained with individual isolates. These amplicons are separated by agarose gelelectrophoresis (AGE) and the obtained fingerprints are compared to each other to determine the epidemiological relatedness of *C. difficile* isolates. Although easy to perform, for interexperiment and interlaboratory comparisons, AGE is not an ideal medium since it is difficult to standardise. Capillary electrophoresis (CE) has a much higher potential to standardise electrophoresis conditions and might be more suitable for interexperiment as well as interlaboratory comparisons. We investigated whether the use of CE might overcome the disadvantages of AGE in ribotyping experiments.

Methods. Ribotyping was performed on 90 C. difficile isolates. Amplicons were analysed by standard AGE or by CE using fluorescent labelled PCR primers and electrophoresis on an automated DNA analysis platform using extended running conditions in order to separate the relatively large DNA amplicons.

Results. In every aspect investigated (sensitivity, resolution and repeatability) CE proved to yield superior results compared to AGE.

Conclusion. Ribotyping of *C. difficile* isolates should be performed in combination with CE. This is especially recommended when large numbers of isolates are to be compared.

P18

Increased treatment failure after 3-days courses of nitrofurantoin and trimethoprim for urinary tract infections in women

W.G. Goettsch[1], R. Janknegt[2], R.M.C. Herings[1]
[1]*PHARMO Institute, Utrecht, the Netherlands,* [2]*Maasland Hospital, Sittard, the Netherlands*

Background. Recently, in Europe a debate is started on the numbers of treatment failures after antimicrobial therapy of urinary tract infections. However, at this time-point there is no substantial information on the magnitude and the determinants of treatment failure after antimicrobial therapy of these infections.

Objectives. To assess the incidence rate and the determinants of treatment failure after antimicrobial therapy of urinary tract infections in women.

Methods. The rate and the determinants of treatment failure after antimicrobial therapy of urinary tract infections were assessed in a cohort of 16703 Dutch women who received a course (3, 5 or 7 days) of trimethoprim, nitrofurantoin or norfloxacin between January 1, 1992 through December 31, 1997 and who were between 15 and 65 years at the day of first use. A further prescription for one of these three antibiotics or for cotrimoxazole, amoxicillin, ciprofloxacin or ofloxacin, within 31 days after the end of the initial therapy was defined as an indicator for failure of the initial treatment. Determinants of treatment failure were identified one year prior to the start of the first dispensing.

Results. Treatment failure rate was 14.4% in patients treated with trimethoprim and nitrofurantoin and 9.6% in patients treated with norfloxacin. A multivariate analysis showed that 5- (RRnit 0.67, 95% CI 0.57-0.82, RRtri 0.82, 95% CI 0.73-0.91) and 7-days (RRnit 0.64, 95% CI 0.53-0.77, RRtri 0.85, 95% CI 0.71-1.02) trimethoprim and nitrofurantoin treatment appeared to be more effective than a 3-day treatment (reference category). Other factors increasing treatment failure rates were the age of the patient, the year of therapy and previous hospitalisation.

Conclusions. Our data indicate that for certain groups of female patients (for example, the older and recently hospitalised) a three days course may be not sufficient. Therefore, we suggest that the optimal antimicrobial therapy for the treatment of urinary tract infections in women is not only based on predefined standards but also on background information of the patient.

P19

Great diversity within the Staphylococcal chromosome cassette *mec* (SCC*mec*) types of methicillin-resistant Staphylococci

A.C. Fluit, A.T.A. Box, J. Verhoef, C.L.C. Wielders
Eijkman-Winkler Centre, University Medical Centre, Utrecht, the Netherlands

Background. The *mecA* gene in methicillin-resistant staphylococci (MRS) is encoded on the Staphylococcal Chromosome Cassette *mec* (SCC*mec*). Four SCC*mec* types and two additional subtypes have been described.[1,2]

Aim. To establish the SCC*mec* (sub)types in coagulase-negative staphylococci (CNS) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains typed by riboprinting. The strains were obtained from North America and Europe and represent isolates from the 1960 - 2000 including the major epidemic clones and sporadic ribotypes.

Methods. Thirty four MRSA (8 different ribotypes)[3] and 7 CNS were characterised for the presence of SCC*mec* specific sequences (locus A-H) by PCR[2,4] (locus A: downstream of *pls*; locus B: *kpd*; locus C: *mecI*; locus D: *dcs*; locus E: between pI258 and Tn*554*; locus F: between Tn*554* and *orfX*; locus G: pUB110/IS*431*; locus H: pT181/IS*431*).

Results and discussion. Loci A, G, and D were associated with SCC*mec* type I-IV, whereas Oliveira and de Lencastre[2] found these loci only in SCC*mec* type I, II, and I, II & IV resp. Locus B was associated SCC*mec* type I and II (ref. 2: type II); loci C and E with type II-IV (ref. 2: type II & III, and III, resp.); locus H was associated with SCC*mec* type III & IV (ref 2: type III). Identical loci patterns were present among different SCC*mec* types, e.g., loci ABCDG were present in both SCC*mec* type I and II. SCC*mec* type IV was described to contain only locus D[2], but we also found this type with loci DA, DE, and DG and DACHE and DAGHE were present in CNS.

Conclusion. An unexpectedly great diversity of sequences within the SCC*mec* types of MRS was observed. SCC*mec* type IV was described to contain only locus D, but could contain at least 5 loci.

References

1. Ma, et al. Antimicrob Agents Chemother 2002;46:1147.
2. Oliveira, de Lencastre. Antimicrob Agents Chemother 2002;46:2155.
3. Wielders, et al. J Clin Microbiol 2002;40:3970.
4. Ito, et al. Antimicrob Agents Chemother 2001;45:1323.

P21

Pseudobacteraemia caused by contaminated erythrocyte sedimentation rate tubes: investigation of an outbreak

E.P.M. van Elzakker[1], J.A. Severin[1], A. Molijn[2], R. Bax[2], A. Ott[3], L. de Groot[1]
[1]*Reinier de Graaf Group, Medical Microbiology, Delft, the Netherlands,* [2]*Delft Diagnostic Laboratory, Delft, the Netherlands,* [3]*Erasmus Medical Centre, Bacteriology, Rotterdam, the Netherlands*

Objectives. Contamination of blood culture (BC) bottles may occur at any stage of inoculation and culturing. Pseudobacteraemia (PB) can lead to unnecessary use of antibiotics, a prolonged admission period and wasting of valuable resources. We investigated an outbreak of PB with non-fermentative Gram-negative rods (nf-GNR) in order to find and eliminate the source.

Methods. The study design was case-control. Patients with a contaminated BC during the year 2000 were included. Controls were randomly selected from a group of patients with a negative BC matched by date of sampling. SPSS software was used for analysis. Data related to patients and sampling technique were recorded. The content of several commercial blood collection tubes was cultured. Species identification was performed using Phoenix automated microbiology system (Becton Dickinson). For molecular typing AP-PCR with primer sets M13 core, DAF4 and ERIC1R/ERIC2R was used.

Results. Forty patients and forty controls were included. Sex and age were equally distributed. Cases were more likely to be admitted with fever (OR 3.61) and their BC were taken more often at the emergency room (ER) (OR 8.5), by a nurse (OR 8.3) in combination with haematological tests (OR 69.9). From the liquid content of erythrocyte sedimentation rate (ESR) tubes nf-GNR were cultured. Fifty isolates from BC bottles, seven from ESR tubes and sixteen epidemiologically unrelated strains were examined. Based on AP-PCR five clonal groups (I-V) could be identified. The predominating species in these groups were *O. anthropi* (I,II), *S. maltophilia* (III), *P. stutzeri* (IV) and *C. meningosepticum* (V). In three groups identical isolates from ESR tubes and BC bottles were identified. All sixteen unrelated strains showed different fingerprints.

Conclusion. ESR tubes were the source of an outbreak of PB in our hospital. BC were most likely infected by the Vacutainer system needle after it was contaminated by the non-sterile liquid content of the ESR tubes. After the implementation of a blood-drawing protocol for nurses at the ER, the problem was reduced considerably.

P22

Antimicrobial susceptibilities of *Salmonella* strains isolated from humans, cattle, pigs and chickens in the Netherlands from 1984-2001

E. van Duijkeren[1], W. Wannet[2], D.T. Mevius[3], D. Houwers[1], W. van Pelt[4]
[1]*Veterinary Microbiological Diagnostic Centre, Utrecht University, Utrecht, the Netherlands, RIVM,* [2]*LIS,* [4]*CIE, Bilthoven, the Netherlands,* [3]*CIDC-Lelystad, Section Infectious Diseases, Lelystad, the Netherlands*

We monitored antimicrobial susceptibility data of *Salmonella* strains isolated from humans (n=45,198), cattle (n=5,892), pigs (n=5,822) and chickens (n=32,326) in the Netherlands over the period 1984 to 2001. The strains were tested for susceptibility to 7 antimicrobial agents using the agar diffusion method. In humans, pigs and chickens, resistance of *S. Typhimurium* strains to tetracycline, ampicillin, chloramphenicol and trimethoprim/sulfonamides increased. This increase could be attributed to the emergence of the multidrug resistant *S. Typhimurium* DT 104. In cattle, the level of resistance of *S. Typhimurium* strains was high in the 1980s, but it declined during the study period to the levels common in the other species in 1996-2001. *S. Enteritidis* isolates remained susceptible during the entire study period. In *S. Paratyphi B* variation Java isolated from chickens resistance to furazolidone, flumequine, trimethoprim/

sulfamethoxazole and ampicillin emerged, although furazolidone was not used after 1990. The level of resistance varied considerably between different serovars isolated from the same host species. Together, our data indicates that the presence of certain sero- and phage types greatly influences the overall resistance to antimicrobials within an animal species, implicating that resistance data of the genus *Salmonella* should be interpreted with caution.

P23

Diversity and spread of multiresistant *Acinetobacter baumannii* strains in Europe

H. van Dessel[1], L. Dijkshoorn[2], T. van der Reijden[2], N. Bakker[2], A. Paauw[1], P.J. van den Broek[2], J. Verhoef[1], S. Brisse[1]

[1]*University Medical Centre, Eijkman-Winkler Institute for Microbiology, Infectious Diseases and Inflammation, Utrecht, the Netherlands,* [2]*Leiden University Hospital, Medical Microbiology, Leiden, the Netherlands*

Introduction. In a previous study, 145 sitafloxacin-resistant *Acinetobacter* isolates were recognised amongst a set of 400 isolates collected from 23 European hospitals; 92 out of these 145 isolates were allocated to three major ribogroups (1, 2 and 3 with 17, 52 and 23 isolates respectively) using automated ribotyping. The aim of this study was to identify representative isolates from the set of 145 isolates and to explore the diversity at strain level.

Methods. Fifty geographically representative isolates of ribogroups 1, 2 and 3 were selected. Species identification was performed by amplified ribosomal DNA restriction analysis (ARDRA), and by AFLP fingerprinting and cluster analysis with fingerprints of reference strains of all described (genomic) species using a clustering level of 50% to delineate species. Diversity at strain level was investigated by high resolution fingerprinting using pulsed field gel electrophoresis (PFGE) and AFLP using a 90% level for strain delineation.

Results. All isolates were identified as *Acinetobacter baumannii*, confirming the predominance of this species in clinical settings. Combined AFLP and PFGE led to the distinction of 23 genotypes, with PFGE being the most discriminatory method. Cluster analysis of PFGE and AFLP profiles showed a good agreement with ribogroup categorisation, except for 2 PFGE types. Most genotypes corresponded to a single hospital, indicating the local spread of a single strain, whereas three genotypes were found in different geographic locations. The AFLP fingerprints of these strains were distinct from the previously described identified NW European clones 1 and 2. Isolates of these three widespread genotypes showed different antibiotic susceptibility profiles in different hospitals.

Conclusions. Grouping by ribotyping seems a useful method for rough typing of strains, whereas AFLP and/or PFGE reveal additional genotypes and AFLP and/or ARDRA are more useful for strain identification. The combined use of the methods revealed the existence of several widespread multiresistant strains.

P24

Genotyping of toxin A-negative *Clostridium difficile* strains from outbreaks in different countries

R.J. van den Berg[1], D.H. Oyib[1], L. Dijkshoorn[1], C.H.W. Klaassen[2], E.C.J. Claas[1], E.J. Kuijper[1]

[1]*Leiden University Medical Centre, Medical Microbiology, Leiden, the Netherlands,* [2]*CWZ, Medical Microbiology, Nijmegen, the Netherlands*

C. difficile causes nosocomial diarrhea and pseudo-membranous colitis. The enteropathogenicity is associated with the production of enterotoxin A (308 kDa) and cytotoxin B (270 kDa), but an increasing number of reports mention severe infections caused by toxin A-negative, toxin B-positive (tox A-neg) strains. The aim of this study was to investigate tox A-neg strains from 8 different countries.

Reference strains of *C. difficile* (29 known serotypes) were included in this study as control strains. Tox A-neg strains (n=59) were obtained from 8 countries. Two to three tox A-neg strains of each country were used (total n=18) for genotyping and for determination of MLS resistance. In addition, 10 unrelated toxin A-positive, toxin B-positive (tox A-pos) isolates of *C. difficile* were included. Tox A-neg strains were defined as strains with a deletion in the toxin A gene. PCR-ribotyping was used as the standard typing technique. This technique was compared to amplified fragment length polymorphism (AFLP). Clindamycin (MLS) resistance was tested by a PCR for the ErmB gene.

Using PCR ribotyping, 29 reference strains yielded 25 genotypes. Indistinguishable were: serotypes H and K, A7 and A11, and A14 and S4. AFLP discriminated 25 types of 27 different serotypes, but was not able to separate type A7 from type A11, and A14 from S4. PCR-ribotyping produced 8 genotypes among the 10 tox A-pos strains, and 5 types for the 18 tox A-neg strains. AFLP yielded 8 genotypes among the 10 tox A-pos strains, and 6 types for 16 tox A-neg strains. Clindamycin resistance was found in 13 of 18 tox A-neg strains, and in 2 of the 10 tox A-pos strains (p=0.008).

In conclusion: (I) Outbreaks of tox A-neg strains in different countries are not due to clonal spread of a specific *C. difficile* strain. (II) The results also indicate that AFLP has a higher discriminatory power than PCR-ribotyping especially for tox A-neg *C. difficile* strains. (III) In addition, a remarkable high percentage (72%) of tox A-neg *C. difficile* strains shows resistance to clindamycin due to the presence of the ErmB gene.

P25

Multiple-locus variable-number tandem repeat analysis of *Bordetella pertussis*

J.G.J. van der Heide, F.R. Mooi, S.C.M. van Amersfoorth, L.M. Schouls

National Institute of Public Health and the Environment, Laboratory for Vaccine Preventable Diseases, Bilthoven, the Netherlands

Introduction. Molecular typing methods have played an important role in analysing whooping cough epidemics. The gold standard for typing the causative agent, *Bordetella pertussis*, is the pulse field gel electrophoresis (PFGE). In addition, strains can be typed by determining restriction fragment length polymorphism (RFLP) using the IS*1002* insertion element as a probe in Southern blot hybridisation.

These methods require purified genomic DNA, are labour intensive and are difficult to interpret as small differences in band mobilities may result in incorrect assignment of genotypes. Latter problem compromises interlaboratory comparisons of patterns. For that reason we assessed whether variable-number of tandem repeats (VNTRs) in the *B. pertussis* genome could be used to differentiate strains.
Method. We analysed a set of 13 strains isolated in the pre-vaccination era in the early 1950s, 180 strains from the period after the introduction of the vaccine and strains isolated prior to and during the recent epidemics in the 1990s. Five VNTR regions were amplified using fluorescent labelled primer sets flanking the repeat regions. The resulting fragments were separated on an ABI 3700 DNA sequencer with internal molecular markers, band sizes were determined and translated into number of repeats. This resulted in data expressed as a telephone number with 5 digits which was used in cluster analysis.
Results and conclusions. The clustering of the VNTR profiles showed that strains from the pre-vaccination period had a high degree of genotypic diversity and that they were distinct from the post-vaccination strains. The majority of strains isolated during the epidemic in the 1990s comprised a limited number of related lineages, suggesting clonal expansion. *Bordetella parapertussis* strains appeared to form a separate very homogenous group with patterns distinct from the *B. pertussis* strains. The VNTR analysis appears to be powerful tool for discrimination of strains. It does not require purified DNA but instead can be performed directly on cell-lysates and throat swabs.

P26

The sequels of a school excursion to a dairy farm

J.A. Wagenaar[1,2], W. van den Brandhof[3], J.R. Dijkstra[2], A.H.W. Schoormans[1], J.H.T.C. van den Kerkhof[4]
[1]Veterinary Microbiological Diagnostic Centre, Faculty of Veterinary Medicine, Utrecht,the Netherlands, [2]Institute for Animal Science and Health (ID-Lelystad), Lelystad, the Netherlands, [3]Institute for Public Health and the Environment, Centre for Infectious Dieases Epidemiology, Bilthoven,the Netherlands, [4]GGD Zuid-Holland Zuid, Dordrecht, the Netherlands

A group of 96 children and their teachers visited a dairy farm. The farmer offered a cup of fresh milk (not treated) from the milktank which was accepted by 57 children and 3 teachers (one swig up to two cups).
Six days after the farm visit, 29 children (51%) were ill with watery diarrhoea, in some cases slimy with blood and fever >40 C. Only 2 out of 36 children (6%) that did not drink the milk had diarrhoea. There was a clear dose-response relation: 27% of the children that took 1 swig became ill whereas 100% of the children that drunk 2 cups fell ill.
Seventeen days after the visit, faecal samples were collected for *Campylobacter* isolation from 18 children (patients and controls). Eleven samples were positive for *C. jejuni* by direct plating; all were patients. One sample was positive after enrichment; a non-diseased child (control due to case definition) that had drunk one swig of milk. There was no sample available from the batch of the bulk milk from which the children drunk. Five colonies per patient were typed by AFLP and all showed the same pattern.

P27

DMS degradation in freshwater sediment with nitrate as terminal electron acceptor

B.B. Meijerink, A. Pol, M.S.M. Jetten, H.J.M. op den Camp
University of Nijmegen, Microbiology, Nijmegen, the Netherlands

In the global sulfur cycle, dimethyl sulfide (DMS) plays an important role in sulfur transport between the aquatic and terrestrial environment. In contrast with the marine environment, DMS does not originate from algae in the freshwater environment. In these ecosystems the main sources of DMS are the methylation of sulfides and MT (methanethiol), during the degradation of methoxylated aromatic compounds.
In the anaerobic freshwater sediments, the production and degradation of DMS appeared to be well balanced, resulting in steady-state concentrations of 10-40 nM. However, sharp declines in concentrations were found at the oxic/anoxic interface, suggesting a high microbial activity. In this project the degradation of DMS was studied in a sulfate rich sediment of De Bruuk, a nature reserve in the eastern part of the Netherlands
Sediment slurries were used in enrichment cultures with DMS as carbon and energy source, and nitrate as terminal electron acceptor. After several DMS additions the slurry was 1:10 diluted and transferred to agar plates. Single colonies were transferred to a liquid medium and subsequently the DMS degradation was tested. Sequence analysis of clones from the enrichment cultures revealed high homology to *Thiobacillus denitrificans* and *Hyphomicrobium facilis*. FISH analysis also gave positive results for these bacteria, indicating that both organisms are involved in DMS degradation.

P28

Measurement of intracellular primary metabolites in *Penicillium chrysogenum* using LC-MS-MS

U.D.M. Nasution, J.C. van Dam, C. Ras, A. Proell, R.J. Kleijn, W.M. van Gulik, J.J. Heijnen
Delft University of Technology, Bioprocestechnology, Delft, the Netherlands

Detailed knowledge of the metabolic flux distribution in primary metabolism is important for successful metabolic engineering of micro-organisms. These fluxes can be obtained from labelling studies by measuring the mass-isotopomer distributions of the intracellular metabolites of that micro-organism after feeding with labelled substrates. In this research project fluxes in primary metabolism of *Penicillium chrysogenum* will be measured using 13C labelled substrates and measurement of mass-isotopomer distributions with liquid chromatography ? mass spectrometry (LC-MS-MS). In this poster, preliminary result of intracellular metabolites measurement in *Penicillium chrysogenum* is presented. These are the metabolites from glycolysis and the TCA cycle. LC-MS-MS is chosen because of its sensitivity and ability to measure intracellular metabolites directly.

P29

Linlog kinetic modelling of glycolysis in *Saccharomyces cerevisiae*

M.T.A.P. Kresnowati, L. Wu, W.A. van Winden,
W.M. van Gulik, J.J. Heijnen
Delft University of Technology, Kluyver laboratory for Biotechnology, Delft, the Netherlands

Modern recombinant DNA technology allows precise modification of DNA sequences and, thus, engineering of metabolic activities for novel and improved functions of industrially relevant micro-organisms. However, cells contain a large number of mRNA-transcripts, enzymes, control proteins and metabolites that perform numerous simultaneous reactions and interactions, which creates an elaborate and high dimensional network. Such a network provides so many possible alternative modifications that it is infeasible to experimentally determine whether each of them leads us to the desired goal. Hence, mathematical modelling is required to provide guidance and insight into metabolic engineering. The developed model should be simple and accurate. The aim of our research is to develop such a model and to analyse its performance.

We applied the concept of linear logarithmic ('linlog') kinetic modelling to the glycolysis in *Saccharomyces cerevisiae*. This modelling approach expresses the rate of enzymatic reactions as a product of the enzyme concentration and a linear combination of terms that are the logarithm of concentrations of intracellular and extracellular metabolites and other possible effectors. All concentrations are normalised towards a chosen reference state.

The model parameters (elasticities) were derived either directly from kinetic equations in a literature model by Teusink *et al.* {Eur J Biochem 267:5313-29} or from data generated by computer-simulated experiments with the same model. The system was disturbed by a step change in substrate concentration to give dynamic metabolite concentrations within a time span of 3 minutes. The model performance was tested in terms of stability, approximative quality, simplicity and the range of applicability.

We found that the developed linlog kinetic model could approximate the (simulated) experimental data very well. It proved both to be stable over a wide range of disturbances and to be simple as it employed less parameter than the mechanistic model (66 parameters instead of 85 parameters employed by Teusink's model).

P30

Towards a more sustainable waste water treatment system: implementation of Anammox technology

M.S.M. Jetten[1], M.C. Schmid[2], I. Schmidt[1], J.W. Mulder[3],
W. Abma[4], K. van de Pas[1], U. van Dongen[2],
M.C.M. van Loosdrecht[2], G. Kuene[2], M. Strous[1]
[1]KU Nijmegen, the Netherlands, [2]University of Technology, Delft, the Netherlands, [3]Zhew Dordrecht, the Netherlands, Paques Balk, the Netherlands

A combination of two novel systems for the removal of ammonia from wastewater - SHARON and ANAMMOX - have been investigated at pilot plant scale. The SHARON (Single reactor for High Ammonium Removal Over Nitrite) process is ideally suited to convert ammonia into nitrite in waste streams with high ammonia (>0.5 g N/l) concentrations. The SHARON process is performed in a single, stirred tank reactor without any biomass retention, which results in a stable nitrification with nitrite as end-product. The nitrifying community is dominated by a bacterium closely related to *Nitrosomonas eutropha*. The SHARON process is currently in operation at two Dutch wastewater treatment plants. The ANAMMOX (anaerobic ammonium oxidation) process is a novel anaerobic process in which ammonia and nitrite are directly converted into dinitrogen gas, with hydrazine as an important intermediate. The dominant bacterium in Anammox bioreactors has been purified using density gradient centrifugation. The 16S rDNA sequence of the cells grouped deep inside the Order Planctomycetales. The key physiological parameters of the Anammox bacteria have been determined and used to design suitable reactor systems for the treatment of high strength waste waters. The combination of the Anammox process and the SHARON process has been successfully tested using sludge digester effluent. The SHARON reactor was operated without pH control with a total nitrogen load of about 1.2 kg N/m^3 per day and 53% conversion. In this way an ammonium-nitrite mixture suitable for the Anammox process was generated. In the nitrite limited Anammox reactor all nitrite was removed, some surplus ammonium remained. During the test period the nitrogen load was 0.75 kg N per m^3 per day. The activity reached values as high as 0.8 kg N per kg dry weight per day. Presently the full-scale implementation of the combined SHARON-Anammox process is implemented at the WWTP Rotterdam Dokhaven.

P31

Epitope structure of Pertactin: A model for immune-escape

M. Hijnen, P.G.M. van Gageldonk, F.R. Mooi,
W.A.M. Berbers
Laboratory for Vaccine Preventable Diseases, RIVM, Bilthoven, the Netherlands

Introduction. The *Bordetella pertussis* protein Pertactin (Prn) mediates adherence to host cells through a RGD motif and is a component of most a-cellular pertussis vaccines. Although antibodies against Prn are known to be important for protection, little is known about the structure and location of its epitopes. Such knowledge may facilitate the rational improvement of pertussis vaccines, and the development of a serological correlate for protection. Prn contains two polymorphic regions, designated region 1 and 2. Antibodies against both regions have been shown to confer protection in a mouse model. Polymorphism in Prn is, however, mainly found in region 1. Significantly, region 1 contains the RGD sequence involved in adherence. The aim of our study is to elucidate the role of region 1 and 2 in immune escape.

Methods. Prn epitopes were mapped using the PEPSCAN method. Overlapping, linear, 15 and 30-mers, and circular 15-mers peptides spanning the entire amino acid sequence of Prn were used. Epitopes were mapped using monoclonal antibodies (mAb's) (n=16) and sera from vaccinated (n=5), naturally infected (n=9) and uninfected (n=3) children.

Results. Mab's were found to recognise peptides derived from several Prn regions, especially region 1 and 2. Interestingly a number of mAb's recognised 2 or 3 non-

overlapping peptides. These peptides were located in, or close to, region 1 and region 2. In contrast to mAb's, human sera predominantly recognised region 2 peptides and did not bind to region 1 peptides.
Conclusions. Combined our results suggest that region 1 and 2 mainly induce antibodies against conformational epitopes or linear epitopes, respectively. This may explain why region 1 is more polymorphic compared to region 2, as variation in the number of repeats is expected to affect conformational epitopes, but not linear. Further, our results suggest that region 1 and 2, although separated by 280 amino acids in the primary structure, may be proximal in the folded protein. Thus the 2 regions may form a single polymorphic domain masking the functional part of the molecule from the immune system.

P32
Biogeochemistry and microbial ecology of metal-sulphur-nitrogen interactions in wetlands

S.C.M. Haaijer[1], M.E.W. van der Well[2], L.P.M. Lamers[2], M.S.M. Jetten[1], J.G.M. Roelofs[2], H.J.M. op den Camp[1]
University of Nijmegen, [1]Microbiology, [2]Enviromental Biology, Nijmegen, the Netherlands

Wetland ecosystems in the Netherlands suffer from pollution with nitrogen and sulfur compounds (especially nitrate and sulfate) originating from industrial and agricultural activities. Increased sulfate influxes can have several effects. Under anoxic conditions, sulfate-reducing bacteria are stimulated which results in high levels of sulfide. Sulfide in turn can be immobilised by binding with ferrous iron or other metal ions forming metal sulfides. When the pool of free metal ions is exhausted, sulfide can break apart iron-phosphate complexes forming iron sulfides and releasing phosphate. The released phosphate will lead to internal eutrophication.

P33
The thioamide prodrugs activator EtaA from *Mycobacterium tuberculosis* is a Baeyer-Villiger monooxygenase

M.W. Fraaije, N.M. Kamerbeek, A.J. Heidekamp, R. Fortin, D.B. Janssen
Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Laboratory of Biochemistry, University of Groningen, Groningen, the Netherlands

Introduction. Several thioamides are widely used as effective antitubercular agents. Surprisingly, the mode of action of these antibiotics is still unknown. Recently, a gene (*etaA*) from *M. tuberculosis* was identified that was shown to be responsible for the toxic effect of ethionamide. It was established that the toxic effect of this thioamide can be fully attributed to the action of the *etaA* gene product, EtaA. The thioamide appears to be activated by EtaA after which the activated product exerts a lethal effect. Analysis of ethionamide-resistant isolates revealed cross-resistance to several other widely-used thioamides. These results suggest that all above-mentioned thioamide drugs are activated by EtaA and that clinically observed resistance to thioamides is associated with functional impairment of EtaA. A thorough study of this newly identified prodrug activator EtaA will improve our understanding of the action of thioamide anti-tuberculosis drugs and the associated drug-resistance mechanism. In this study we have focussed on substrate and reactivity profiling of EtaA.
Methods. For efficient expression the *EtaA* gene was cloned into a pBAD*myc*-HisA vector. The enzyme was purified to homogeneity by affinity chromatography. All subsequent studies were conducted in standard buffer (25 mM Tris/HCl, pH 7.5) at 25°C.
Results. The enzyme was purified from cell extracts in the presence of Triton X-100. Elimination of the detergent from the purification buffer resulted in very low yields suggesting that the enzyme is membrane associated. The purified enzyme migrated as a single band in SDS-PAGE corresponding to a mass of about 56 kDa. This value is close to the theoretical mass of the recombinant protein: 58216.3 Da.
Spectral analysis of the purified recombinant enzyme showed a typical flavin spectrum displaying absorbance maxima at 364 nm and 438 nm. Upon phosphodiesterase treatment of the isolated flavin cofactor the flavin fluorescence increased indicating that EtaA contains FAD as cofactor. Quantification of cofactor and protein content indicates that the purified enzyme contains stoichiometric (1:1) amounts of the flavin cofactor.
Using ethionamide and NADPH as substrates, oxidation of the thioamide was observed confirming the activating role of EtaA. It is known that monooxygenases can, except for catalysing oxygenations, also generate hydrogen peroxide, which can initiate chemical oxidation reactions. To test whether the observed sulfoxidation reactions are enzyme mediated we examined the EtaA-mediated conversion of methyl-p-tolylsulfide. It was found that the enzyme catalyses an enantioselective oxygenation of this sulfide yielding mainly the S-sulfoxide. This finding confirms a direct role of EtaA in prodrug activation.
We noted that the EtaA sequence contains a sequence motif that typifies Baeyer-Villiger monooxygenases. This suggests that the enzyme *in vivo* catalyses Baeyer-Villiger reactions. We have found that EtaA indeed efficiently converts several ketones via a Baeyer-Villiger reaction (insertion of an oxygen atom in a C-C bond).
Conclusions. We have expressed, purified and characterised the newly identified antitubercular prodrug activator EtaA. We have found that the activator is a NADPH-dependent FAD-containing monooxygenase. The enzyme catalyses enantioselective sulfoxidation reactions indicating that the enzyme is directly involved in activating thioamide prodrugs. Product analysis has also shown that EtaA is able to catalyse Baeyer-Villiger reactions and therefore represents a Baeyer-Villiger monooxygenase. These substrate-profiling studies provide valuable information concerning this newly identified prodrug target from *Mycobacterium tuberculosis*.

P34

Respiration, a checkpoint of the morphological differentiation of *Streptomyces*

A.V. Cherepanov, B.J.F. Keijser, G.W. Canters, E. Vijgenboom
Leiden University, Leiden Institute of Chemistry, Leiden, the Netherlands

Streptomyces, filamentous gram-positive bacteria, are ubiquitous in nature and constitute an essential part of the bacterial soil community. They display a complex three-stage cycle of the morphological differentiation, which includes (a) growth of vegetative mycelium, (b) formation of the aerial hyphae, and (c) production of spores. The control of the developmental process has obvious extracellular components as shown by cross feeding of developmental mutants and the complete block of development by the chelation of copper ions in the medium. The latter phenomenon allows us to study and fully control the onset of development.

In this presentation we discuss the Cu-dependence of the morphological development of Streptomycetes in relation to respiration. The formation of aerial hyphae occurs under conditions of high oxygen tension and requires an efficient respiratory terminal oxidase such as the Cu-containing cytochrome *c* oxidase (COX). Cu-depletion suppresses the activity of the cytochrome *c* oxidase and as a consequence aerial hyphae will not be produced. However, vegetative growth occurs under conditions of low oxygen tension, where a terminal oxidase with a high affinity for oxygen is required. A *bd*-type oxidase, that does not need Cu as a co-factor, can operate as the terminal oxidase during this growth phase. This would explain why the removal of copper has no effect on vegetative growth.

Data will be presented that support this model and that are obtained from the following experiments: (a) analysis of the extracellular proteome has shown that Cu-depletion induces the expression of Sco1, which is involved in Cu-scavenging and Cu-incorporation in COX, (b) studies of the morphology of the *cox1* disruption mutant (cytochrome *c* oxidase, subunit 1), and (c) morphological differentiation of streptomycetes under micro-aeration conditions.

P35

Diet-related changes in the bacterial community structure in the gut of weaning piglets

S.R. Konstantinov[1], A.D.L. Akkermans[1], B.A. Williams[2], H. Smidt[1], W.M. de Vos[1]
Wageningen University, [1]Laboratory of Microbiology, [2]Animal Nutrition Group, Wageningen, the Netherlands

The present study was initiated to assess the alterations in the bacterial composition in the terminal ileum and colon of piglets after the addition of a diet containing four fermentable carbohydrates, namely lactulose, inulin, wheat starch and sugar beet pulp. A comparative molecular microbiological analysis was made on four differentially reared sub-groups of piglets: fasting 48h after weaning and non-fasting, and fermentable and non-fermentable carbohydrate containing diets. Ileum and colon lumen samples were taken from piglets at weaning (n=12), and 4 (n=48) and 10 days (48) afterwards. Total genomic DNA was isolated from the samples followed by bacterial 16S rRNA gene amplification and denaturing gradient gel electrophoresis (DGGE) analysis, fluorescent *in situ* hybridisation (FISH), and cloning and sequencing. Analysis of the results showed that the principal microbiological difference between the ileum and colon lumen was the occurrence of a higher number of DGGE bands in the colon compared to the ileum. Moreover, both the number of bacteria and the composition of bacterial communities in the small intestine of weaned piglets were affected by the diet containing fermentable carbohydrates at day 10 of the experiment. Quantitative FISH showed that both, the total bacterial count and the number of lactobacilli, were increased 4-fold compared with the control. 16S rDNA sequence analysis revealed that *Lactobacillus amylovorus* and *L. reuteri* were the most predominant in piglets that were fed with the fermentable carbohydrate-containing diet, while *Lactobacillus acidophilus* was most predominant in the control group. Based on this evidence we concluded that addition of fermentable carbohydrates supports the growth of lactobacilli in the proximal ileum and in the colon of weaned piglets. The results also suggest that the presence of fermentable carbohydrates may stimulate microbial diversity in the colon.

P36

Biogeochemical constraints for sustainable development of floodplains in riverine regions: The role of sulfate-reducing prokaryotes in wetlands restoration

M. Miletto[1], P.L.E. Bodelier[1], A. Loy[2], H. J. Laanbroek[1]
[1]NIOO-KNAW Centre for Limnology, Department of Microbial Ecology, the Netherlands, [2]University of Technology, Microbiology, München, Germany

The Dutch policy for river management is changing from exclusion strategies by the reinforcement of damns and dikes to the development of a more natural riverine system by allowing more access of the rivers to their floodplains. Heavily altered hydrological and hydrochemical factors however form important constraints for sustainable ecological development of typical vegetation types in riverine wetlands. The ongoing project on areas for water retention and conservation has not let to the desired biodiverse vegetation types. Sulfate-reducing prokaryotes (SRP) might have an important role in the restoration process, being the main producers of the phytotoxic S_2^- in $SO4^{2-}$-enriched environments. This work aims at the study of the structure of sulfate-reducing microbial communities in riverine freshwater ecosystems. Nucleic acid micro-arrays are an ideal tool to assess the sequence diversity of 16S rRNA in natural environmental samples, enabling the parallel detection of 16S rRNA genes within phylogenetically diverse microbial populations. As a preliminary analysis a screening of the sulfate-reducing community has been performed by means of a SRP-PhyloChip grouping 16S rRNA probes hierarchical targeting all recognised lineages of SRP. The hybridisation pattern revealed the presence of δ-proteobacteria and low GC Gram-positive bacteria. Among δ-proteobacteria, genera widespread in soil were detected in most of the samples (*Desulfovirga, Desulforhabdus, Syntrophobacter*) as well as genera previously reported in marine, brackish and freshwater habitats (*Desulfovibrio, Desulfonema,*

Desulfomonile and genera detected by the probes DSACI175, DSB706 and DSS658). δ-Proteobacteria often observed in soil (i.e. *Desulfomicrobium*, *Desulfobacter*, *Desulfococcus*, *Desulfobotulus* and *Desulfoarculum*) were absent. Finally, among low GC Gram-positive bacteria there was no evidence for the presence of *Desulfotomaculum*, a genus finding its growth optimum in freshwater sediments, paddy soils and rice roots.

P37
Experimental and modelling studies on biological Fe(II) and S(-II) oxidation at near-neutral pH

J.S. Geelhoed, M.C.M. van Loosdrecht, J.G. Kuenen
Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, the Netherlands

Since the demonstration of autotrophic Fe(II) oxidation at near-neutral pH, interest has developed in the role of Fe(II) oxidising micro-organisms in the cycling of Fe in sediments and soils. Microaerophilic Fe-oxidisers are present at the oxic-anoxic boundary at a position in the oxygen and the opposing Fe gradient where biological conversion can outcompete the chemical oxidation of Fe(II) with oxygen, the rate of which is relatively high at near-neutral pH. Fe-oxiders are able to utilise aqueous Fe(II) or Fe(II) complexes but also a number of solid phases with lower solubility such as FeS.
Whereas for sulfide oxidation it has long been recognised that biological sulfide oxidation may enhance oxidation rates by several orders of magnitude, for Fe oxidation merely the geometry of the Fe profiles in the oxic-anoxic interface is affected.
The aim of our research is to study the kinetics and the mechanism of biological Fe(II) and S(-II) oxidation at near-neutral pH. For this, experimental and modelling work will be combined. We are growing Fe- and sulfide oxidising organisms in agarose stabilised systems with opposing oxygen and Fe(II) and/or S(-II) gradients, and are testing the response to different oxygen concentrations and Fe(II) or S(-II) substrates.
We have also developed a mathematical model describing the above system, in which chemical speciation is combined with diffusive transport and kinetic expressions for chemical and biological oxidation.

P38
Cyanophages in freshwater environments: their influence and interactions

M. Tijdens, H. Gons, H.J. Laanbroek
NIOO-KNAW Centre for Limnology, Department of Microbial Ecology, Nieuwersluis, the Netherlands

The goal of this research project is to study cyanophages, viruses which infect cyanobacteria, in eutrophic lakes. Viruses are minute genetic particles only able to replicate or express their genes within a host cell, making them obligate parasites. Recently it was shown that viruses occur in much higher densities in aquatic environments than initially thought. Densities of 10^7 or 10^8 viruses per ml are common. These high viral abundances imply that viruses might have a large influence on the structure of their host community by inducing high mortality within their host community and on gene transfer within the host community. It is thought that viral lysis of the host community creates a whole new microbial loop within the classic food web.
Cyanobacteria can be found in high densities in Dutch eutrophic lakes and form the host for cyanophages. They often occur in dense blooms, which can be a nuisance because of their smell, toxicity or mere physical occurrence. In a study observing marine cyanobacteria, 30% of the cyanobacterial mortality was found to be cyanophage induced. In a laboratory study imitating the bloom of freshwater cyanobacteria in eutrophic shallow lakes, a complete collapse of the cyanobacterial bloom was observed. This collapse was probably caused by viral lysis. These two studies indicate that also cyanophages might have a significant effect on their host community.
Even though cyanophages have been observed to occur in eutrophic lakes and the complete collapse of cyanobacterial blooms has been observed to occur repeatedly within laboratory experiments, this phenomenon has still not been reported to occur in natural freshwater environments. Cyanophages in these environments are probably influenced by several factors resulting in a regulation of their virulence. The aim of this study is to find out which factors play a role in the virulence of freshwater cyanophages and to obtain a better understanding of the influence of cyanophages on the cyanobacterial community in Dutch eutrophic lakes.

P39
Isolation, identification and characterisation of a denitrifying *Bacillus* strain from a biodenox reactor

R. Kumaraswamy, G. Muyzer, M.C.M. Loosdrecht,
J.G. Kuenen
Kluyver Laboratory for Biotechnology, University of Technology, Delft, the Netherlands

BioDeNOx is a process to remove nitrogen oxides (NOx) from flue gas at thermophilic conditions. It consist of two steps, (i) wet absorption of NO_x to FeII(EDTA), and (ii) biological reduction of NOx to N_2 by denitrifying bacteria. The following reaction takes place in the absorption step, when the NO_x-containing flue gas is mixed with an anaerobic $FeII(EDTA)^{2-}$ solution:

$Fe^{II}(EDTA)^{2-} + NO \leftrightarrow Fe^{II}(EDTA)(NO)^{2-}$

The NOx absorbed is subsequently reduced to di-nitrogen gas in a process, in which ethanol is used as the electron donor:

$6\ Fe^{II}EDTA(NO)^{2-} + C_2H_5OH \rightarrow Fe^{II}(EDTA)^{2-} + 3\ N_2 + 2\ CO_2 + 3\ H_2O$

The main goal of the project is to isolate and characterise the organism(s) responsible for NOx removal. Denitrifying enrichments were grown with NO^-_2 as electron acceptor and iron, ethanol, or acetate as electron donors. A moderately thermophilic, denitrifying bacterium was isolated from a BioDeNOx reactor running at 50°C. The organism is a Gram-positive, spore-forming rod growing in a temperature range from 40 to 60°C. Comparative sequence analysis of the 16S rRNA showed an affiliation with the species *Bacillus azotoformans*. The denitrifying *Bacillus* used NO^-_2 and N_2O as electron acceptors, and ethanol or acetate as electron donors under chemoorganoheterotrophic conditions, while Fe^{2+} was used as an electron donor under mixotrophic conditions with 0.001% (w/v) yeast extract.

Molecular tools, such as PCR-DGGE and fluorescence In situ hybridisation (FISH), were used to determine the dominance of this *Bacillus* strain in the BioDeNOx process. Preliminary results indicated the presence of this organism among others in both laboratory and pilot scale bioreactors. Further isolation and chemostat experiments will be conducted to characterise the role of the *Bacillus* strain and other major players in the BioDeNox process.

P40

Kinetic parameter determination in dynamic chemostat experiments: Ferrous iron oxidation by *Leptospirillum ferrooxidans*

R. Kleerebezem, M.C.M. van Loosdrecht
Kluyver Laboratory for Biotechnology, University of Technology, Delft, the Netherlands

This work is conducted within the framework of a project on the biotechnological removal of hydrogen sulfide (H_2S) from gasses. H_2S removal is achieved by washing with a ferric iron (FeIII) solution. The absorption of H_2S is enhanced by a chemical redox reaction with FeIII under formation of elemental sulfur (S_o) and ferrous iron (FeII). The process is operated at a pH of 1-2 to avoid the formation of FeIII-precipitates. Elemental sulfur can be separated from the medium and for reuse of the FeIII solution biological oxidation of FeII with molecular oxygen needs to be achieved. However, the biological S_o oxidation needs to be avoided.
In order to investigate the interaction between the biological iron and sulfur cycle in acidic environments, kinetic experiments are conducted in chemostat reactors. Model organisms that are utilised are *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*. Contrary to *A. ferrooxidans*, *L. ferrooxidans* is not capable of oxidation of reduced sulfur compounds. The objective of our work is to define the conditions that favor growth of *L. ferrooxidans* over *A. ferrooxidans* to avoid S_o oxidation in an aerobic reactor fed with S_o and FeII.
The FeII oxidation kinetics by *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* were investigated in dynamic chemostat experiments that allow for rapid and accurate determination of kinetic parameters. By on-line measurement of oxygen and carbon dioxide in the off gas and the FeII:FeIII concentration ratio in the medium, mass balances for electron donor, acceptor and biomass can be established. Results of these measurements and the methods developed for parameter identification are presented in the poster.

P41

Comparative genotyping of *Saccharomyces cerevisiae* CEN.PK 113-7D and S288C using oligonucleotide microarrays

P. Daran-Lapujade[1], J-M. Daran[2], P. Kötter[3], T. Petit[2], M.D.W. Piper[4], J.T. Pronk[1]
[1]*Kluyver Laboratory of Biotechnology, University of Technology, Delft, the Netherlands,* [2]*Beijerinck Laboratory, DSM-GIST, Delft, the Netherlands,* [3]*Institute for Microbiology, University J.W. Goethe, Frankfurt, Germany,* [4]*University College London, Department of Biology, London, UK*

High-density oligonucleotide micro-arrays have been used to genotype two popular *S. cerevisiae* laboratory strains: S288C, the sequenced strain, and CEN.PK113-7D. The micro-array results showed that these strains' genomes share strong similarities (96% of the *ca.* 6300 yeast genes with the same hybridisation intensity) as confirmed by two other genotyping techniques, RAPD and electrophoretic Karyotyping.
Microarrays have the unique ability to scan at once the whole genome and locate genetic polymorphisms. We identified 288 genes with significantly different hybridisation intensities that could be classified as duplicated, absent or with sequence polymorphism in CEN.PK113-7D compared to S288C. We chose to focus on the subset of 25 genes called absent in CEN.PK113-7D. Among these absent genes, 17 were clustered together on 5 chromosomes, mainly in subtelomeric regions. Thorough analysis of these regions by PCR and RFLP confirmed the absence of these genes in CEN.PK113-7D. Surprisingly 3 of these regions where not smaller in CEN.PK113-7D chromosomes, thus harbouring unidentified and potentially new sequences. In addition, 8 genes called absent by the micro-arrays were scattered over the chromosomes. Using diagnostic PCR most of these genes were actually found present in CEN.PK113-7D, but after sequencing were found to differ significantly at the DNA level from S288C, explaining the poor hybridisation to the arrays. In addition to their power to quantify genome-wide gene expression, micro-arrays should thus be considered as useful tool for genotyping.

P42

Transcriptional responses of *Saccharomyces cerevisiae* to limitations of carbon, nitrogen, phosphorus or sulfur

V.M. Boer[1], J.H. de Winde[1,2], J.T. Pronk[1], M.D.W. Piper[1]
[1]*Kluyver Laboratory of Biotechnology, University of Technology, Delft, the Netherlands,* [2]*DSM Life Sciences, Division Bakery Ingredients, Technology Cluster, Delft, the Netherlands*

Growth of micro-organisms in their natural environment and in many industrial applications is often limited by nutrient availability. To cope with nutrient limited conditions, micro-organisms have evolved a multitude of strategies. These strategies involve the induction of high affinity transport systems, the use of alternative sources of the limiting element and the sparing of the limiting element by changing biomass composition. With the use of micro-arrays we have investigated the genome-wide transcriptional

response of *Saccharomyces cerevisiae* to limitations of glucose, ammonium, phosphate or sulfate. We used chemostat cultivation which enabled us to study the effects of one particular limitation while other growth parameters (growth rate, pH, temperature, dissolved oxygen tension) remained constant. In addition, the concentration of non-limiting nutrients was constant and comparable between conditions. In total, 1881 transcripts (31% of the genome) had altered expression levels in at least one condition, whereas 3558 (58%) were unchanged, and 645 (11%) remained below reliable detection in all four conditions. Of the 1881 transcripts that showed change between conditions, 484 were significantly higher or lower in one limitation only. The functional annotations of these genes indicated cellular metabolism was altered to meet the growth requirements for nutrient-limited growth. Furthermore, we identified responses for several active transcription factors with a role in nutrient assimilation. Finally, 54 genes were identified that showed 10-fold higher or lower expression in a single condition only. The transcription of these genes can be used as indicators for the characterisation of nutrient-limited growth conditions and provide information for metabolic engineering strategies.

P43
Differentiation of *Streptomyces coelicolor* at the air interface of standing liquid cultures and the involvement of gas vesicles

G. van Keulen[1], D. Claessen[1], H.M. Jonkers[2], L. Dijkhuizen[1], H.A.B. Wösten[3]
[1]*University of Groningen, Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Groningen, the Netherlands,* [2]*Microsensor Technology, Max-Planck-Institute for Marine Microbiology, Bremen, Germany,* [3]*Utrecht University, Department of Microbiology, Utrecht, the Netherlands*

Streptomycetes are Gram-positive soil bacteria that colonise their substrate by forming a substrate mycelium. These bacteria differentiate by forming hydrophobic aerial hyphae which septate into chains of grey-pigmented spores. Formation of aerial structures has only been studied in *Streptomyces coelicolor* grown on solid substrata. We describe here the morphology and differentiation events that take place when *S. coelicolor* is grown in standing liquid cultures. Initially a submerged feeding substrate mycelium is formed. Biofilm assays showed that a subset of submerged hyphae grown on minimal media strongly adhere to the solid bottom and walls. Oxygen profiles measured with a sensitive microsensor showed that standing glucose minimal medium cultures became completely anoxic within one week. The oxygen concentration of standing mannitol minimal medium cultures decreased to 70 µM. *S. coelicolor* was able to grow under the anoxic conditions tested despite being classified as an obligate aerobic organism. Colonies are formed at the air interface of standing minimal media cultures at day three of growth and they fully differentiate. Interestingly, these colonies are surrounded by a rigid protein film. After mechanical disruption the film is reformed within 2 hours indicating the presence of film-forming protein in the supernatant. This protein was enriched by subjecting the supernatant to electrobubbling. A stable foam was formed, collected, and analysed on SDS-PAGE. The major constituent was a protein band of 20 kDa. Current experiments aim to elucidate the role of this protein in film formation. The function of the film may be to fix colonies at the air interface to enable hyphae to grow into the air. Alternatively, it serves as a surface-active compound that lowers the water surface tension. The *S. coelicolor* genome contains two gene clusters coding for gas vesicle proteins (*gvp*). Gas vesicles are bacterial organelles that provide buoyancy to aquatic bacteria such as cyanobacteria. Since *S. coelicolor* is a soil bacterium, the presence of gas vesicle genes was unexpected. We investigated whether gas vesicles were present in colonies at the air interface of standing cultures. Electron microscopy showed vesicular structures in protein-stained sections of the submerged fraction of air interface colonies. Vesicles were isolated from lysozyme-treated and osmotically-shocked colonies. SDS-PAGE of lyophilised vesicles revealed five low molecular weight proteins with one very abundant protein of 15 kDa. This molecular weight agrees with that of the *gvpA* gene product. *gvpA* resides in both gas vesicle gene clusters and its homologues in cyanobacteria and halophilic archaea constitute the major structural gas vesicle protein. We are currently determining the roles of the *gvp* clusters in formation of colonies at the air interface of standing liquid cultures by introducing gene deletions.

P44
Engineering of steroid biotransformation in *Rhodococcus*

R. van der Geize, G.I. Hessels, L. Dijkhuizen
University of Groningen, Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Haren, the Netherlands

Steroid pathway intermediates, formed during sterol catabolism, are widely recognised as pharmaceutically important precursors for drug synthesis. Production of steroids via biotransformation, however, encounter difficulties due to the capability of micro-organisms to effectively degrade the steroid poly-cyclic ring structure. 3-Ketosteroid 9α-hydroxylase (KSH) and 3-ketosteroid Δ^1-dehydrogenase (KSTD) are key-enzymes in the degradation of the ring system of steroids. Different approaches have been developed by others, each having specific drawbacks, to block the undesired degradation of the steroid ring structure, enabling selective degradation of the aliphatic side chain of the sterol molecule in biotransformations. These approaches include the use of chelating agents inhibiting KSH activity (e.g. 2.2'-dipyridyl), gene inactivation through classical mutagenesis (e.g. UV irradiation), or by chemically preventing ring opening using steroid derivatives (e.g. 19-hydroxylated steroids).
Our study focusses on the bioengineering of molecularly defined mutant strains of *R. erythropolis* blocked at the level of steroid Δ^1-dehydrogenation and steroid 9α-hydroxylation. The study includes a molecular and physiological characterisation of the steroid catabolic pathway of *R. erythropolis*. A substantial molecular toolbox has been developed for this actinomycete, including protocols for electrotransformation, conjugation, targeted gene disruption and unmarked gene deletion, as well as the

development of a *Rhodococcus-Escherichia coli* shuttle vector pRESQ for genomic library construction and functional complementation.

P45

Construction of environmental BAC libraries for the detection of antimicrobial agents with pharmaceutical applications

R. van der Geize, L. Dijkhuizen
University of Groningen, Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Haren, the Netherlands

High-molecular weight community DNA (100-200 kb) will be cloned and characterised by preparing gene libraries from four different environmental habitats in bacterial artificial chromosome (BAC) vectors. *E. coli-Streptomyces* artificial chromosome vectors (ESACs) are available and will be used to screen gene libraries of community DNA in a *Streptomyces lividans* host strain for the expression of bio-active or anti-microbial agents. Molecular tools developed within the EU-project Actapharm will be applied to additionally screen for genetic diversity and the presence of (novel) secondary metabolite gene clusters.
A cloning system for BAC library construction using HMW DNA will be optimised using the ESAC vectors (e.g. pPAC-S1). Other BAC vectors may be constructed to improve excisting ESAC vectors. Such vectors will allow screening of community DNA in a broader actinomycetal host-range. Two test strains will be used to produce known secondary metabolites of these two strains. As a heterologous production host strain *S. lividans* strain is available. This strain has been manipulated to have improved properties for the synthesis of secondary metabolites. Actinorhodin producing strain *Streptomyces coelicolor*, of which the nucleotide sequence of the complete genome is known, and the streptomycin producing strain *Streptomyces griseus* will be used as test strains. Once a suitable and reliable system for gene library construction has been set-up, the system will be used to clone and screen community DNA of four carefully selected habitats.
We hope to capture the genetic diversity and biosynthetic potential of uncultured actinomycetes.

P46

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

J. Siebring, G. van Keulen, L. Dijkhuizen, A.M.C.R. Alves
University of Groningen, Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Haren, the Netherlands

Streptomyces coelicolor A3(2) is the best studied *Streptomyces* species at the genetic level. Like other members of the order *Actinomycetales* it has a complex life cycle involving mycelial growth and spore formation. Streptomycetes are medically and industrially important because they produce pharmaceutically useful compounds including over two-thirds of naturally derived antibiotics currently in use for which several precursors are retrieved from the primary metabolic pathways. Therefore studies on central metabolism are a pre-requisite for a rational approach for the optimisation of antibiotic production.
With the availability of the complete genome sequence[1] it became interesting to elucidate the numerous duplication events found in central metabolism in particular glucose degradation. This project focuses on the four (putative) phosphofructokinase (PFK) genes annotated in the S. coelicolor genome, whereas related organisms usually contain up to 2 pfk genes. PFKs catalyze an important regulatory step in glycolysis - the reaction fructose-6-phosphate Æ fructose-1.6-phosphate - making the enzyme an interesting subject concerning antibiotic production. Furthermore, the duplicated pfk gene sets may be differentially expressed throughout the complex life cycle of Streptomycetes and/or when grown on different carbon sources.

Reference

1. Bentley SD, Hopwood DA. Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 2002;417:141-7.

P47

Anaerobic ammonium oxidation (ANAMMOX)

K.T. van de Pas-Schoonen[1], J.A. Fuerst[2], J.S.S. Damste[3], M. Wagner[4], M.C. Schmid[5], H.J.M. op den Camp[1], J.G. Kuenen[5], M.S.M. Jetten[1], M. Strous[1]
[1]KU Nijmegen, Microbiology, Nijmegen, the Netherlands, [2]University of Queensland, Microbiology, Brisbane, Australia, [3]NIOZ, Biogeochemistry, Texel, the Netherlands, [4]University of Technology Munich, Microbiology, Freising, Germany, [5]University of Technology Delft, Biotechnology, Delft, the Netherlands

The ANAMMOX (anaerobic ammonium oxidation) process is a novel anaerobic process in which ammonia and nitrite are directly converted into dinitrogen gas, with hydrazine as an important intermediate. The dominant bacterium in anammox bioreactors was purified using density gradient centrifugation, and was named *Candidatus* Brocadia anammoxidans. The 16S rRDNA gene sequence of B. anammoxidans grouped deep inside the Order Planctomycetales (Strous *et al.* 1999). Based on the 16S rRNA gene sequence several probes have been developed specific for the detection of anammox cells. Survey of many natural and man made ecosystems has revealed several new anammox sequences, indicating a very high biodiversity in this group of bacteria.
Electron microscopic analysis showed a complex ultrastructure of the planctomycete anammox bacteria. The cells contained several internal compartments. Immuno-gold labelling demonstrated that the innermost compartment contained the enzyme hydrazine oxido-reductase. Further analysis of this ?anammoxosome-compartment revealed the presence of very unusual membrane lipids (Damste *et al.* 2002). The lipids contained up to five linearly fused cyclobutane moieties with *cis* ring junctions. Such 'ladderane' molecules were unprecendented in Nature but are known as promising building blocks in opto-electronics. Stacking of ladderane moieties in a biomembrane will result in an exceptionally dense arrangement of carbon atoms, a tight barrier against

diffusion. Such a membrane may be required to maintain concentration gradients during the exceptionally slow anammox metabolism and to protect the remainder of the cell from the toxic anammox intermediate hydrazine.

References

1. Strous M, Fuerst J, Kramer E, Logemann S, Muyzer G, van de Pas K, Webb, R, Kuenen J, Jetten M. Missing lithotroph identified as new planctomycete. Nature 1999;400:446-9.
2. Damste JS, Strous M, Rijpstra I, Hopmans E, Geenevasen J, Duin A van, Niftrik LA van, Jetten MSM. Linearly concatenated cyclobutane lipids form a dense bacterial memebrane Nature 2002;419:708-12.

P48
Origin of variability in heat resistance of *Bacillus subtilis* endospores

A. O'Brien[1], J-W. Veening[2], K.J. Hellingwerf[3], S.Oomes[4], S. Brul[1,4]

University of Amsterdam, SILS, Departments of [1]Molecular Biology and Microbial Food Safety, [3]Microbiology, Amsterdam, the Netherlands, [2]University of Groningen, Department of Molecular genetics, Groningen, the Netherlands, [4]Food processing group, Unilever research, Vlaardingen, the Netherlands

In the food processing industry, heat resistance of bacterial spores and their ability to germinate to form vegetative cells is a major cause of food spoilage and safety problems. Studies of the resistance of *Bacillus* spores to a variety of heat treatments commonly used in food processing have identified a number of factors important in determining the level of spore heat resistance. Most important are sporulation temperature, and the presence of a cocktail of calcium, magnesium, iron, manganese and potassium upon sporulation in batch cultures grown in nutrient broth.
The aim of this study was to define spore heat resistance as a function of sporulation conditions using defined media and culture conditions. The well-characterised *B. subtilis* 168 (laboratory strain) and a food product isolate, *B. subtilis* A163, (with naturally high heat resistant spores) were used for sporulation experiments. Homogenous spore crops from *B. subtilis* chemostat cultures were produced in a defined sporulation medium. Sporulation could be induced under both carbon and nitrogen limiting conditions and it was observed that a variation in the dilution rate could be used for triggering sporulation. Increased heat resistance of spores was shown to be dependent on the addition of extra calcium ions to the sporulation medium. *B. subtilis* reporter strains, which have GFP under the promoters of early-late sporulation genes, are currently used to monitor the homogeneity of sporulation in chemostats and shake-flasks.

P49
Microbial diversity in peatlands

A.A. Raghoebarsing[1], F. Smolders[2], H. op den Camp[1], M. Jetten[1], M. Strous[1]

KU Nijmegen, [1]Microbiology, [2]Aqutic Ecology, Nijmegen, the Netherlands

Bog mosses, i.e. *Sphagnum* species, are an important component of the peat forming vegetation. Peatlands are ombotrophic and have a low pH due to *Sphagnum* exudates. In these peatlands, methane is biologically produced by methanogenic Archaea and biologically consumed by methanotrophs. Therefore, these ecosystems are both a source and a sink for methane and carbon dioxide. The emission of methane from peatlands is lower than the production, and it is possible that methanotrophic bacteria play an important role in this process. They might oxidise the CH_4 forming CO_2 and H_2O. Recently, methanotrophic bacteria which oxidise methane at low pH were isolated from peat (Dedysh *et al.*, 2000). Our research focuses on the diversity of the different groups of bacteria using molecular ecological methods. The different bacterial species will be quantified with the help of fluorescence in situ hybridisation (FISH). Also, the identity of the different bacteria species will be analysed by screening of a 16S rDNA library of total peat bacterial DNA. This approach will provide new 16S rDNA sequences, which can be used for making new probes for FISH. Another approach is the cloning of functional genes. Methanotrophs use a conserved methane monooxygenases (MMO) to catalyze the oxidation of methane to methanol. The genes of MMO are useful as biomarker to detect the presence of methanotrophs in different environments.

P50
The structure and expression of the ribosomal DNA operons of *Lactobacillus plantarum* WCFS1

M.C. de Vries[1,2], E.E. Vaughan[1,2], M. Kleerebezem[1], W.M. de Vos[1,2]

[1]Wageningen Centre for Food Sciences, Wageningen, the Netherlands, [2]Wageningen University, Laboratory of Microbiology, Wageningen, the Netherlands

The human GI tract represents a dynamic ecosystem harbouring a great variety of micro-organisms. To study the functionality of these micro-organisms and their interaction with the host we are aiming to determine their *in situ* activity based on culture-independent approaches. We have chosen as a model micro-organism, *Lactobacillus plantarum* WCFS1 that is of human origin, shows considerable survival following oral consumption, and whose complete genome sequence has been determined. Comparison of the five ribosomal DNA operons (rDNA operons) on the chromosome of *L. plantarum* WCFS1 revealed highly similar rRNA genes, but showed that the number and kind of tRNA genes present differed considerably. Also the rDNA promoters showed major differences. In the tRNA genes following one of the operons a promoter can be found. This is in contrast to the rDNA promoters studied in *E. coli*, where both the operons and the promoters show high similarity. The expression of the five operons was invested by cloning the promoters under the chloramphenicol acetyl transferase (*cat*) reporter gene in a low copy vector in the *L. plantarum* WCFS1 background. Promoter activity was studied during different growth phases. So far, those experiments showed differential expression during growth. Expression of the promoters appeared maximal during the log-phase, decreased in the late log-phase, and was in the stationary phase hardly expressed. Similar results were obtained for the tRNA promoter. Differential rDNA promoter activities of the different rDNA promoters could be confirmed based on a minimal inhibitory concentration (MIC) determination, using chloramphenicol.

P51

Characterisation of a subtilisin-like endoprotease of *Aspergillus niger*

R. Jalving, P.J. Schaap
Fungal Genomics, Laboratory of Microbiology, Wageningen University and Research Centre, Wageningen, the Netherlands

In filamentous fungi an effective method to enhance the secreted yields of foreign proteins is the use of a translational fusion between the target protein and an endogenous secreted carrier protein. Removal of the carrier protein is usually achieved *in vivo* through cleavage of an engineered KEX2 endoprotease recognition site at the fusion junction. We have cloned the kexin-encoding gene of *Aspergillus niger* (*kex*B). Disruption of *kex*B resulted in a transformant with a hyper-branching morphology. Using fluorogenic substrates kexin-like activity was measured in membrane-protein fractions of the wild type strain and a KexB overexpressing strain. In contrast, no kexin specific activity was detected in the similar protein fractions of the *kex*B-disrupted strain. Expression in this loss of function strain of a glucoamylase human interleukin-6 fusion protein with an engineered KEX2 dibasic cleavage site at the fusion junction resulted in secretion of unprocessed fusion protein.

P52

Chasing the Red Queen: genomics of coevolution

B.W. Ibelings[1], A. de Bruin[1], M. Rijkeboer[1], M. Brehm[1], E. van Donk[1], J.A.G.M. de Visser[2], H.C.P. Matthijs[3], J. Huisman[3]
[1]*Centre for Limnology, NIOO(KNAW), Nieuwersluis, the Netherlands,* [2]*Laboratory of Genetics, WUR, Wageningen, the Netherlands,* [3]*Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands*

The Red Queen hypothesis describes coevolution, the reciprocal adaptive genetic changes within populations of interacting species. We are testing the Red Queen's central prediction, that of accelerated evolution under strong biological interactions. We have selected resource competition between the unicellular green alga *Chlamydomonas* and the cyanobacterium *Synechocystis* as the biological interaction to be studied. By comparing the rate of adaptation during coevolution, to controls where coevolution is minimised the Red Queen hypothesis can be (dis)proved. In the controls one of the competitors is repeatedly replaced by the ancestral strain, taken from the freezer, disallowing coevolution in both. Evolution is allowed for >1000 generations in chemostats. The evolution of fitness - as the most important phenotypic trait - is assayed in a direct head to head competition between ancestor and evolved strains. Both competitors have been fully sequenced enabling a genomics approach to the molecular evolution. DNA microarrays are used to screen the evolving populations of both species for significant changes in gene expression. Oligonucleotide probes of 60 mer length have been designed for every of the 3235 open reading frames in the 3.7Mb genome of *Synechocystis. Chlamydomonas* will follow. Genes that significantly alter their expression are the first (but not the only) candidates to be involved in coevolutionary adaptation, and will be sequenced at regular intervals to look for mutations. Phylogenetic trees will be constructed - in which the ancestor forms the root of the tree - from which the rate of evolution can be inferred. Mutations are highly frequent events, but beneficial mutations are extremely rare. However we carried out a theoretical analysis which shows - on basis of a large effective population size and an enhanced value of s (the selective advantage of a mutation) under coevolutionary adaptation - that we may expect some 50 adaptive sweeps during 1000 generations of experimental evolution. Sufficient for a successful chase of the Red Queen.

P53

Growth of haloalkaliphilic sulfur-oxidising bacteria in continuous culture at high salt concentration

H. Banciu[1], D.Y. Sorokin[2], R. Kleerebezem[1], G. Muyzer[1], J.G. Kuenen[1]
[1]*Kluyver Laboratory for Biotechnology, University of Technology, Delft, the Netherlands,* [2]*Institute of Microbiology, RAS, Moscow, Russia*

Recently a new group of chemolithoautotrophic sulfur-oxidising bacteria has been discovered in soda lakes of central Asia, East Africa and North America. They belong to 3 distinct genera, *Thioalkalimicrobium, Thioalkalivibrio* and *Thioalkalispira* in the gamma subdivision of the *Proteobacteria*[1,2]. Among the most distinct properties of these bacteria are their halotolerance, and their potential to grow at extremely high pH (up to 10.6). They use thiosulfate, sulfide, sulfur and polysulfide as energy source and HCO_3^- as the only carbon source.

So far, only a few data are available on the growth kinetics of chemolithoautotrophic bacteria in continuous cultures, and even fewer data are known for growth under high pH and high salt concentration. Herewith the present experimental results provide new information on the eco-physiology of extremophilic organisms.

A typical representative of the genus *Thioalkalivibrio, Tv. versutus* ALJ 15, is able to grow aerobically at high pH and within a broad range of salt concentration (0.6 to 4M of total Na^+). In batch culture with thiosulfate as energy source, the strain grows optimally at pH 10 and between 0.6 and 2 M of total Na^+. At 4 M of total Na^+ the cells can grow and oxidise thiosulfate at 50% of the maximum growth and oxidation rate respectively. For a better characterisation of its growth kinetics, *Tv. versutus* ALJ 15 was grown aerobically in thiosulfate-limited continuous culture at pH 10 and at three different salt concentrations (0.6, 2 and 4 M of total Na^+). The maximum specific growth rates were 0.29, 0.21 and 0.1 h^{-1}, respectively. The highest molar yield (7.9 g protein/mol thiosulfate) was observed at 0.6 M Na^+. The yield decreased substantially towards more extreme salt conditions. Similar to that observed in the batch culture, the maximum thiosulfate- and sulfide-oxidising potentials were higher at 0.6 and 2 M than at 4 M. Elemental sulfur production was prominent at 0.6 and 2 M, but not at 4 M total Na^+. The apparent affinity constant (Ks) for thiosulfate increased with dilution rates from 3 to 10 μM.

The oxidation rates and biomass yields of the haloalkaliphilic bacteria described here are comparable to those found under

moderate conditions. The potential of these chemolithoautotrophic bacteria for stable growth at pH >10 and at moderate to high salt concentrations can be exploited in certain industrial waste purification technologies. In particular, the possible application of extremely salt-tolerant *Thioalkalivibrio* strains to remove H_2S from the industrial off-gases is currently under investigation

References

1. Sorokin DY, Lysenko AM, Mityushina LL, Tourova TP, Jones BE, Rainey FA, Robertson LA, Kuenen JG. Int J Syst Evol Microbiol 2001;51:565-80.
2. Sorokin DY, Tourova TP, Kolganova TV, Sjollema KA, Kuenen JG. Int J Syst Evol Microbiol 2002;52:2175-82.

P54

Functional analysis of the transcriptional activator XlnR from *Aspergillus niger*

A.A. Hasper, L.H. de Graaff

Fungal Genomics section, laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands

The transcriptional activator XlnR from *A. niger* is a Zn binuclear cluster transcription factor that belongs to the Gal4 superfamily. Besides the fact that it harbours a functional N-terminal DNA-binding domain, not much is known about structure-function relations in this protein. Based on the amino acid sequence, two coiled-coil regions were predicted, the first near the DNA-binding domain and a second one in the C-terminal part of the protein. C-terminal deletion mutants of XlnR showed that this second coiled-coil region is involved in nuclear localisation, after deletion of this domain XlnR resides in the cytoplasm. Identification of C-terminal amino acid substitutions in XlnR mutants led to the suggestion that this part of the protein fulfils a regulatory role. A mutant *A. niger* strain NW147, which is constitutive in xylanase expression, was isolated using a pathway specific selection mechanism based on the bidirectional marker gene *pyrA*. One single amino acid substitution V756F in XlnR was found responsible for the constitutive phenotype. In a CreA depressed strain there is no transcription of the XlnR target genes when grown on a non-inducing carbon source, in this strain the presence of inducer is necessary for transcription. When the V756F mutation in XlnR is combined with the CreA mutation in a single strain, high levels of transcript were found when grown on a non-inducing carbon source. This indicates two levels of regulation, at the level of the structural genes by repression of transcription by CreA, and at the level of XlnR by post-translational modification. By visualising XlnR *via* a fluorescent tag we show that XlnR is constitutively present in the nucleus and not *de novo* synthesised upon addition of inducer.
Based on these mutations partial C-terminal deletion mutants were constructed and analysed. This resulted in a regulatory model of XlnR in which the C-terminus responds to repressing signals that result in an inactive masked state of the protein.

P55

Niche differentiation of ammonia-oxidising bacteria along a salinity gradient in microcosm experiment

M. Coci, P.L.E. Bodelier, D. Riechmann, H.J. Laanbroek

NIOO-KNAW Centre for Limnology, Department of Microbial Ecology, Nieuwersluis, the Netherlands

Ammonia-oxidising bacteria convert ammonia to nitrite, playing a key role in the nitrogen cycle. The functioning of these bacteria in their natural habitat will mainly depend on ammonia and oxygen availability. However, in estuaries also salinity may be a key factor driving their community composition and functioning.
In this study the effect of salinity was tested using samples from an inter-tidal freshwater sediment of the river Schelde, Belgium. Microcosms were set up allowing to sample sub-cores during the incubation time and to obtain pore water samples at different depths. The systems were filled with freshwater sediment (top 5 cm) and flooded twice a day (6 hours each) with a 1mM NH_4^+ medium of different salinity (freshwater, brackish and marine) for a period of 5 weeks.
16s rRNA and *amo*A gene PCR-DGGE analyses of community composition showed that in the freshwater sediment organisms related to *Nitrosomonas urea* were dominant. Flooding with freshwater medium resulted in replacement of the *N. urea* related ammonia oxidiser by a strain affiliated with the *Nitrosomonas* 6a sequence cluster. The shift occurred already in the first week. It occurred up to 5 cm sediment depth.
Exposure to salt also resulted in replacement of *N. urea* but by a *Nitrosomonas marina*- related strain. This shift occurred after 4 and 3 weeks for the brackish and marine treatment respectively and did not extend deeper than 1 cm.
These experiments show that changing the environmental conditions lead to rapid shifts in the ammonia oxidising community. This confirms that different species inhabit different niches.
Microcosm, flux systems, model for aquatic environment in which nutrient concentration can be a limiting factor.

P56

Prolonged glucose limited chemostat cultivation affects the metabolic response of *Saccharomyces cerevisiae* to a glucose pulse

L. Wu, M.R. Mashego, J.L. Vinke, W.M. van Gulik, J.J. Heijnen

Kluyver laboratory for biotechnology, Bioprocess Technology, Delft, the Netherlands

In order to investigate whether the *in vivo* kinetic properties of the central metabolic pathways of *S. cerevisiae* are influenced by prolonged chemostat cultivation, pulse response experiments have been carried out. Glucose pulses were given to a long-term (approximately 70 generations) and a short-term (approximately 10 generations) glucose limited chemostat culture of *S. cerevisiae*. After addition of glucose, the intra- and extracellular metabolite concentrations and the off-gas oxygen and carbon dioxide content were followed in a time window of approx. 100 seconds. The latter were used to calculate the oxygen uptake rate and the carbon dioxide evolution rate during transient. It was found that the difference in culture age is clearly reflected in distinct pulse

responses of the two cultures. Much less glucose is taken up by cells in the long-term culture for example, and much less ethanol and acetate is produced. The observed differences can be accounted for by changes in enzyme activities during long term cultivation.

P57

The metabolic response of *Saccharomyces cerevisiae* to heat stress

F.I.C. Mensonides, J.M. Schuurmans, M.J. Teixeira de Mattos, K.J. Hellingwerf, S. Brul
University of Amsterdam, Swammerdam Institute for Life Sciences (SILS)-Microbiology, Amsterdam, the Netherlands

A study has been initiated to integrate physiological and molecular responses of *Saccharomyces cerevisiae* to heat stress conditions. We focus our research on a quantification of the energetics of the stress response by time-resolved metabolic flux analysis of cultures that are subject to various temperature shifts (28°C → 37°C, 38°C....43°C). Our analyses include anabolic, catabolic and energy fluxes, internal polymer (trehalose) breakdown and viability changes. In addition, changes in the posphorylation level of slt2p, an intermediate in the cell integrity-pathway involved in the yeast's stress response, are monitored.
We observed a shift from exponential growth to growth arrest and ultimately to cell death within a very narrow range of growth temperatures. Furthermore, the data presented here show that a non-lethal continuous heat stress invokes a redistribution of catabolic and anabolic fluxes and that the initial response is rather different from the long-term one. Finally, growth arrest is accompanied by increased slt2p phosphorylation whereas the capacity to resume growth seems to relate to a decrease in phosphorylation. These results are discussed in view of the increased energetic demands for growth at elevated temperatures.

P58

Anaerobic ammonium oxidation? A view beyond the rRNA phylogeny

M.C. Schmid[1], H.J.M. op den Camp[2], M. Strous[2], I. Cirpus[1], L.A. van Niftrik[1], H. Harhangi[2], D. Le Paslier[3], J.G. Kuenen[1], J. Weissenbach[3], M. Wagner[4], M.S.M. Jetten[2]
[1]*University of Technology Delft, Biotechnology, Delft, the Netherlands,* [2]*KU Nijmegen, Microbiology, Nijmegen, the Netherlands,* [3]*Genoscope, Evry, France,* [4]*University of Technology Munich, Microbiology, Freising, Germany*

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

P59

Fermentative capacity of *Saccharomyces cerevisiae* strains altered in expression of global catabolic regulators

J.M. Schuurmans, K.J. Hellingwerf, M.J. Teixeira de Mattos
SILS-Microbiology, University of Amsterdam, Amsterdam the Netherlands

Fermentative capacity (specific ethanol production in an anaerobic environment) is an important quality parameter of *Saccharomyces cerevisiae* for (industrial) dough rising. The fermentative capacity will depend on the capacity and activity of glycolysis and on the capacity of the specific fermentative enzymes, pyruvate decarboxylase and alcohol dehydrogenase. Catabolic fluxes, *i.e.* glycolysis and respiration in the aerobically growing yeast cell is under control of a set of global regulators (*e.g.* Hxk2p, Mig1p and Hap4p) and mutations in the expression level of these regulators often result in a redistribution of respiro-fermentative fluxes towards respiration. Thus, the growth yield can be significantly increased. The question then arises how such mutants perform under anaerobic conditions with respect to fermentative capacity. Here we present data showing that deletion of *hxk2* results in a lowered capacity when glucose is the energy source but an increased capacity with maltose. We discuss these results in view of glucose repression mechanisms. Also data on the performance of a hap4p overproducing and a Δmig1 strain will be presented.

P60

Hepatitis C virus specific immunity after extracorporeal whole body hyperthermia in patients with chronic hepatitis C virus infection

G.J. Boland[1], S. Pamporis-Moschatsis[1], C. Huijben[1], H. van Soest[2], A.M. van Loon[1], J. van Hattum[2]
University Medical Centre Utrecht, [1]*Medical Microbiology, Virology,* [2]*Gastroenterology, Utrecht, the Netherlands*

Besides the use of extracorporeal whole body hyperthermia (EWBH) as a treatment for several forms of malignancies, EWBH has shown to induce an antiviral effect in patients co-infected with HIV and HCV. Since this effect might start several weeks after the procedure, it is assumed that induced HCV-specific T-cell immunity is (one of) the effector mechanism(s).
A single application of EWBH (body core temperature raised to 41,8°C ± 0.15°C for 2 hours, Temet™, First Circle Medical, Minneapolis, MN, USA) was performed in 13 patients with HCV genotype 1 and previous non-response to antiviral therapy to study the safety, early viral effect and immunity

pattern. All 13 patients have been followed for 24 weeks. Blood samples were collected pre-treatment, at day 1, weeks 1, 2, 3, 4, 8, 12, 16, 20 and 24.
General and HCV specific immunity was studied by measuring serum cytokines and T-cell proliferation to HCV- and recall antigens. Moreover, HCV-specific T-cells were quantified and characterised by immunofluorescent detection after stimulation of PBMCs with different HCV-peptides restricted to HLA-A2 or -B7. The cells were stained for cell surface markers (CD4, CD8 and CD69) and intracellular cytokines (ICC: IL-2, IFN-gamma and TNF-alpha).
The serum cytokines TNF-alpha, IL-1beta, IL-6 and IL-10 increased during the procedure in all patients. Also, increased cytokine levels were observed in all of the patients ranging from 4-20 weeks after EWBH. The T-cell proliferation to HCV antigens significantly increased in 10/13 patients with an optimum at 2 to 6 weeks after EWBH. The ICC results show that preferently CD8-positive T-cells are activated in response to HCV peptide stimulation. Little IFN-gamma is produced, whereas IL-2 production was detected in CD4 and CD8 T-cells. Strongest was the TNF-alpha response to nearly all HCV peptides in both CD4 and CD8 T-cells.
In conclusion: a strong general and HCV specific immunity is induced after EWBH in chronic hepatitis C patients non-responding to former therapy. This might be a clue for developing new (combination) treatment modalities.

P61

Isolation and characterisation of the septal pore cap structure in the plant pathogen *Rhizoctonia solani*

K.G.A. van Driel[1], W.H. Müller[2], H. Yigittop[1],
A.J. Verkleij[2], H.A.B. Wösten[3], T. Boekhout[1]
[1]Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, University of Utrecht, [2]Molecular Cell Biology, [3]Molecular Microbiology, Utrecht, the Netherlands

The septal pore cap (SPC) structure is situated at both sides of the dolipore septum within the hyphae of many Basidiomycetous fungi. Though ultrastructural studies on the SPC demonstrated different SPC morphologies, the function of the SPC is still not well understood. In this study, we used the plant pathogen *Rhizoctonia solani* as a model organism to isolate and characterise the SPC structure. Further, we used a number of (fluorescent) biomarkers to visualise the SPC. These biomarkers may possibly be used to monitor the fate of the SPC during isolation procedures. The used biomarkers were: Calcofluor white, wheat germ agglutinin, DIOC, ER-tracker and protein stains. This resulted in different patterns of staining of the SPC region, thus illustrating the different biochemical properties of the SPC. Moreover, these markers can be used to isolate the SPC structure.

P62

Differential protein secretion in *Aspergillus niger*

A.M. Levin, R.P. de Vries, H.A.B. Wösten
Univerity of Utrecht, Molecular Microbiology, Utrecht, the Netherlands

Aspergillus niger is considered an excellent system for protein production due to its ability to secrete large amounts of enzymes into the medium. Secretion of certain enzymes of industrial relevance, such as glucoamylase, has been studied in detail and improved very successfully in some cases. The strategies followed were creation of multicopy transformants, improved fermentations and/or mutagenesis and screening. Currently we are studying secretion from a mycelial point of view.
From previous studies glucoamylase was shown to be exclusively secreted at the periphery of the colony. In this zone only few hyphae secreted the protein. From these observations we believe that the production of a certain enzyme can be improved increasing the number of hyphae that secrete it. This can be done by expressing glucoamylase behind promoters that are active in hyphae normally not expressing this enzyme. We have designed and optimised a ring plate system, which allows taking concentrical liquid samples within the colony. Enzymes secreted in different zones of the colony can be visualised by SDS-PAGE and their activity measured by PNP assays. Several zone specific proteins were detected using five different carbon sources and two different strains. Currently these proteins are being N-terminal sequenced which will allow us to clone the corresponding genes. By cloning the glucoamylase coding sequence behind the promoters of these genes it is expected that glucoamylase secretion will mimic the zone specific secretion pattern of the selected proteins. In this way we will be able to see whether glucoamylase can be produced by hyphae that normally do not secrete the enzyme

P63

The role of SC15 in the formation of aerial hyphae in *Schizophyllum commune*

J.F. de Jong, H.A.B. Wösten, L.G. Lugones
University of Utrecht, Microbiology, Utrecht, the Netherlands

The SC3 hydrophobin plays a pivotal role in the formation of aerial hyphae in the fungus *Schizophyllum commune*. It lowers the surface tension of the medium, enabling aerial growth, and it coats the aerial hyphae rendering them hydrophobic. SC3 also allows hyphae to attach to hydrophobic surfaces.
A 15 kDa protein (SC15) co-purifies with self-assembling SC3. SC15 is secreted into the medium but is also found in the cell walls of aerial hyphae. Both *SC3* and *SC15* are regulated by the *B* mating type genes and the *thn* gene.
To investigate whether SC15 could also have a function on aerial hyphae formation and attachment of hyphae to hydrophobic substrates, the *SC15* gene was deleted.
Three days old colonies of *S. commune* strains 4-40, Ä*SC15*, Ä*SC3* and Ä*SC3*Ä*SC15* were checked for their appearance and wettability.
Wildtype *S. commune* formed aerial hyphae which were hydrophobic and gave the colony a white appearance. Disrupting *SC15* showed no effect whereas disruption of *SC3*

reduced the amount of aerial hyphae formed and increased their wettability. The aerial mycelium of a Ä*SC3*Ä*SC15* strain was completely hydrophilic and was less whitish than that of Ä*SC3*. SC15 might help SC3 in reducing the surface tension which is currently being studied by means of ADSA-P experiments.
The same strains were allowed to grow on Teflon for 5 days after which stripping with increasing percentages of agarose was performed. The strength of attachment was deduced from the percentage of agarose which removed all hyphae.
Wildtype and Ä*SC15* hyphae remained attached to Teflon after stripping with high percentages of agarose. Very low percentages of agarose were sufficient to remove all hyphae of Ä*SC3*Ä*SC15*. Ä*SC3* showed values in between.
From these data we conclude that SC15 has a role in aerial hyphae formation and attachment, although the underlying mechanism is not yet clear.

P65

Localization of glucoamylase expression in *Aspergillus niger*

A. Vinck[1], M. Terlou[2], E.P. Martens[3], A.F. Ram[4], C.A.M.J.J. van den Hondel[5], H.A.B. Wösten[1]
University of Utrecht, [1]Microbiology, [2]Design and Image Analysis, [3]Biostatistics, Utrecht, the Netherlands, [4]University of Leiden, Molecular Plant Sciences, Leiden, the Netherlands, [5]TNO Nutrition and Food Institute, Molecular Genetics and Gene Technology, Rijswijk, the Netherlands

Filamentous fungi grow by means of hyphae that branch subapicaly, does forming a mycelium. Growth and secretion of proteins occur at the extreme tip of these hyphae.
Previously, it was shown that only a restricted number of hyphae at the periphery of the colony secrete glucoamylase (Gla). We here studied whether this is due to regulation at the transcriptional level or at the level of translation or secretion.
The green fluorescent protein (GFP) coding sequence was fused to Gla promoter of *Aspergillus niger* and introduced in this fungus. The glucose-6-phosphate dehydrogenase (GPD) promoter served as a control.
Transformants were grown for 5 days on non-inducing xylose medium and then transferred to inducing maltose medium. After 6-8 hours the GFP fluorescence was studied by confocal microscopy.
Optical sectioning followed by projecting the 3D image stack into a 2D image allowed for analysis of the hyphae at the peripheral zone of the colony. Fluorescence was determined by measuring the mean pixel values of selected hyphae. Data was analysed with the statistical program SPSS.
Variation of fluorescence was significantly larger when GFP was expressed behind the Gla promoter when compared to GPD driven expression.
Two groups of hyphae could be distinguished, those expressing Gla highly and those that express this gene only to a low level. Interestingly, the hyphae in former group have a larger diameter than those in the latter group.
It is thus concluded that differential secretion of Gla occurs at the transcriptional level and thick hyphae are more active in Gla expression than thin hyphae.

P66

Type IV fimbriae of *Actinobacillus pleuropneumoniae* are expressed *in vivo*

B.K.H.L. Boekema, J.P. van Putten, N. Stockhofe-Zurwieden, H.E. Smith
ID-Lelystad, Division of Infectious Diseases and Food Chain Quality, Lelystad, the Netherlands

The type IV fimbrial gene cluster of *Actinobacillus pleuropneumoniae* serotype 1 containing *apfA*, *apfB*, *apfC* and *apfD* was cloned and characterised. Sequence analysis of the *apfA* gene showed a characteristic alanine residue at the position -1 from the cleavage site in ApfA which is different from the consensus glycine at this position in type IV subunits. Electron microscopy demonstrated fimbriae protruding from the bacterial surface of *A. pleuropneumoniae* when the entire fimbriae cluster was cloned behind a constitutive promoter. Experiments with reporter constructs of the fimbriae promoter region revealed that fimbriae expression was tightly regulated. Fimbriae promoter activity depended on the bacterial growth phase and was seen only when bacteria were grown in chemically defined medium. The activity of the fimbriae promoter was tested in the presence of lung epithelial cells (LEC) and was found only in bacteria adhering to LEC ($p<0.05$). Non-adherent bacteria in the culture supernatant exhibited no promoter activity. To test the activity of the fimbriae promoter *in vivo*, pigs were endobronchially inoculated. The fimbriae promoter activity showed *in vivo* a 15-fold higher activity compared to *in vitro* conditions. In conclusion, the type IV fimbriae promoter in *A. pleuropneumoniae* is active upon cell contact and *in vivo*.

P67

Local antibiotic guidelines to improve prudent use of antibiotics: a survey in Dutch secondary care hospitals

J. Schouten[1,2], R. Jellema[1], S. Natsch[3], M. Hulscher[1], R. Grol[1], J. van der Meer[2]
University Medical Centre St Radboud, [1]Centre for Quality of Care Research, Departments of [2]Infectious Diseases and [3]Clinical Pharmacy, Nijmegen, the Netherlands

Background. Despite attempts at control, clinical misuse of antibiotics is widespread and increasing. Consequences are financial waste and antimicrobial resistance. Many hospitals attempt some degree of control of prescribing by means of an antibiotic policy
Aim of the study. Responding to a lack of information on the use of local antibiotic guidelines, we performed a survey in Dutch secondary care hospitals.
Material and methods. In all 91 Dutch secondary care hospitals a medical microbiologist was asked to fill in a written questionnaire.
Results. 88 of 91 contacted hospitals agreed to receive a written questionnaire, 62 (70%) completed questionnaires were returned.
A large proportion of respondents (95%) reported use of antibiotic a written policy for antibiotic therapy. Local antibiotic policies were generally composed by consensus procedure (78%) in a group consisting of a medical microbiologist, a hospital pharmacist and clinical specialists, invited depending on the purpose of the guideline. In the

composition process, international guidelines (37%), national guidelines (37%), reference books and literature review (12%) were used. In 32% local practice guidelines from other hospitals were used, 9/19 deducted from academic hospitals, 11/19 from surrounding hospitals or regional guidelines. Only 12% of the hospitals referred to use of the national guidelines developed by SWAB.

In 81% (n=48/59) of hospitals guidelines were revised with an average frequency of once per 2.64 (2.27-3.0) years. In 64% (n=38/59) local resistance surveillance data were taken into account, when updating a practice guideline.

Local practice guidelines were published as an antibiotic booklet in 90% of respondent hospitals. Apart from disseminating these booklets amongst users, 9 respondents organised a presentation on the subject, 3 used mailings and 12 intranet applications.

Conclusion. 1. National guidelines are not widely used in the development of local antibiotic guidelines in Dutch secondary care hospitals. This asks for improvement of the implementation process of these national guidelines in clinical practice. 2. Dissemination of local guidelines is concentrated on passive spreading of an antibiotic booklet in written form. Active implementation methods are rarely used.

P68

Association between human papillomavirus infection and cutaneous squamous cell carcinoma

L. Struijk[1], J.N. Bouwes Bavinck[2], P. Wanningen[1], E. van der Meijden[1], R.G.J. Westendorp[3], J. ter Schegget[1], M.C.W. Feltkamp[1]

Leiden University Medical Centre, Departments of [1]Medical Microbiology, Centre of Infectious Diseases, [2]Dermatology and [3]Internal Medicine, Leiden, the Netherlands

Background. A role for cutaneous human papillomaviruses (HPV) has been proposed in the development of skin cancer. Well-designed epidemiological studies to demonstrate an association between HPV infection and skin cancer are extremely rare. To identify HPV infection as a potential risk factor, we investigated the association between HPV infection and cutaneous squamous cell carcinoma.

Methods. A case-control study was designed consisting of 155 immunocompetent individuals with a history of squamous cell carcinoma and 371 controls without skin cancer. DNA extracted from plucked eyebrow hairs collected from the study population was analysed for the presence of cutaneous HPVs. To this purpose, samples were tested with a cutaneous HPV subgroup PCR and newly designed HPV type-specific PCRs for HPV 2, 5, 8, 15, 16, 20, 24 and 38.

Results. HPV infection was detected in 63.1% of the study population. HPV infection was associated with age (p=0.0002) and male sex (p=0.02), but not with sun exposure, skin type and smoking. After adjustment for age and sex, HPV infection, in particular multiple infection, was associated with squamous cell carcinoma (odds ratio 1.7, 95% CI 1.1; 2.7). HPV type specific analysis revealed that no HPV type stood out. The high-risk mucosal type HPV 16 and the skin wart type HPV 2 were rarely found in this study (<0.2%). Conclusion. The positive association found between HPV infection and squamous cell carcinoma suggests a role for HPV infection in the development of cutaneous squamous cell carcinoma.

P69

Application of mupirocin in the nose postpones nosocomial *Staphylococcus aureus* infections

H.F.L. Wertheim[1], M.C. Vos[1], A. Ott[1], C.M.J. Vandenbroucke-Grauls[2], H.H.M. Meester[2], A. Voss[3], J.A.J.W. Kluytmans[4], P.H.J. van Keulen[4], H.A. Verbrugh[1]

[1]Erasmus Medical Centre, Rotterdam, the Netherlands, [2]Free University Medical Centre, Amsterdam, the Netherlands, [3]University Medical Centre St Radboud, Nijmegen, the Netherlands, [4]Amphia Hospital, Breda, the Netherlands

Staphylococcus aureus (SA) is a frequent cause of nosocomial infections (NI) of which most are endogenous and originate from the nose. Elimination of SA from the nose of SA nasal carriers (SAC) may thus be a good strategy to prevent these infections and has been used in surgical settings. Application of mupirocin (Mu) in the nose is effective in eradicating SA. We studied whether Mu application in the nose at admission can prevent NI in non-surgical settings.

From 1999 to 2001 17,529 patients admitted to non-surgical departments (dialysis excluded) in four Dutch hospitals were screened for SAC. SACs were randomised to receive either mupirocin or placebo (Pb) (twice daily for five days, double-blinded). All NI were recorded according to CDC criteria. Nasal and clinical SA isolates were typed by Pulsed Field Gel Electrophoresis. Fisher's exact, Student-t test and Kaplan Meier survival were used for statistical analysis where appropriate.

4,479/17,529 (25.6%) Patients were found positive for SAC. 1,627 Patients were randomised to Mu (n=805) or Pb (n=822) (mean age: 57 years, 57% male). NI occurred in 2.6% (Mu) versus 2.8% (Pb) (p=0.88); bacteremias in 0.9% (Mu) versus 1.2% (Pb) (p=0.63). Mortality was 2.9% (Mu) versus 3% (Pb). Mean duration of admission was 11.6 days (Mu) versus 12.8 days (Pb) (p=0.1). Survival analysis showed that the time to NI is delayed from 20 to 32 days (p=0.029) for Mu patients. 80% Of all NI were endogenous.

We conclude that mupirocin prophylaxis did not affect the cumulative rate of nosocomial SA infections in this study population. However, mupirocin significantly delayed time to nosocomial SA infection. Future studies should consider to use mupirocin repetitively in prolonged admissions. Since 80% of the nosocomial SA infections of SACs are endogenous, eliminating SA carriage remains an attractive preventive strategy.

Auteur	Code
Aarts H.J.M.	N08
Abee T.	N08
Abma W.	P30
Advani A.	P15
Aerts P.C.	D10, E09
Akkermans A.D.L.	E07, N05, P35
Alves A.M.C.R.	P46
Amersfoorth S.C.M. van	P15, P25
Appelmelk B.J.	D04, S04
Appels C.	J05
Arnold M.	B02
Asselt G.J. van	R04
Assen S. van	L16
Bakker B.	P11
Bakker N.	P23
Bakker X.R.	J01
Banciu H.	P53
Bart A.	D07, D11, V01
Bartelink I.H.	R04
Bax R.	P21
Beaumont H.J.E.	E02
Beek A. ter	N13
Beekhuizen H.	S01
Beersma M.F.C.	W05, P04
Beerthuizen G.I.J.M.	V04
Belkum A. van	P16
Ben-Amor K.	N05
Benne C.A.	H03
Berbers G.	B01
Berbers W.A.M.	P31
Bereswill S.	D08
Berg H.F.	R01
Berg R.J. van den	P24
Bergen M.A.P. van	P11
Bergman M.P.	D04
Bergmans A.M.C.	C01
Berning M.	R05
Bindels P.J.E.	R03
Bitter W.	S02, S04, S05
Bloemen H.H.J.	E10
Blok H.E.M.	B05, V05, P09
Bodelier P.L.E.	P36, P55
Boekema B.K.H.L.	P66
Boekhout T.	F06, P61
Boel C.H.E.	C05, T03-04
Boer A.S. de	V03
Boer R.F. de	W04
Boer V.M.	P42
Boermans P.A.M.M.	R04
Bogaard A.E.J.M. van de	P10, P12
Bohlmann R.	F07
Boland G.J.	P60
Bolhuis H.	N07
Bolscher J.G.M.	R02
Bondeson K.	P15
Bonsdorff C. von	H01
Bonten M.J.M.	V05, P09
Boom R.	P05
Bormann C.	N01
Bosma F.	J02, L16
Bosman A.	L12
Bosman D.K.	P08
Bottiger B.	H01
Boucher C.A.B.	H05, L15, P14
Bouwes Bavinck J.N.	P68
Box A.T.A.	J03, P19
Boxma B.	E08
Brandenburg A.H.	W06
Brandhof W. van den	P26
Brehm M.	P52
Brisse S.	P23
Broek P.J. van den	P23
Brons J.K.	N07
Bruggeman C.A.	D03, H02
Brüggemann R.J.M.	R04
Bruijn R. de	R02
Bruin A. de	P52
Bruinsma N.	P10
Bruisten S.	P06
Brul S.	N06, N13, P48, P57
Brule A.J.C. van den	C05
Bruns H.M.	J04
Buckling A.	A03
Burger M.C.	D06
Camp H.J.M. op den	E01, E04, N04, P27, P32, P58
Canters G.W.	P34
Caro V.	P15
Celik G.	D06
Cherepanov A.V.	P34
Cirpus I.	N04, P58
Claas E.C.J.	C03, W01, W05, P04, P24
Claessen D.	N01, P43
Clercq P.A. de	T03-04
Coci M.	P55
Coenjaerts F.E.J.	F04-05
Crevel R. van	L10
Crielaard W.	N06
Dam J.C. van	E03, E11, P28
Damste J.S.S.	P47
Dankert J.	C03, D07, V01
Daran J-M.	P41
Daran-Lapujade P.	P41
Declerck S.	N14
Deelstra H.J.	F07, N01
Degener J.	P10
Dessel H. van	J04, P23
Diavatopoulos D.A.	B02
Diepen A. van	S01
Dierdorp M.	P06
Dijk H. van	D10
Dijk S.R. van	V04
Dijkhuizen L.	N01, N10, N11, P43, P44, P45, P46
Dijkshoorn L.	P23, P24
Dijksterhuis J.	F07
Dijkstra J.R.	P26
Dinkelaar W.	H04
Dissel J.T. van	N03, S01

Dongen U. van P30
Donk E. van P52
Doorn H.R. van C03
Doornum G.J.J. van L11
Drent M. P01, P03
Driel K.G.A. van P61
Driessen C. J06
Drift C. van der E04
Duijkeren E. van J03, P22
Duijnhoven Y. H01
Duim B. V01, P05
Eadie K. P16
Eeden F. van S05
Elburg R.M. van V02
Ellerbroek P.M. F04-05
Elsacker-Niele A.M. W06
Elzakker E.P.M. van P21
Ende A. van der D05, D07, D11, P08
Engering A. D04
Ernst F.D. D08
Ettema T. N10
Ezzahiri R.R. D03
Faden H. P16
Fell J.W. F06
Felske A. E09
Feltkamp M.C.W. P68
Fetter W.P.F. V02
Fikkers B.G. L16
Filius P.M.G. P10
Fisher M.C. F08-09
Fluit A.C. J03, P19
Fortin R. P33
Fraaije M.W. P33
Fuerst J.A. E06, P47
Gadikota R.R. S04
Gageldonk P.G.M. van P31
Galama J.M.D. J02, L10, L13, L16
Gavilanes A.W.D. J06
Geelhoed J.S. P37
Geel-Schutten G.H. van N11
Geest S. van der P01, P03
Geijtenbeek T. S04
Geize R. van der P44, P45
Gerrits M.M. R05
Gevel J.S. van de S01
Gielis-Proper S.K. D06
Gijswit G. F06
Goedegebuur F. N01
Goeij B. de D04
Goettsch W.G. P18
Gons H. P38
Gooskens J. D02
Goossens H. W01
Graaf L.H. de P54
Graaf-Miltenburg L.A.M. de B05
Grauls G. H02
Griensven L.J.L.D. van E04
Griethuysen A. van L14
Groenink J. R02
Grol R. P67
Groot L. de P21
Gruteke P. P06
Gucht K. van der N14
Guiso N. P15
Gulik W.M. van E03, E10, E11, N02, P28, P29, P56
Gyssens I.C. V03
Haaijer S.C.M. P32
Haase G. F03
Habets M. N10
Hackstein J.H.P. E08
Hallander H.O. P15
Hannen E. van N14
Hannen E.J. van P07, P13
Harhangi H. P58
Harmsen T. D10
Hasper A.A. P54
Hattum J. van P60
Have R. ten N12
Hays J. P16
Hazen M. P12
He Q. P15
Heeswijk W.C. van E05
Heide H.G.J. van der B01, B02, P15
Heide J.G.J. van der P25
Heidekamp A.J. P33
Heijens A. D01, D09
Heijkamp-de Jong I.G.A. N05
Heijnen J.J. E03, E10, E11, N02, P28, P29, P56
Heilmann F.G.C. J04, P02
Hellingwerf K.J. E05, N06, N13, P48, P57, P59
Herings R.M.C. P18
Herk C.M.C. van C05
Hermans A.P.H.M. N08
Herngreen S.B. D03, H02
Herpers B.L. P07
Hessels G.I. P44
Heuvelman C. B01
Hijnen M. B01, P31
Hoek B. van C02
Hoepelman A.M. F04-05
Hokke C.H. S04
Homuth G. D08
Hondel C.A.M.J.J. van den P65
Hoog G.S. de A02, F02
Horrevorts A.M. P17
Horst H.J. ter R04
Houwevers D. P22
Huijben C. P60
Huijsdens-van Amsterdam K. D05
Huisman J. P52
Hulscher M. P67
Ibelings B.W. P52
Jacobs J.A. J06, P01, P03
Jager D. N01
Jalink K.P. V05, P09
Jalving R. P51
Janknegt R. P18
Janse I. N09
Jansen M.L.A. N02
Janssen C.M. N03
Janssen D.B. P33
Janssen R. S01
Jellema R. P67

Jetten M.S.M. E01, E04, E06, N04, P27, P30, P32, P47, P58
Jol-van der Zijde C.M. H04
Jong D. de H05
Jong J.F. de P63
Jong M.D. de C03, V01, P05
Jonge E. de V01
Jonge R. de S03
Jongh B.M. de P07, P13
Jonkers H.M. P43
Kahmann R. F07
Kaiser A.M. V02
Kamerbeek N.M. P33
Kamp E.E.H.M. van de H06
Kamp-Hopmans T.E.M. V05, P09
Kamst-van Agterveld M.P. N09, N14
Kaper T. N10
Kardinaal E. N09
Kasteren M.E.E. van V03
Keijser B.J.F. P34
Kerkhof J.H.T.C. van den P26
Keulen G. van P43, P46
Keulen P.H.J. van B03, R01, P69
Kimman T.G. H06
King A. B01
Kissing J. J04
Kist M. D08
Kitslaar P.J.E.H.M. D03
Kiviet D.J. E05
Klaassen C.H.W. V06, P17, P24
Kleerebezem M. E07, P50
Kleerebezem R. P40, P53
Kleijn R.J. P28
Klip H. C04
Kluytmans J.A.J.W. B03, R01, P69
Koch G. H06
Koenig C.H.W. von P15
Kohli E. H01
Koletzko S. P08
Konstantinov S.R. P35
Kooistra A.M.D. V05, W03, W04
Koopmans M.P.G. H01, H03, L12
Kooyk Y. van D04, S04
Koppele-Vrije A. te C03, P02
Koppelman M.H.G.M. B04
Korsten H.H.M. T03-04
Koster T. J05
Kötter P. P41
Kraan M.I.A. van der R02
Kralj S. N11
Kresnowati M.T.A.P. P29
Kroes A.C.M. H04, W05, P04
Kuene G. P30
Kuenen J.G. E01, E06, N04, P37, P39, P47, P53, P58
Kuijper E.J. C03, D02, P24
Kuiken T. P11
Kuipers E.J. D01, D09, R05, S03
Kuipers H.D.R. L11
Kuipers S. D10, L11
Kullberg B.J. L16, V03
Kumaraswamy R. P39
Kurtzman C.P. F06
Kurvers H.A.J.M. D03
Kusters J.G. D01, D08, D09, R05, S03
Laanbroek H.J. P36, P38, P55
Laijendecker D. W02
Lamers L.P.M. P32
Lammens M. L16
Lauridsen T. N14
Leeflang C. N01
Lepaslier D. N04
Leroy P.L.J.M. J06
Levin A.M. P62
Li Y. E09
Lieshout L. van C02
Lindström E.S. N14
Linssen C.F.M. P01, P03
Llamas M. S02
Loffeld R.J.L.F. S03
London N. J06
Loo I. van B02
Loogman A. P16
Loon A.M. van P60
Loosdrecht M.C.M. van P30, P37, P39, P40
Lopman B. H01
Lowary T.L. S04
Loy A. P36
Lugones L.G. P63
Maarel M.J.E.C. van der N10, N11
Maas E. van der P05
Maaskant J.M. D04
Maiden M.C. B02
Manniën J. V03
Martens E.P. P65
Mascini E.M. V05, P09
Mashego M.R. E11, N02, P56
Matthijs H.C.P. P52
Meer J. van der P67
Meester M.H.M. B03, P69
Meijden E. van der P68
Meijer A. S16
Meijer B.C. W06
Meijerink B.B. P27
Meima M. N09
Meis J.F.G.M. F02
Melchers W.J.G. L16
Mensonides F.I.C. P57
Mertsola J. P15
Mevius D.T. N03, P22
Miletto M. P36
Molijn A. P21
Mooi F.R. B01, B02, P15, P25, P31
Mook W.N.K.A. van P01, P03
Mouton J.W. V06
Mulder J.W. P30
Müller W.H. P61
Munster J. van N10
Musters R.J.P. S05
Muyzer G. N15-17, P39, P53
Naiemi N. al V01
Nannini-Bergman M.G. P13
Nasution U.D.M. P28

Auteursindex

Auteur	Code
Natsch S.	P67
Neeleman C.	V06
Neeling A.J. de	D02
Nieuw Amerongen A.V.	R02
Niftrik L.A. van	E06, P58
Nijhuis M.	H05
Njamkepo E.	P15
Nys S.	P10
O'Brien A.	P48
Onland G.H.W.	C05
Oomes S.	P48
Oostvogel P.M.	R04
Ossewaarde J.M.	D06
Osterhaus A.D.M.E.	P11
Ott A.	B03, P21, P69
Overdiek J.W.P.M.	R04
Oyib D.H.	P24
Paauw A.	P23
Pamporis-Moschatsis S.	P60
Pas K. van de	P30
Paslier D. le	P58
Pas-Schoonen K.T. van de	E01, P47
Passel M.W.J. van	D11
Peeters M.F.	R01
Pelt W. van	L12, P22
Penninga N.A.	N01
Persoons M.C.J.	C04
Petit T.	P41
Piet J.	D07
Pilon J.W.	P06
Piper M.D.W.	P41, P42
Pol A.	P27
Polderman A.M.	W02
Poppelaars S.W.	D01, D09
Pot R.G.J.	S03
Prick J.J.W.	L11
Proell A.	E11, P28
Pronk J.T.	N02, P41, P42
Pruszczynski M.	J02
Putten J.P. van	P66
Ram A.F.	P65
Ramsay G.	P01, P03
Ras C.	E03, E11, P28
Raymond J.	P08
Reijden T. van der	P23
Richter C.	L14
Ridwan B.U.	L15
Riechmann D.	P55
Riet D. van der	P05
Rietveld R.P.	R03
Riffelman M.	P15
Rijkeboer M.	P52
Robert V.	F06
Roberts I.	F06
Roelofs J.G.M.	P32
Roerig B.	C04
Roholl P.J.M.	D06
Rolink A.	L14
Salas J.	N01
Sar A.M. van der	S05
Savelkoul P.H.M.	V01
Schaap P.J.	N12, P51
Schee C. van der	P16
Schegget J. ter	P68
Schellekens J.F.P.	J04
Scheltinga S.A.	W01, W05, P04
Schinkel J.	H04
Schinkel L.	W02
Schipper P.	H05
Schmid M.C.	N04, P30, P47, P58
Schmidt I.	E01, N04, P30
Schneider M.	L15
Scholts M.C.	W03
Schoormans A.H.W.	J05, P11, P26
Schouls L.M.	B01, C01, P02, P15, P25
Schouten J.	P67
Schouten J.S.A.G.	P01, P03
Schreier E.	H01
Schülin T.	J01
Schulpen T.W.J.	B05
Schuurman R.	H05, L15, P14
Schuurman T.	W03
Schuurmans J.M.	P57, P59
Seeldrayers S.	P12
Severin J.A.	P21
Siebring J.	P46
Siekman J.H.	D06
Sloos J.H.	R03
Slootjes I.M.	C05
Smeets C.	J01
Smidt H.	P35
Smit J.A.H.	J03
Smith H.E.	P66
Soest H. van	P60
Soolingen D. van	Q04-06, P02
Sorokin D.Y.	P53
Spanjaard L.	V01
Spanning R.J.M. van	E02
Spauwen P.H.M.	J01
Spijkerman I.J.B.	C02
Staals L.M.E.	L16
Stalpers J.	F06
Stassen F.R.M.	D03, H02
Steendam E.	W06
Stender H.	F03
Stevens S.	H02
Stobberingh E.E.	R01, P10, P12
Stockhofe-Zurwieden N.	P66
Stoof J.	D01, D04, D08, D09
Straaten R.J.H.M. van der	N03
Straatsma G.	N12
Strien E. van	H01
Strous M.	E01, E06, N04, P30, P47, P58
Struijk L.	P68
Sturme M.H.J.	E07
Svensson L.	H01
Swanink C.	L14
Taylor J.W.	A05
Teixeira de Mattos M.J.	P57, P59
Templeton K.E.	C03, W01, W05, P03
Terlou M.	P65
Thewessen E.A.P.M.	J05
Thunnissen F.B.J.M.	F02
Tijdens M.	P38

Tjaden J. E08
Tjhie J.H.T. R01
Tol M.J.D. van H04
Troelstra A. B05, V05, P09
Vahrenholz C. P15
Valk J.A. de V06, P17
Vandenbroucke-Grauls C.M.J.E. B03, D04, S04, S05, V02, P69
Vaughan E.E. E07, E09, N05, P50
Veening J.W. P48
Veerman E.C.I. R02
Ven A. van der L10
Vennema H. H01
Verbrugh H.A. B03, V04, P16, P69
Verduin C. P16
Verhaegen M.H.G. E10
Verhargh S. N05
Verhoef J. V05, P09, P19, P23
Verkleij A.J. P61
Verschoor L. L14
Verweij J.J. C02, W02
Viëtor H.E. F02
Vijgenboom E. P34
Vijver D.A.M.C. van den P14
Vinck A. P65
Vink C. J06
Vinke J.L. E11, P56
Visser J.A.G.M. de P52
Vlaminckx B.J.M. P13
Vliegen I. H02
Vliegen J.H.R. L11
Vliet A.H.M. van D01, D08, D09, R04, S03
Vogels W.H.M. V04
Voncken F. E08
Voorn G.P. P13
Vos M.C. B03, P69
Vos P. L16
Vos W.M. de E07, E09, N05, P35, P50
Voss A. B03, P69
Vossen J.M. H04
Vreede W.B. P06
Vries M.C. de P50
Vries R.P. de P62
Waar K. H03
Wagemaker M.J.M. E04
Wagenaar J.A. J05, P11, P26
Wagner M. N04, P47, P58
Waidner B. D08
Wannet W. J03, P22
Wanningen P. P68
Weemaes C.M.E. J02
Weert H.C.P.M. van R03
Weissenbach J. N04, P58
Well M.E.W. van der P32
Westendorp R.G.J. P68
Westerhoff H.V. E02
Wertheim H.F.L. B03, P69
Wever B. de P05
Wielders C.L.C. P19
Wielen P.W.J.J. van der N07
Wijngaard K. van den L12
Wilbrink B. L13
Wille J.C. V03
Willems R.J.L. D02
Williams B.A. P35
Winde J.H. de P42
Winden W.A. van E03, E11, P29
Wintermans R.G.F. C01
Winther M.P.J. de D03
Wirth H.P. D04
Wolbers F. F04-05
Wösten H.A.B. F07, N01, P43, P61, P62, P63, P65
Wu L. E10, E11, P29, P56
Wulf M.W.H. J01, L10
Yigittop H. P61
Zaaijer H.L. B04
Zanden A.G.M. van der C03, P02
Zeijl J.H. van L13
Zwaluw K. van der P07
Zwart G. N09, N14
Zwet A.A. van C04, V04, W04
Zwet W.C. van der V02

Nederlands Tijdschrift voor Medische Microbiologie
Het Nederlands Tijdschrift voor Medische Microbiologie is het officiële orgaan van de Nederlandse Vereniging voor Medische Microbiologie (NVMM). Het doel van het tijdschrift is de lezers te informeren over ontwikkelingen betreffende het vakgebied. In het tijdschrift worden zowel fundamentele als klinische aspecten van de Medische Microbiologie belicht. Daarnaast biedt het plaats voor promoties e.d., nieuws over evenementen en mededelingen uit de Vereniging.

NVMM-secretariaat
Postbus 21020, 8900 JA Leeuwarden
Telefoon (058) 293 94 95, fax (058) 293 92 00
E-mail nvmm@knmg.nl
Internet http://www.nvmm.nl

Redactie
Dr. A.M. Horrevorts, hoofdredacteur
Mw. Dr. I.A.J.M. Bakker-Woudenberg/
Dr. A. Fleer/Dr. T. van Gool/
J.A. Kaan/Mw. L.M. Kortbeek/
Dr. J.G. Kusters/Dr. J.F.G.M. Meis/Dr. M.F. Peeters/
Dr. M. van Rijn/Prof. dr. H.A. Verbrugh

Eindredactie
Mw. G. Brouwer
Van Zuiden Communications B.V.
Postbus 2122, 2400 CC Alphen a/d Rijn
Telefoon (0172) 47 61 91, fax (0172) 47 18 82
E-mail brouwer@zuidencomm.nl

Oplage
800 exemplaren, 4 x per jaar

Abonnementen
€ 35,– per jaar voor niet-leden van de NVMM,
Europa € 42,50 per jaar, losse nummers € 10,20.
Opgave abonnementen: telefoon (0172) 47 61 91

Advertentie-exploitatie

Van Zuiden Communications B.V.
Telefoon (0172) 47 61 91

Auteursrecht en aansprakelijkheid
©Van Zuiden Communications B.V., 2003
Alle rechten voorbehouden. Niets uit deze uitgave mag worden verveelvoudigd, opgeslagen in een geautomatiseerd gegevensbestand of openbaar gemaakt, in enige vorm of op enige wijze, hetzij elektronisch, mechanisch, door fotokopieën, opnamen, of enige andere manier, zonder voorafgaande schriftelijke toestemming van de uitgever. Uitgever en auteurs verklaren dat deze uitgave op zorgvuldige wijze en naar beste weten is samengesteld; evenwel kunnen uitgever en auteurs op geen enkele wijze instaan voor de juistheid of volledigheid van de informatie. Uitgever en auteurs aanvaarden dan ook geen enkele aansprakelijkheid voor schade, van welke aard ook, die het gevolg is van bedoelde informatie. Gebruikers van deze uitgave wordt met nadruk aangeraden deze informatie niet geïsoleerd te gebruiken, maar af te gaan op hun professionele kennis en ervaring en de te gebruiken informatie te controleren.

Algemene voorwaarden
Op alle aanbiedingen, offertes en overeenkomsten van Van Zuiden Communications B.V. zijn van toepassing de voorwaarden welke zijn gedeponeerd bij de Kamer van Koophandel te Amsterdam.

ISSN 0929-0176