SUPPLEMENT BIJ ACHTSTE JAARGANG, APRIL 2000

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) in samenwerking met: de SLW Werkgemeenschap Algemene en Moleculaire Microbiologie, de Nederlandse Vereniging voor Medische Mycologie, de NWO Werkgemeenschap Microbiële Pathogenese, de Werkgroep Epidemiologische Typeringen, de Werkgroepen Oost, West en Noord Medische Microbiologie, de Nederlandse Werkgroep Klinische Virologie en de Stichting Kwaliteitsbewaking Medische Microbiologie

Veldhoven, 17 - 19 april 2000
Conference Hotel Koningshof, Locht 117, Veldhoven
Tel.: 040 - 235 74 75

Programma-overzicht

Abstracts

Auteursindex

Hoofdsponsor:
Aventis

Sponsors en exposanten:
Abbott
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Biomedical Diagnostics
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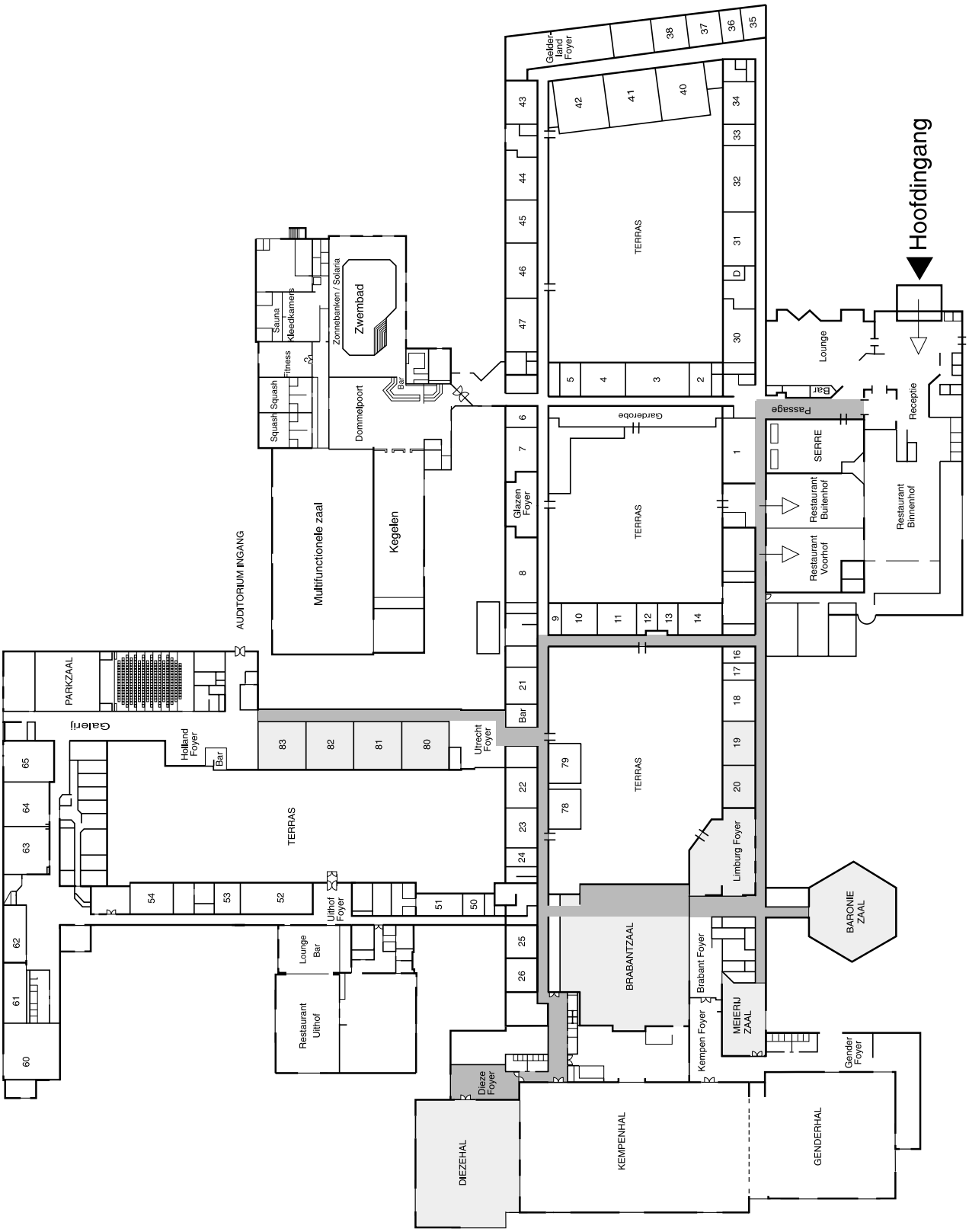
Hoofdingang
Gelder-land Foyer
TERRAS
Lounge
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Receptie
Garderobe
SERRE
Restaurant Buitenhof
Restaurant Voorhof
Restaurant Binnenhof
Zwembad
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DIEZEHAL
KEMPENHAL
GENDERHAL

MAANDAG 17 APRIL 2000

13:30 - 14:00	Registratie en koffie/thee		
14:00 - 16:00	Parallelsessies:		
	Stichting Kwaliteitsbewaking Medische Microbiologie	A	Baroniezaal
	Werkgroep Epidemiologische Typeringen	B	Meierijzaal
15:40 - 16:00	Koffie/thee		
16:00 - 18.00	Ledenvergadering NVMM		Baroniezaal
18:30 - 20:00	Diner		Restaurant
20:00 - 21:30	Uitreiking NVMM/Aventis Pharma Prijs Voordracht Laureaat		Baroniezaal
21:30	Borrel		

DINSDAG 18 APRIL 2000

Expositie & Posters in de Brabantzaal, hier wordt doorlopend koffie en thee geserveerd

09:00 - 09:30	Registratie en koffie/thee			
09.30 - 11:00	Plenaire sessie	**Legionellose**	C	Diezezaal
11:00 - 11.30	Koffie/thee			
11:30 - 13:00	Plenaire sessie	vervolg	C	Diezezaal
13:00 - 14:00	Lunchsymposium Aangeboden door Pharmacia & Upjohn			Baroniezaal
14:00 - 15:20	Parallelsessies:			
	Nederlandse Vereniging voor Medische Microbiologie (Werkgroepen)		D	Baroniezaal
	Sectie Algemene Virologie		E	Meierijzaal
	Nederlandse Vereniging voor Microbiologie (Secties)		F	Diezezaal
	Nederlandse Vereniging voor Medische Microbiologie		G	Zaal 80
15:20 - 15:50	Koffie/thee			
15:50 - 16.50	Parallelsessies	vervolg D, E, F, G		
17:00 - 18:00	Borrel			Brabantzaal
18:00 - 20:00	Diner			Restaurant
20:00 - 22:00	Posterpresentaties			Brabantzaal
22:00	Uitreiking Yakult Posterprijzen			Brabantzaal

WOENSDAG 19 APRIL 2000

Expositie & Posters in de Brabantzaal, hier wordt doorlopend koffie en thee geserveerd

07:15 - 09:00	Ontbijtsymposium Aangeboden door Imphos/Abbott		Baroniezaal
09:00 - 10:20	Parallelsessies:		
	Nederlandse Werkgroep Klinische Virologie	H	Zaal 81
	Nederlandse Vereniging voor Medische Microbiologie	K	Zaal 80
	Nederlandse Vereniging voor Microbiologie (secties)	L	Diezezaal
	NWO Werkgemeenschap Microbiële Pathogenese	M	Baroniezaal
	Sectie Levensmiddelenmicrobiologie	N	Meierijzaal
10:20 - 10:50	Koffie/thee		
10:50 - 12:10	Parallelsessies vervolg H, K, L, M, N		
12:10 - 12:30	Uitreiking Kiemprijs en Ledenvergadering NVvM		Diezezaal
12:30 - 13:30	Lunchsymposium Aangeboden door Roche		Baroniezaal
13:30 - 14:30	Parallelsessies:		
	Nederlandse Vereniging voor Medische Mycologie	R	Zaal 81
	Nederlandse Vereniging voor Medische Microbiologie	K	Zaal 80
	Nederlandse Vereniging voor Microbiologie (secties)	L	Diezezaal
	NWO Werkgemeenschap Microbiële Pathogenese	M	Baroniezaal
	Sectie Levensmiddelenmicrobiologie	N	Meierijzaal
14:30 - 15:00	Koffie/thee		
15:00 - 16:00	Parallelsessies vervolg R, K, L, M, N		

ALGEMENE INFORMATIE

Voorbereidingscommissie:
Prof. dr. C.M.J.E. Vandenbroucke-Grauls, voorzitter
Mw. drs. L.M. Kortbeek
Dr. B.P. Overbeek
Dr. M. Rutgers
Ir. W.A. Scheffers

Jury Yakult Posterprijzen:
Prof. dr. M.C. Horzinek, voorzitter
Dr. G.J.J. van Doornum
Mw. drs. L.M. Kortbeek
Prof. dr. B. Oudega
Prof. dr. B. Poolman

PROGRAMMA MAANDAG 17 APRIL 2000

A Baroniezaal **Stichting Kwaliteitsbewaking Medische Microbiologie**

Voorzitters: L.J.M. Sabbe en J.W. Mouton

14:00 – 14:20	H. Endtz *Campylobacter,* isolatie en identificatie anno 2000	A01
14:20 – 14:40	W.J.H.M. Wolfhagen Diagnostiek van toxigene *Clostridium difficile:* het blijft lastig	A02
14:40 – 15:00	T. van Gool Triple Faeces Test (TFT): een efficiënte nieuwe methode voor parasitologisch faecesonderzoek	A03
15:00 – 15:20	M.P.G. Koopmans Virale gastro-enteritis	A04
15:20 – 16:00	Jaarvergadering van de SKMM	

B Meierijzaal **Werkgroep Epidemiologische Typeringen**

Voorzitters: T. Boekhout en L. Dijkshoorn

14:00 – 14:20	T. Boekhout, B. Theelen, M. Diaz, J.W. Fell Moleculaire epidemiologie en infraspecifieke classificatie van de pathogene gist *Cryptococcus neoformans*	B01
14:20 – 14:40	E.M. Bik De WET op het WEB	B02
14:40 – 15:00	W.C. van der Zwet, G.A. Parlevliet, P.H.M. Savelkoul, J. Stoof, A.M. Kaiser, A.M. van Furth, C.M.J.E. Vandenbroucke-Grauls Genomic typing by Amplified Fragment Length Polymorfism (AFLP) identified a cluster of *Bacillus cereus* infections in a neonatal intensive care unit (NICU) accociated with balloons used in manual ventilation	B03
15:00 – 15:20	J. Spaargaren, J. Stoof, J.S.A. Fennema, S. Bruisten, P.H.M. Savelkoul 'Amplified Fragment Length Polymorphism' als identificatietechniek voor de 'core group' van *Neisseria gonorrhoeae* in de populatie van de Amsterdamse geslachtsziektenpolikliniek	B04
15:20 – 15:40	S.A. Morré, L. Rozendaal, I.G.M. van Valkengoed, A.J.P. Boeke, P.C. van Voorst Vader, J. Schirm, S. de Blok, J.A.R. van den Hoek, G.J.J. van Doornum, C.J.L.M. Meijer, A.J.C. van den Brule Urogenital *Chlamydia trachomatis* serovars in men and women having either a symptomatic or an asymptomatic infection: an association with clinical manifestations?	B05
15:40 – 16:00	J.L.W. Rademaker, B. Hoste, F.J. Louws, K. Kersters, J. Swings, L. Vauterin, P. Vauterin, F.J. de Bruijn Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system	B06

Baroniezaal	**Nederlandse Vereniging voor Medische Microbiologie**	
16:00 – 18:00	Ledenvergadering NVMM	
20:00 – 21:30	NVMM/Aventis Pharma Prijs Uitreiking Voordracht door de laureaat	
	Voorzitter: J. Dankert	

PROGRAMMA DINSDAG 18 APRIL 2000

C Diezezaal	**Symposium 'Legionellose'**	
	Voorzitter: H.A. Verbrugh	
09:30 – 10:15	V.L. Yu Legionnaires' disease: new insights and controversies	C01
10:15 – 11:00	D. van der Kooij Aanwezigheid en gedrag van *Legionella*-bacteriën in (drink)waterinstallaties	C02
11:00 – 11:30	Koffie/thee	
	Voorzitter: J.E. Degener	
11:30 - 12:15	J.W. den Boer Een epidemie van *Legionella*-pneumonie onder bezoekers van de Westfriese Flora in Bovenkarspel	C03
12:15 – 13:00	E.P.F. IJzerman, J.P. Bruin, J.F.P. Schellekens, R.J. van Ketel, M.F. Peeters Bacteriologische speurtocht naar de bron van de *Legionella*-epidemie te Bovenkarspel	C04
Baroniezaal	**Lunchsymposium aangeboden door Pharmacia & Upjohn**	
13:00 – 14:00	Gram positive infections – future solutions Linezolid, de eerste uit een nieuwe klasse, de oxazolidinonen	
	Voorzitter: J. Verhoef	
	J. Verhoef Epidemiologie	
	J.W. Mouton Farmacokinetiek/Farmacodynamiek	
D Baroniezaal	**Nederlandse Vereniging voor Medische Microbiologie (Werkgroepen)**	
	Voorzitter: M.J.H.M. Wolfhagen	
14:00 – 14:20	A.A. van Zwet, M.M.P. Haalebos, M.J.H.M. Wolfhagen Native valve endocarditis caused by *Staphylococcus lugdunensis:* the importance of an accurate identification of the microorganism	D01

14:20 – 14:40	J.P. Arends, G.A. Kampinga, G. Gezelle Meerburg, W. Postma, W. Baas, C. vd Ploeg An outbreak of a cefotaxime, tobramycin and amikacin resistant *Klebsiella pneumoniae*	D02
	Voorzitter: E.A.P.M. Thewessen	
14:40 – 15:00	M. Tersmette, J.H. Schagen-van Leeuwen, W.J. Duits, H.P.T. van Helden, B.M. de Jongh Onderzoek naar nosocomiale hepatitis-B-virustransmissie bij patiënten van een HBsAg-positieve arts op een afdeling gynaecologie	D03
15:00 – 15:20	R.W. Vreede, W. Goessens, H. Dekker Vermindering van de gevoeligheid voor Augmentin: het vervolg	D04
15:20 – 15:50	Koffie/thee	
	Voorzitter: A. Buiting	
15:50 – 16:10	A. van der Zee, H. Verbakel, C. de Jong, M.F. Peeters, A. Bergmans Molecular diagnosis and typing of *Legionella (non-) pneumophila*	D05
16:10 – 16:30	A.M. Horrevorts, M. Wolfhagen Nocardiosis in Nederland: voorlopige bevindingen tot 1 januari 2000	D06
16:30 – 16:50	J.W. Mouton, A.M. Horrevorts Aanpassing van het antibiotica-doseringsregime op basis van pharmacokinetische en pharmacodynamische eigenschappen bij patiënten met ernstige infecties	D07

E Meierijzaal — Sectie Algemene Virologie

Voorzitter: P.J.M. Rottier

14:00 – 14:20	G. Vreugdenhil, W. Melchers, N. Schloot, D. Pipeleers, C. Rongen, J. Galama Acute onset of type 1 diabetes mellitus caused by severe echo virus type 9 infection: investigation of putative pathogenic pathways	E01
14:20 – 14:40	A.D.M.E. Osterhaus, G.F. Rimmelzwaan, B.E.E. Martina, T.M. Bestebroer, R.A.M. Fouchier Influenza B virus in seals	E02
14:40 – 15:00	M.G.J. Tacken, P.J.M. Rottier, A.L.J. Gielkens, B.P.H. Peeters Interactions *in vivo* between the proteins of infectious bursal disease virus: capsid protein VP3 interacts with the RNA dependent RNA polymerase, VP1	E03
15:00 – 15:20	E. Kooi, N. Huijkman, M. Lucassen, J. Thijssen,W. Spaan, P. Bredenbeek Yellow fever virus as an expression vector for the hepatitis C virus envelope proteins	E04
15:20 – 15:50	Koffie/thee	
15:50 – 16:10	N. Renwick, M. Cornelissen, G. Weverling, N. Dukers, M. Bakker, R. Coutinho, T. Schulz, J. Goudsmit Disease association, transmission and natural history of HHV8/KSHV infection in the Amsterdam Cohort Studies on HIV infection and AIDS	E05
16:10 – 16:30	J.M. Smit, K. Ryan, W. Klimstra, B. Waarts, R. Johnston, R. Bittman, J. Wilschut Receptor interaction and membrane fusion activity of alphaviruses	E06
16:30 – 16:50	C. Vink, E. Beuken, C.A. Bruggeman The complete DNA sequence of the genome of rat cytomegalovirus	E07

F Diezezaal — Nederlandse Vereniging voor Microbiologie (Secties)

Voorzitter: B. Poolman

14:00 – 14:20	S. Alexeeva, W. Laan, K. Hellingwerf, M.J. Teixeira de Mattos Molecular physiology of Arc-dependent regulation of catabolism in *Escherichia coli*	F01
14:20 – 14:40	N.A.E. Ariës-Kronenburg, L. Fischer, J.C. Verdoes, J.A.M. de Bont Bio-imprinting of the epoxide hydrolase from *Rhodotorula glutinis*	F02
14:40 – 15:00	J. Baar, W.H. Konijnenbelt, J.M. van Groenendael Are ectomycorrhizal fungi of importance for wet Alder carr forests?	F03
15:00 – 15:20	M. Bandell, J. Lolkema Substrate binding and selectivity of the 2-hydroxycarboxylate transporter family	F04
15:20 – 15:50	Koffie/thee	
15:50 – 16:10	A.R. Bellu, J.A.K.W. Kiel, I.J. van der Klei, M. Veenhuis Peroxisome degradation in the yeast *Hansenula polymorpha*	F05
16:10 – 16:30	A. Bollmann, H.J. Laanbroek Influence of oxygen partial pressure (pO2) and salinity on the community shift of the ammonia oxidizing bacteria in the river Schelde	F06
16:30 – 16:50	U. van Dongen, M. Strous, K. van de Pas-Schoonen, M. van Loosdrecht, J.G. Kuenen, M.S.M. Jetten Combination of partial nitrification and anaerobic ammonium oxidation for the removal of ammonia from concentrated wastewater	F07

G Zaal 80 — Nederlandse Vereniging voor Medische Microbiologie

Voorzitter: G.J.J. van Doornum

14:00 – 14:20	J.M. Orendi, F.C.H. Abbink, A.J. de Beaufort Een tropische verrassing in een Hollandse neonaat	G01
14:20 – 14:40	G.A. Kampinga, F.P. Schröder, I.J.R.Visser, J.M.E. Anderson, D. Buxton, J.E. Degener, A.V.M. Möller Ovine *Chlamydia psittaci:* een belangrijke oorzaak van abortus bij schapen en een gevaarlijke ziekteverwekker voor zwangeren	G02
14:40 – 15:00	A. van der Ende, O.J. van Doorn, B.W.M. van 't Hoff, J.A.J.M. Taminiau, J. Dankert, D.K. Bosman Evaluation of the *Helicobacter pylori*-Specific-Antigen (HpSA) test to assess *Helicobacter pylori* infection in children	G03
15:00 – 15:20	T.G. Mank, T. van Gool De Triple Faeces Test (TFT) en ELISA bij de laboratoriumdiagnose van Giardiasis	G04
15:20 – 15:50	Koffie/thee	

Voorzitter: C.M.J.E. Vandenbroucke-Grauls

15:50 – 16:10	N.L.A. Arents, A.A. van Zwet, J.C. Thijs, J.H. Kleibeuker, A. de Jong, J.G. Kusters Serological screening for antibody response to the CagA virulence factor of *Helicobacter pylori:* validation of an ELISA based test	G05

16:10 – 16:30	N.L.A. Arents, L.C. Smeets, A.A. van Zwet, J.C. Thijs, E.J. van der Wouden, J.E. Degener, J.G. Kusters No evidence for multiple strain infections in patients carrying both metronidazole susceptible and resistant *Helicobacter pylori*	G06
16:30 – 16:50	R.P. Verkooyen, J. Harkema, M.F. Peeters, J.H. van Rijsoort-Vos, W.I. van der Meijden, W.H.F. Goessens, J.W. Mouton Evaluation of three peptide-based EIA assays for the diagnosis of tubal factor infertility caused by *Chlamydia trachomatis*	G07

P Brabantzaal **Postersessie en uitreiking Posterprijzen**

20:00 – 22:00 Posterpresentaties

22:00 Uitreiking Yakult Posterprijzen
Voorzitter van de jury: M.C. Horzinek

PROGRAMMA WOENSDAG 19 APRIL 2000

Baroniezaal **Ontbijtsymposium aangeboden door Imphos / Abbott**

07:15 – 09:00 The art of *Helicobacter pylori*-diagnostiek en resistentiebepaling

Voorzitter: W.A. de Boer

W.A. de Boer
Inleiding

J.G. Kusters
Resistentiemechanisme

A.A. van Zwet
Praktische aspecten van de kweek met resistentiebepaling

W.A. de Boer
Helicobacter-diagnostiek vanuit klinisch perspectief

Baroniezaal **Lunchsymposium aangeboden door Roche**

12:30 – 13:30 Nailing down to a moving target

Voorzitter: A.D.M.E. Osterhaus

Sprekers:
A.D.M.E. Osterhaus (Erasmus Universiteit Rotterdam)
K.G. Nicholson (The Leicester Royal Infirmary, Leicester)

H Zaal 81 **Nederlandse Werkgroep Klinische Virologie**

Voorzitter: G.J.J. van Doornum

09:00 – 09:40	E.J.H.J. Wiertz Immuunevasie en herpesvirussen	H01

09:40 – 10:00	F. Miedema Immuunevasie en HIV	H03
10:00 – 10:20	M.C.M. Feltkamp, J. ter Schegget, J.N. Bouwes Bavinck, W. Spaan Immuunevasie en HPV	H04
10:20 – 10:50	Koffie/thee	
10:50 – 11:30	J.M. Middeldorp Epstein-Barr-virus en immuunevasie: een onlosmakelijke eenheid	H05
11:30 – 12:10	A.D.M.E. Osterhaus Immuunevasie en influenzavirus	H07

K Zaal 80 Nederlandse Vereniging voor Medische Microbiologie

Voorzitter: J. Dankert

09:00 – 09:20	S. van den Hof, C.M.E. Meffre, F. Woonink, M.A.E. Conyn-van Spaendonck, R.S. van Binnendijk An outbreak of measles in a primary school in the Netherlands, June 1999	K01
09:20 – 09:40	W. Goettsch, E. Geubbels, W. Wannet, M.G.R. Hendrix, J.H.T. Wagenvoort, A.J. de Neeling MRSA in nursing homes in the Netherlands 1989-1998	K02
09:40 – 10:00	H.J. Wisselink, U. Vecht, N. Stockhofe-Zurwieden, H. Smith MRP and EF protect pigs against challenge with virulent *Streptococcus suis* serotype 2 strain	K03
10:00 – 10:20	S. van den Hof, G.A.M. Berbers, H.E. de Melker, M.A.E. Conyn-van Spaendonck Antibodies to capsular polysaccharide of *Haemophilus influenzae* type b (Hib) in the Dutch population a few years after introduction of vaccination	K04
10:20 – 10:50	Koffie/thee	

Voorzitter: J.E. Degener

10:50 – 11:10	H.E. de Melker, F.G.A. Versteegh, M.A.E. Conyn-van Spaendonck, L.H. Elvers, G.A.M. Berbers, A. van der Zee, S.E. Neppelenbroek, J.F.P. Schellekens Pertussis serodiagnosis with single serum and estimation of frequency of *Bordetella pertussis* infection in the population	K05
11:10 – 11:30	N. van den Braak, A. van Belkum, Working Party Hospital Infection Epidemiology Netherlands (WHEN), H.Ph. Endtz Prevalence and determinants of fecal colonization with vancomycin-resistant enterococci in hospitalized patients in the Netherlands	K06
11:30 – 11:50	C.M.J.E. Vandenbroucke-Grauls, H.H.M. Meester, L.E.A. Donkers, H.L. Zaaijer, G.J. Timmer, P.C. Huijgens, R.J.L. Willems, P.H.M. Savelkoul Succesvolle bestrijding van een epidemie met vancomycineresistente *Enterococcus faecium* op een hematologische afdeling	K07
11:50 – 12:10	I.K. Veldhuijzen, S.L.A.M. Bronzwaer, J. Degener, J. Kool, EARSS participants European Antimicrobial Resistance Surveillance System: *Streptococcus pneumoniae* susceptibility test results	K08
12:10 – 13:30	Lunch	

Voorzitter: H.A. Verbrugh

13:30 – 13:50	J.W. Dorigo-Zetsma, B. Wilbrink, J. Dankert, S.A.J. Zaat Identification of eight M. *pneumoniae* P1 cytadhesin gene PCR-RFLP subtypes, and a new variable region in the P1 gene sequence	K09
13:50 – 14:10	H.R. van Doorn, E.J. Kuijper, A. van der Ende, A.G.A. Welten, D. van Soolingen, P.E.W. de Haas, J. Dankert Isoniazid resistance in *Mycobacterium tuberculosis:* the mutation on amino acid position 463 of the *katG* gene is not a causative factor	K10
14:10 – 14:30	L.C. Smeets, N.L.A. Arents, A.A. van Zwet, C.M.J.E. Vandenbroucke-Grauls, J.G. Kusters Transfer of metronidazole resistance between two *Helicobacter pylori* strains	K11
14:30 – 15:00	Koffie/thee	
15:00 – 15:20	P. de Boer, B. Duim, A. Rigter, W.F. Jacobs-Reitsma, J. van der Plas, J.A. Wagenaar Comparative analysis of four genotyping techniques for the *Campylobacter* species *C. jejuni* and *C. coli*	K12
15:20 – 15:40	X.W. Huijsdens, R.K. Linskens, M. Mak, J. Stoof, S.G.M. Meuwissen, C.M.J.E. Vandenbroucke-Grauls, P.H.M. Savelkoul Real-time quantitative PCR of *Bacteroides vulgatus* and *Escherichia coli* in mucosal samples from healthy humans and patients with IBD	K13

L Diezezaal — Nederlandse Vereniging voor Microbiologie (Secties)

Voorzitter: W.M. de Vos

09:00 – 09:20	B. Boxma, T. van Alen, G. Vogels, J. Hackstein Mitochondrial chaperonine Hsp60 encoding genes in anaerobic chytrids	L01
09:20 – 09:40	C. van der Does, A.J.M. Driessen Studies on the bacterial protein-conducting pore: the SecYEG complex	L02
09:40 – 10:00	M. Gunnewijk, B. Poolman Phosphorylation state of HPr determines expression and activity of the lactose transport protein from *Streptococcus thermophilus*	L03
10:00 – 10:20	W.T.M. Jansen Development and evaluation of synthetic pneumococcal vaccines	L04
10:20 – 10:50	Koffie/thee	
10:50 – 11:10	J.D.H. Jongbloed, U. Martin, G. Venema, S. Bron, J.M. van Dijl, J. Müller Tat-dependent secretion of the phosphodiesterase PhoD of *Bacillus subtilis*	L05
11:10 – 11:30	B. Keijser, G. Canters, E. Vijgenboom The role of *ram* cluster during morphological differentiation of *Streptomyces lividans*	L06
11:30 – 11:50	G. van Keulen, L. Dijkhuizen, W.G. Meijer Regulation of autotrophic growth by the chemoautotrophic bacterium *Xanthobacter flavus*	L07
11:50 – 12:10	F. Leroy, L. De Vuyst Predictive microbiology as a tool for the estimation of the functionality of a sausage starter culture	L08
12:10 – 12:30	Uitreiking Kiemprijs en Ledenvergadering NVvM	
12:30 – 13:30	Lunch	

Voorzitter: J.G. Kuenen

13:30 – 13:50	E. van Lierop, M.E.O.C. Heck, H. Brunings, W.J. van Leeuwen, W.J.B. Wannet, J.F.P. Schellekens Molecular typing of *Legionella pneumophila*	L09
13:50 – 14:10	K.M. Overkamp, P. Kötter, B.M. Bakker, J.P. van Dijken, J.T. Pronk Mechanisms for respiratory oxidation of cytosolic NADH by *Saccharomyces cerevisiae* mitochondria	L10
14:10 – 14:30	J.L.W. Rademaker, R. Fulthorpe, C.L. Moyer, M.H. Schultz, F.J. de Bruijn, J. Tiedje Taxonomic characterization of strains from a microbial endemism study of 3-chlorobenzoate mineralizers	L11
14:30 – 15:00	Koffie/thee	
15:00 – 15:20	J.E. Tuininga, C.H. Verhees, J. van der Oost, A.J.M. Stams, S.W.M. Kengen, W.M. de Vos The modified Embden-Meyerhof pathway of *Pyrococcus furiosus* and other hyperthermophilic Archaea	L12
15:20 – 15:40	C.H. Verhees Glycolytic enzymes and their control in *Pyrococcus furiosus*	L13
15:40 – 16:00	A. Vos, W. Eeuwema, L. Dijkhuizen, M.J.E.C. van der Maarel Isolatie van genen coderend voor natief aardappelzetmeel afbrekende enzymen van *Bacillus firmus/lentus* en *Microbacterium* sp.	L14

M Baroniezaal — NWO Werkgemeenschap Microbiële Pathogenese

Voorzitter: P.W.M. Hermans

09:00 – 09:20	A.H.M. van Vliet, S. Bereswill, N. de Vries, E.J. Kuipers, M. Kist, C.M.J.E. VandenbrouckeGrauls, J.G. Kusters Regulation of iron uptake in *Helicobacter pylori* by the Fur protein	M01
09:20 – 09:40	J.J. de Soet, J. de Graaff, A. Herscheid, L. Frenken Antimicrobial activity of single chain antibodies against *S. mutans* coupled to glucose oxydase	M02
09:40 – 10:00	N. de Vries, D. Duinsbergen, E.J. Kuipers, P. Wiesenekker, C.M.J.E. Vandenbroucke-Grauls, J.G. Kusters Characterization of transcriptional phase variation in a type III restriction modification system of *Helicobacter pylori*	M03
10:00 – 10:20	W.B. van Leeuwen, W. van Nieuwenhuizen, C. Gijzen, H.A. Verbrugh, A. van Belkum Genetic polymorphism in the *agr*-locus of *Staphylococcus aureus* strains	M04
10:20 – 10:50	Koffie/thee	
10:50 – 11:10	J.J.E. Bijlsma, M. Lie-a-Ling, I.C. Nootenboom, J.Y. Jeong, C.M.J.E. Vandenbroucke-Grauls, D.E. Berg, J.G. Kusters *Helicobacter pylori* genes needed for growth under acidic conditions typical of the gastric mucosa	M05
11:10 – 11:30	A. de Greeff, L. van Alphen, H.E. Smith Selection of recombinant antibodies specific for pathogenic *Streptococcus suis* by subtractive phage display	M06
11:30 – 11:50	T. van der Straaten, L. Zulianello, R. Jansen, J.T. van Dissel A novel *Salmonella typhimurium* protein involved in resistance against macrophages oxidative stress	M07

11:50 – 12:10	T. Ó Cróinín, S. Brands Heerma, B. Drumm, C.M.J.E. Vandenbroucke-Grauls, B. Bourke, J.G. Kusters Identification of *Helicobacter mustelae* virulence factors by screening of a random insertional mutant library	M08
12:10 – 13:30	Lunch	
	Voorzitter: G.W. Welling	
13:30 – 13:50	S.A. Morré, C. Kuijl, J.M. Ossewaarde, C.J.L.M. Meijer, A.J.C. van den Brule Expression analysis by NASBA of the *Chlamydia trachomatis* virulence genes Hsp60, Hsp70 and CT-MIP in symptomatically versus asymptomatically infected patients	M09
13:50 – 14:10	L.C. Smeets, J.J.E. Bijlsma, S.Y. Boomkens, C.M.J.E. Vandenbroucke-Grauls, J.G. Kusters Identification of *comH*: a novel, *Helicobacter pylori* specific transformation gene	M10
14:10 – 14:30	J. Krijgsveld, J. Dankert, P.S. Hiemstra, S.P. Mannesse-Lazeroms, A.J. Kuijpers, G.H.M. Engbers, J. Feijen, S.A.J. Zaat Recombinant H-TC, a thrombocidin-derived peptide antibiotic	M11
14:30 – 15:00	Koffie/thee	
15:00 – 15:20	K. van Amsterdam, J. Dankert, A. van der Ende *Helicobacter pylori* gene expression induced upon interaction with gastric epithelial cells	M12
15:20 – 15:40	F.E.J. Coenjaerts, A. Walenkamp, J. Scharringa, B. Dekker, J. van Strijp, A. Hoepelman Impaired mobility of human neutrophils in response to cryptococcal mannoproteins	M13
15:40 – 16:00	J.J.E. Bijlsma, M. Sparrius, L.C. Smeets, F. Namavar, C.M.J.E. Vandenbroucke-Grauls, J.G.Kusters The neutrophile activating protein of *H. pylori* is a DNA binding protein essential for growth at pH 5	M14

N Meierijzaal **Sectie Levensmiddelenmicrobiologie**

Voorzitter: S.H.W. Notermans

09:35 – 09:40	Inleiding	
09:40 – 10:20	A.S.J.P.A.M. van Miert Risks involved in the veterinary use of antibiotics	N03
10:20 – 10:50	Koffie/thee	
10:50 – 11:30	F.M. Rombouts, A.W. van de Giessen Health risks of foodborne pathogens	N05
11:30 – 12:10	J. Kinderlerer Estimation of risks of GMO's	N07
12:10 – 13:30	Lunch	
13:30 – 13:50	P. Bijker Factoren die een rol spelen bij de besmetting van productiedieren met *Escherichia coli* O157:H7	N09
13:50 – 14:10	B.J. Hartog Microbiële risico's bij de verwerking van levensmiddelen	N10

14:10 – 14:30	M.A.S. de Wit, M.P.G. Koopmans, L.M. Kortbeek, W.J. van Leeuwen, A.I.M. Bartelds, Y.T.H.P. van Duynhoven Beschrijving van gevallen van gastro-enteritis waarvoor de huisarts wordt geconsulteerd	N11
14:30 – 15:00	Koffie/thee	
15:00 – 15:40	P.F.M. Teunis Dosis-respons analyses van infectieuze micro-organismen	N12
15:40 – 16:00	G.J. Medema *Cryptosporidia, Giardia* en *Cyclospora:* nieuwe risico's?	N14

R Zaal 81 — Nederlandse Vereniging voor Medische Mycologie

Voorzitter: J.F.G.M. Meis

13:30 – 13:50	M. de Boer, M. Mensink, W. Schaftenaar Aspergillose bij vogels in de collectie van Diergaarde Blijdorp	R01
13:50 – 14:10	H. Peltroche-Llacsahuanga, N. Schnitzler, S. Jentsch, G. Haase Comparison of phagocytosis of black yeasts by human neutrophils	R02
14:10 – 14:30	A.O.A. Ahmed, M.M. Mukhtar, M. Kools-Sijmons, A.H. Fahal, S. de Hoog, B. Gerrits van den Ende, E.E. Zijlstra, H.A. Verbrugh, El Sir A.M. Abugroun, A.M. Elhassan, A. van Belkum Eumycetoma due to *Madurella mycetomatis:* current laboratory experience	R03
14:30 – 15:00	Koffie/thee	
15:00 – 15:20	A. Borst, M.A. Leverstein-Van Hall, P. Haima, P. Sillekens, J. Verhoef, A.C. Fluit NASBA-based detection of *Candida* spp. in blood cultures	R04
15:20 – 15:40	R.C. Summerbell, A.K. Gupta A new look at *Acremonium* nail infection	R05
15:40 – 16:00	A. van Duin 'Rotterdamse' schimmels	R06
16:00 – 16:20	J. Meletiadis, J. Meis, J. Kerremans, J.P. Donnelly, P. Verweij Evaluation of different media for the growth of filamentous fungi with a microdilution kinetic model	R07
16:20 – 16:40	J. Kerremans, J. Meis, P. Verweij *Aspergillus* in tabak	R08

B01
Moleculaire epidemiologie en infraspecifieke classificatie van de pathogene gist *Cryptococcus neoformans*

T. Boekhout[1], B. Theelen[1], M. Diaz[2], J.W. Fell[2]
[1]*Centraalbureau voor Schimmelcultures, Afdeling Gisten, Delft,* [2]*RSMAS, University of Miami, Key Biscayne, USA*

Cryptococcus neoformans is een pathogene basidiomycete gist die voornamelijk infecties veroorzaakt bij mensen met een verstoorde afweer. Schattingen van de incidentie bij AIDS-patiënten lopen uiteen van 5% tot 30%, waarbij het hoogste aantal voorkomt in Afrika. Hoewel sexuele vermenigvuldiging is waargenomen in het laboratorium, is dit in de natuur nog niet aangetroffen. Derhalve veronderstelt men dat het pathogeen zich clonaal vermeerdert. Met behulp van AFLP en nucleotidensequenties van de InterGenic Spacer (IGS) van het rDNA kunnen zes genotypen worden onderscheiden. Deze resultaten ondersteunen de eerder gedane suggestie dat de soort uit ten minste twee soorten bestaat met elk drie genotypen. Deze genotypen vertonen aanmerkelijke verschillen in geografie en klinische incidentie, die mogelijk gerelateerd zijn met verschillen in virulentie. Bovendien vertoont één van de genotypen per soort een hybride AFLP-patroon, hetgeen wijst op het voorkomen van hybridizatie. Wij veronderstellen dat, naast clonale vermeerdering, (para)sexuele recombinatie een belangrijk onderdeel vormt in het voortplantingsrepertoire van de gist. Het directe evolutionaire voordeel voor de gist is dat op deze wijze erfelijk materiaal kan worden gerecombineerd, waarbij de nieuwe genotypen vervolgens clonaal worden verspreid.

B02
De WET op het WEB

E.M. Bik
Werkgroep Epidemiologische Typering (WET), Afdeling Medische Microbiologie en Immunologie, St. Antonius Ziekenhuis, Nieuwegein

Eind 1997 is de Werkgroep Epidemiologische Typeringen (WET) opgericht. Een van de belangrijkste taken van de WET is te inventariseren wie-waar-welke micro-organismen typeert, en deze informatie uitwisselbaar te maken. Inmiddels zijn van ca. 200 laboratoria gegevens verzameld en samengebracht in een database. Om deze informatie voor iedereen toegankelijk te maken, is begonnen met de constructie van een Internetpagina. Een voorlopige versie is te bekijken op www.gironet.nl/home/harbersg.

B03
Genomic typing by Amplified Fragment Length Polymorfism (AFLP) identified a cluster of *Bacillus cereus* infections in a neonatal intensive care unit (NICU) associated with balloons used in manual ventilation

W.C. van der Zwet[1], G.A. Parlevliet[1], P.H.M. Savelkoul[1], J. Stoof[1], A.M. Kaiser[1], A.M. van Furth[2], C.M.J.E. Vandenbroucke-Grauls[1]
[1]*Dept of Medical Microbiology and Infection Control,* [2]*Dept of Neonatology, University Hospital Vrije Universiteit, Amsterdam*

Bacillus species are aerobic spore-forming rods which are ubiquitous in nature. *Bacillus cereus* is increasingly recognized as an important opportunistic pathogen in premature neonates. An investigation was initiated when a cluster of serious *B. cereus* infections was identified in the first trimester of 1998 on the NICU of our hospital. Three neonates experienced serious infection, but in most cases neonates were asymptomatically colonized. Colonization with *B. cereus* occurred exclusively in the respiratory tract of mechanically ventilated neonates. Characterization by AFLP of strains isolated in infected neonates, as well as strains isolated in colonized neonates, showed that they were identical. A culture survey was conducted to identify environmental sources of *B. cereus* and carriers among the nursing staff. The percentage of carriers of *B. cereus* on the hands was significantly higher for the NICU than for other wards. Balloons used in manual ventilation that were regularly cleaned with detergent, appeared to be colonized with *B. cereus* strains, showing an identical AFLP-pattern. Sterilization of these balloons put an end to the outbreak.
B. cereus can cause serious infection in neonates and should not be dismissed as a culture contaminant. Colonization of neonates with this bacterium should be prevented. Therefore balloons used in manual ventilation should be free of *B. cereus* spores, which can only be achieved by sterilization. Genomic typing techniques like AFLP can provide good insight in the transmission routes and source of infection in infection control problems in hospitals.

B04
'Amplified Fragment Length Polymorphism' als identificatietechniek voor de 'core group' van *Neisseria gonorrhoeae* in de populatie van de Amsterdamse geslachtsziektenpolikliniek

J. Spaargaren[1], J. Stoof[2], J.S.A. Fennema[3], S. Bruisten[1], P.H.M. Savelkoul[2]
[1]*Streeklaboratorium voor de Volksgezondheid & Bijzonder Instituut voor Virologie, GG&GD, Amsterdam,* [2]*Medische Microbiologie en Infectiepreventie, Academisch Ziekenhuis Vrije Universiteit, Amsterdam,* [3]*Polikliniek voor Geslachtsziektenbestrijding, GG&GD, Amsterdam*

Uit het nationale surveillance-onderzoek van het RIVM blijkt dat de incidentie van gonorrhoe vanaf begin 1980 is gedaald als gevolg van een veranderd sexueel gedrag ten gevolge van de AIDS-epidemie en educatie op het gebied van veilige sex en condoomgebruik. Sindsdien is er sprake van een continue verspreiding bij een lage endemiciteit. Gepostuleerd wordt dat *gonorrhoe* zich op een dergelijk laag niveau kan handhaven als gevolg van het voortdurend onderhouden van overdracht binnen een zgn. 'high-risk core group', een groep individuen met een hoog frequent sexueel contact tussen de leden en een hoge prevalentie van gonorrhoe binnen die groep. Identificatie van deze 'core group' is in het kader van volksgezondheid en interventiemaatregelen van wezenlijk belang. Laboratoriumdiagnostiek van *Neisseria gonorrhoeae* berust op isolatie (kweek) en identificatie (GRAM-preparaat en biochemie). In het kader van epidemiologische studies zijn serotypering en auxotypering tot nu toe gebruikelijk. Deze methoden zijn echter arbeidsintensief en complex, ze hebben specifieke, goed gekarakteriseerde antisera nodig en ze discrimineren waarschijnlijk

onvoldoende. Tegenwoordig worden voor typeringsonderzoek in toenemende mate moleculair-biologische methoden gebruikt. In dit geval is 'Amplified Fragment Length Polymorphism'-analyse, een fingerprintmethode gebaseerd op amplificatie van delen van geïsoleerd genomisch DNA, toegepast voor *N. gonorrhoeae*. De AFLP- analyse van *N. gonorrhoeae* DNA heeft een goede reproduceerbaarheid (>90%) met een onderscheidend vermogen op stamniveau. Ongerelateerde *N. gonorrhoeae*-isolaten kunnen worden onderscheiden op basis van hun AFLP-patroon. Verschillende isolaten van één patiënt en isolaten van bekende contacten kunnen als identiek worden geïdentificeerd. In een prospectieve studie, waarbij gebruik wordt gemaakt van een uitgebreide gerichte enquete, zal op grotere schaal worden nagegaan of het mogelijk is om de leden van 'core group' te identificeren.

B05
Urogenital *Chlamydia trachomatis* serovars in men and women having either a symptomatic or an asymptomatic infection: an association with clinical manifestations?

S.A. Morré[1], L. Rozendaal[1], I.G.M. van Valkengoed[2], A.J.P. Boeke[2], C. van Voorst Vader[3], J. Schirm[4], S. de Blok[5], J.A.R. van den Hoek[6], G.J.J. van Doornum[6], C.J.L.M. Meijer[1], A.J.C. van den Brule[1]

[1]Dept of Pathology, Section of Molecular Pathology, University Hospital Vrije Universiteit Amsterdam, [2]Institute for Research in Extramural Medicine, VU, Amsterdam, [3]STD Clinic, Dept of Dermatology, University Hospital, Groningen, [4]Regional Public Health Laboratory, Groningen, [5]Dept of Obstetrics and Gynecology, OLVG Hospital, Amsterdam, [6]Municipal Health Service, Amsterdam

To determine whether certain *Chlamydia trachomatis* (CT) serovars are preferentially associated with a symptomatic or an asymptomatic course of infection, CT serovar distributions were analyzed in symptomatically and asymptomatically infected persons. Furthermore, a possible association between CT serovars and specific clinical symptoms was investigated. From 440 men and women serovars were determined. The most prominent differences found were: the association of serovar Ga with symptoms in men ($p=0.0027$) specifically dysuria ($p<0.0001$), and serovar Ia was detected more often in asymptomatically infected people (men and women) ($p=0.035$). Furthermore, in women serovar K was associated with vaginal discharge ($p=0.002$) and serovar variants were found only in women ($p=0.045$). The most prevalent CT serovars D, E and F showed no association with either a symptomatic or asymptomatic course of infection.

B06
Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system

J.L.W. Rademaker[1,2], B. Hoste[6], F.J. Louws[2,4,5], K. Kersters[7], J. Swings[6,7], L. Vauterin[8], P. Vauterin[8], F.J. de Bruijn[2,3,4]

[1]The Netherlands Culture Collection of Bacteria (NCCB), Utrecht, [2]MSU-DOE-PRL, [3]Microbiology Dept, [4]NSF-CME, MSU, East Lansing, USA, [5]Plant Pathology Dept, NCSU, Raleigh, USA, [6]BCCM(/LMG Bacteria Collection, [7]Laboratorium voor Microbiologie, Rijksuniversiteit Gent, Belgium, [8]Applied Maths BVBA, Gent, Belgium

The genus *Xanthomonas* is one of the largest groups of bacteria characterized by DNA-DNA homology studies and genomic fingerprinting. This genus has also been well characterized by complementary phenotypic and genotypic methods. Presently, a 70% total genomic DNA-DNA homology value represents an internationally accepted criterion to define bacterial species levels. However, the complexity of DNA-DNA reassociation kinetics methods precludes the rapid analysis of large numbers of isolates, which is imperative in bacterial classification and typing. In this study more facile PCR-based techniques, such as rep-PCR and AFLP genomic fingerprinting were compared to DNA-DNA hybridization studies. Similarity values of rep-PCR (using three different primer sets) and AFLP genomic fingerprint analyses were calculated and used to determine the correlation with corresponding DNA-DNA homology values. A high correlation was observed, highlighting that genomic fingerprinting techniques reveal taxonomic and (phylo)genetic relationships between bacterial organisms. Genomic fingerprinting analysis proved to be effective to classify and type xanthomonad strains.

C01
Legionnaires' disease: new insights and controversies

V.L. Yu

Pittsburgh, PA USA

Diagnosis of Legionnaires' disease requires application of specialized diagnostic testing that is often not routinely available in microbiology laboratories. When diagnostic methods are uniformly applied to all patients with pneumonia, *Legionella pneumophila* is the third or fourth most common cause of community acquired pneumonia in immunocompetent hosts. It can be the cause of up to 40% of hospital-acquired pneumonias if the water supply is contaminated.
New information will be presented on antibiotic therapy (erythromycin should no longer be used for Legionnaires' disease), utility of diagnostic testing (especially PCR and urine antigen), mode of transmission (aspiration is far more common than aerosolization), new extrapulmonary syndromes (prosthetic valve endocarditis and wound infection), new risk groups (nursing home patients, immunosuppressed pediatric patients), and importance of other new species in causing pneumonia (especially *L. dumoffii, L. feelei, L. micdadei*). Finally, we advocate routine environmental cultures of hospital water supplies as a screen for prevention of hospital-acquired Legionnaires' disease; public health authorities, especially US CDC, has strongly opposed such guidelines. Nevertheless, such guidelines are being adopted by individual state health departments in the US and elsewhere in the world.

C02
Aanwezigheid en gedrag van *Legionella*-bacteriën in (drink)waterinstallaties

D. van der Kooij

Kiwa NV/Wageningen Universiteit

Vanaf 1980 werden ook in ziekenhuizen in Nederland gevallen van legionellose waargenomen die een gevolg waren van besmetting met *Legionella* afkomstig uit het warmtapwatersysteem. Bij een landelijk onderzoek (1984-1985) bleek dat *Legionella* aanwezig was in het warmtapwatersysteem van 63% van 72 onderzochte ziekenhuizen. In 1986 adviseerde de Gezondheidsraad om de temperatuur van het warmtapwater in ziekenhuizen boven 60 °C te houden.
Legionella's stellen hoge eisen aan hun voeding; onder meer

is een tiental aminozuren, waaronder cysteïne, nodig voor vermeerdering. Groei in water is alleen mogelijk in aanwezigheid van andere bacteriën, algen en protozoa, die als gastheer kunnen dienen. Biofilmvorming, een watertemperatuur tussen 25 en 45 °C en stilstaand water vormen de voornaamste risicofactoren voor de groei van *Legionella* in (drink)waterinstallaties. Biofilmvorming wordt veroorzaakt door afbreekbare verbindingen afkomstig uit het water of uit (leiding)materialen. De biofilmvormingssnelheid van drinkwater is afhankelijk van de aard van de grondstof en de daarop toegepaste zuivering. (Leiding)materialen op kunststofbasis kunnen een sterke biofilmvorming veroorzaken, maar het meest toegepaste leidingmateriaal (hard PVC) is vrijwel niet groeibevorderend. Onder laboratoriumomstandigheden is voor (leiding)materialen een duidelijke relatie tussen biofilmvorming en groei van *Legionella* aangetoond. Beperking van de vorming van biofilm en sediment door selectie van materialen en goed ontwerp van waterinstallaties kan een wezenlijke bijdrage leveren aan voorkómen van *Legionella*-vermeerdering.

C03
Een epidemie van *Legionella*-pneumonie onder bezoekers van de Westfriese Flora in Bovenkarspel

J.W. den Boer
GGD Zuid-Kennemerland, Haarlem

In Nederland is *Legionella*-pneumonie sinds 1987 aangifteplichtig. Het aantal aangegeven gevallen is sindsdien stabiel: 40-45 per jaar. De helft van deze gevallen is geassocieerd met reizen naar het buitenland, 10-15% is nosocomiaal en de overigen zijn op zichzelf staande gevallen, ergens in Nederland opgelopen. De incidentie van 0,3 per 100.000 is laag vergeleken met andere Westerse landen. Een mogelijke verklaring is het gematigde Nederlandse klimaat, waardoor koeltorens zelden een bron zijn. Daartegenover staat dat in Nederland, in tegenstelling tot bijvoorbeeld de Verenigde Staten, slechts een deel van het drinkwater gechloreerd is. Uitbraken onder Nederlanders zijn sporadisch beschreven: tweemaal werd een grote groep hotelgasten in Zuid-Europa getroffen en tweemaal werd een grote groep ziekenhuispatienten getroffen. In de open bevolking traden tot 1999 geen epidemieën op, afgezien van een sauna-geassocieerde uitbraak in Haarlem, waarbij zes bezoekers *Legionella*-pneumonie opliepen.
In de tweede week van maart 1999 werden 12 patiënten met atypische pneumonie opgenomen in het Westfriese Gasthuis in Hoorn. Uit een oriënterende case-control studie, uitgevoerd door de Landelijke Coördinatiestructuur Infectieziektenbestrijding (LCI) en de GGD Westfriesland, kwam naar voren dat cases vaker dan controls de Westfriese Flora (WF) hadden bezocht. De WF is een jaarlijkse bloemententoonstelling, gecombineerd met een consumentenbeurs en een beurs voor agrarische producten, gehouden in de Veilinggebouwen van de Coöperatieve Nederlandse Bloembollenveiling (CNB) in Bovenkarspel (gemeente Stede Broec). Dit jaar werd de WF gehouden van 19 tot 28 februari. Op 13 maart hadden vijf patiënten een positieve urine-antigeentest voor *Legionella pneumphila* serogroep 1. Landelijk werd alarm geslagen, vooral gericht op adequate behandeling voor nog niet onderkende gevallen van *Legionella*-pneumonie. Het Rijksinstituut voor Volksgezondheid en Milieuhygiëne werd verzocht de bron van de epidemie op te sporen. Assistentie hierbij werd gevraagd aan het Streeklaboratorium voor de Volksgezondheid te Haarlem (SLHa) en de GGD Zuid-Kennemerland, vanwege bestaande expertise en lopende onderzoek naar *Legionella*-pneumonie.
Drie studies werden uitgevoerd: een microbiologisch onderzoek, een case-control studie en een cohort-studie. Uit het microbiologische onderzoek kwam naar voren dat 28 van 29 door een groot aantal Nederlandse microbiologische laboratoria verzamelde patiënten-isolaten genotypisch overeenkwamen met één van drie in drie apparaten aangetroffen omgevingsisolaten. De 29e kwam overeen met een ander omgevingsisolaat. Omgevingsisolaten werden door drie onafhankelijke laboratoria in filtermateriaal aangetroffen: Afdeling Medische Microbiologie van het AMC, Kwaliteitsinstituut voor Water (Kiwa) en SLHa. Genotypering (PFGE en ERIC/REP) werd uitgevoerd door het Laboratorium voor Infectieziekendiagnostiek en perinatale Screening (LIS/RIVM) en door het Streeklaboratorium voor de Volksgezondheid te Tilburg (AFLP). Uit interviews van GGD-artsen (in opdracht van de Inspectie Gezondheidszorg) en inspecteurs van de Inspectie Gezondheidsbescherming met standhouders bleek dat één van de met *Legionella* besmette whirlpools waarschijnlijk het meest aan verspreiding had bijgedragen.
De case-control studie werd uitgevoerd onder 153 cases en 400 controls uit de gemeente Stede Broec, waarbij de interviews van de cases (vragenlijst, schetsen van stands en een plattegrond van de WF) persoonlijk werden afgenomen door artsen en verpleegkundigen van 39 van de 53 GGD's. Met de drie gebruikte meetinstrumenten bleek steeds één whirlpool geassocieerd met een verhoogd risico op *Legionella*-pneumonie. Met andere apparaten was de associatie minder eenduidig.
De cohort-studie werd uitgevoerd onder 884 standhouders, vrijwilligers en werknemers van de WF. Van hen werd tweemaal bloed afgenomen waarin IgG en IgM werd bepaald. Door iedere 30 x 30 cm van de WF een kleur te geven, corresponderend met de gemiddelde antistoftiter (ELISA) van de 35 meest nabije standhouders, ontstond een vlekkenpatroon dat overeenkwam met de lokatie van de whirlpool die het meest geassocieerd was met risio op *Legionella*-pneumonie.
Alledrie de studies wijzen hetzelfde apparaat aan als bron voor deze epidemie. Regelgeving voor tentoonstelling van whirlpools is noodzakelijk om herhaling te voorkomen.

C04
Bacteriologische speurtocht naar de bron van de *Legionella*-epidemie te Bovenkarspel

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Legionella spp. zijn waterbacteriën en aangezien hun primaire habitat wordt gevormd door natuurlijke waterbronnen, behoort bij de concrete speurtocht naar de bron van een epidemie de hele waterketen te worden geanalyseerd. Deze waterketen bestond op de Westfriese Flora in Bovenkarspel uit meerdere schakels: *(ii)* het water aangeleverd via het waterleidingbedrijf (twee aanleverpunten), *(ii)* het lokale waterleidingnet met semipermanente uitbreidingen van polyethyleen, en *(iii)* de water gebruikende apparatuur op de consumentenbeurs en de decoratieve fonteinen. Voor het opsporen van de primaire bron zijn het aanvoerende en

lokale waterleidingnet uitgebreid bemonsterd. Voor het daadwerkelijk optreden van een infectie is echter meer nodig dan het uitsluitend aanwezig zijn van de bacterie: hij moet zich kunnen vermenigvuldigen tot grote aantallen en zich vervolgens middels aërosolen verspreiden. Hierop gebaseerd is een risico-analyse gemaakt van de gebruikte apparatuur. Na het opsporen van deze apparatuur zijn monsters genomen van eventueel aanwezig restwater, van biofilm en, indien van toepassing, van reinigingsfilters. Indien noodzakelijk geacht vond tevens monsterneming plaats na destructie. Watermonsters werden geconcentreerd d.m.v. filtratie. Wattendragers werden geresuspendeerd. Na voorbehandeling volgde enting op gesupplementeerde BCYE-platen. Bovendien werd op 10-20 ml restanten van de onbehandelde watermonsters d.m.v. een voor dit doel nog niet gevalideerde PCR-techniek naar DNA van *Legionella pneumophila* en *non-pneumophila* gezocht.
Alle kweken en PCR-onderzoeken van het aanvoerende en lokale waterleidingnet zijn bij herhaling negatief gebleven. Van de onderzochte apparatuur werden twee whirlpools en een groentenvernevelaar positief bevonden in de kweek en één van deze whirlpools tevens in de PCR. De geïsoleerde *L. pneumophila*-isolaten behoorden tot serotypen 1 en 6. Typering door PFGE en AFLP toonde aan dat binnen de serotype-1-bronisolaten twee genotypen konden worden onderscheiden. Eén genotype kwam overeen met de stam die bij het merendeel van de patiënten werd geïsoleerd. Het andere serotype-1-isolaat werd bij slechts één patiënt aangetroffen.

D01
Native valve endocarditis caused by *Staphylococcus lugdunensis:* the importance of an accurate identification of the microorganism

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Staphylococcus lugdunensis endocarditis is an important cause of destructive native-valve endocarditis frequently requiring valve replacement. An unexplained aspect in the presentation of a considerable number of these patients is a history of vasectomy in the immediate period before the onset of endocarditis. Here we present three cases of *S. lugdunensis* native-valve endocarditis. In two cases immediate cardiac surgery appeared to be necessary. One patient had a recent history of vasectomy. As the clinical outcome of disease often depends upon rapid valve replacement, adequate early identification of the microorganism is required distinguishing it from the often almost identical 'coagulase-negative' staphylococci.

D02
An outbreak of a cefotaxime, tobramycin and amikacin resistant *Klebsiella pneumoniae*

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On 14 August 1999 a patient from Warsaw, Poland was admitted for six days to the neurosurgical ward. Because the patient came from a foreign country, he was nursed in a separate room with Contact Precautions. A multiresistant (cefotaxime, tobramycin, gentamicin, amikacin, sulfa, trimethoprim) *Klebsiella pneumoniae* (MRKLPN) was cultured from a throat and rectal swab. Between 18 and 28 September four patients on the neurosurgical intensive care (NIC) and two former NIC patients on another intensive care were found positive. The NIC was closed for new patients. The hands of ten persons with patient contact were cultured; one nurse was positive. After intensive cleaning, the NIC was reopened at 9 October. Five days later a new patient on the NIC became positive, and the NIC was closed again. Hand cultures of 11 out of 37 persons were positive. Hand hygiene was reinforced, and it was ordered that all patient contact on wards with positive patients should be done with gloves. Thereafter hand cultures of three out of 52 persons were positive. Screening (rectal and throat swab) on wards where positive patients had been nursed yielded nine other patients; seven had been nursed on the NIC. Hand cultures from only two out of at least 800 persons not working on the NIC were positive. All positive patients were cohorted, with a dictated nursing staff. On 12 November the NIC was reopened. All MRKLPN had the same DNA fingerprint. They contained three different beta-lactamases: SHV-1, TEM-1 and CTX-M-3. The CTX-M-3 beta-lactamase, responsible for the cefotaxime resistance, was also found in *Escherichia coli, Enterobacter cloacae* and *Citrobacter freundii*. The sequence was identical to the beta-lactamase previously described in patients from a hospital in Warsaw, Poland (AAC 1998; 42:827-32). This strain was difficult to eradicate from hands and persisted in two nurses for several days, even after disinfection with isopropanol. Contaminated hands of personnel play an imported role in the spread of this MRKLPN. Strict use of gloves with every patient contact seemed to stop the outbreak.

D03
Onderzoek naar nosocomiale hepatitis-B-virustransmissie bij patiënten van een HBsAg-positieve arts op een afdeling gynaecologie

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N.a.v. een negatieve anti-HBs-respons op herhaalde hepatitis-B-vaccinatie werd de hepatitis-B-serostatus bepaald bij een arts die op dat moment een half jaar werkzaam was geweest op de afdeling gynaecologie. De arts bleek positief voor HBsAg, HbeAg en anti-HBc, en negatief voor anti-HBe. De viruslast, bepaald met PCR, was 4,5 x 10^9 genoomequivalenten per ml. De arts bleek voornamelijk verloskundige werkzaamheden te hebben verricht. De arts werd op non-aktief gesteld v.w.b. klinische werkzaamheden, en de Inspectie Gezondheidszorg werd geïnformeerd. Alle vrouwen (n=183) en kinderen (n=181) die met de arts contact hadden gehad, werd onderzoek aangeboden op HbsAg- en anti-HBc-antistoffen drie maanden na het risicocontact. De resultaten van het contactonderzoek, dat begin maart 2000 is afgerond, zullen worden gepresenteerd. Deze resultaten zullen bijdragen aan het inzicht in de risicofactoren voor nosocomiale hepatitis-B-virustransmissie.

D04
Vermindering van de gevoeligheid voor Augmentin: het vervolg

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Na introductie in 1992 voor behandeling van galweginfecties nam het gebruik van amoxicilline/clavulaanzuur (Augmentin) voor andere indicaties geleidelijk toe. In 1997 werd vrij plotseling een duidelijke afname van het aantal gevoelige *Escherichia coli*-stammen waargenomen (van 91% in 1996 naar 81% in 1997). Dit hing samen met een toename van het aantal intermediair-gevoelige isolaten. Ook bij *Klebsiella* spp. werden significant meer intermediair-gevoelige stammen gezien (van 7% in 1997 naar 19% in 1998). Als vervolg op de voordracht in 1998 worden de resultaten getoond van het onderzoek naar het achterliggende mechanisme. Fenotypische analyse vond plaats middels vergelijking van minimaal remmende concentraties van 32 geselecteerde isolaten (31 x *E. coli* en 1 x *Klebsiella pneumoniae*) voor amoxicilline/clavulaanzuur, amoxicilline, cefalotine, cefuroxim, ceftazidim en cefoxitine. CRG-richtlijnen werden als criteria gebruikt. Bij de meeste stammen paste het resistentiepatroon bij hyperproduktie van TEM-1 bèta-lactamasen, maar bij vier *E. coli*-stammen was sprake van chromosomale bèta-lactamaseproductie (derepressie van Amp-C-mutanten). In de voordracht zullen deze resistentiemechanismen bij amoxicilline plus clavulaanzuur worden toegelicht, alsmede de resultaten van de aanpak om het gebruik van Augmentin in de kliniek terug te dringen.

D05
Molecular diagnosis and typing of *Legionella (non-) pneumophila*

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Legionella is the etiologic agent of Legionnaires' disease and Pontiac Fever. Rapid diagnosis is essential to permit efficient treatment. We designed a highly sensitive PCR, which amplifies *Legionella* spp. DNA. By means of two specific probes, *L. pneumophila* is discriminated from *L. non-pneumophila*. We compared the performances of *Legionella* PCR, culture, serology and urine antigen detection. Bronchoalveolar lavage and sputum samples from more than 200 patients were tested with PCR. Sensitivities and specificities of the different diagnostic methods are shown. We also analyzed environmental samples from the *Legionella* outbreak in March 1999 after an exhibition of flowers and consumer goods in Bovenkarspel, the Netherlands. Although the PCR conditions are not yet optimized for analysis of environmental samples, one water sample from a whirlpool was PCR-positive. This result was confirmed by culture about six weeks later. We also developed an amplified fragment length polymorphism (AFLP) typing method for *L. pneumophila,* which could distinguish 21 different types among 108 Dutch *L. pneumophila* isolates. The method was applied on 67 isolates from patients and environmental samples of the exhibition to determine the source of infection. GelCompar analysis results of the AFLP patterns show that AFLP typing of *L. pneumophila* is highly reproducible, highly discriminatory, and applicable to outbreak investigation.

D06
Nocardiosis in Nederland: voorlopige bevindingen tot 1 januari 2000

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Sinds oktober 1998 zijn in Nederland in het kader van een Europese studie naar het voorkomen van infecties met *Nocardia* spp. 16 stammen verzameld. Het betrof 16 isolaten geïsoleerd bij 15 patiënten, vier vrouwen en 11 mannen in leeftijd variërend van 23 tot 88 jaar. Bij negen patiënten was de plaats van infectie de longen, inclusief isolaties uit pleuravocht. Bij vier patiënten was er sprake van een abces (2 x kaak, 1 x huid en 1 x prostaat). Bij één patiënt werd *Nocardia* uit bloed gekweekt en bij een ander uit gewrichtsvocht. In negen gevallen was er sprake van predisponerende factoren, in vijf overige gevallen konden deze niet worden aangetoond (één patiënt niet bekend). Chronisch longlijden al of niet in combinatie met het gebruik van corticosteroïden werd gerapporteerd bij zes patiënten. Verder betrof het een patiënt met een status na niertransplantatie, een patiënt met SLE en een patiënt die i.v. drugs gebruikte. Cotrimoxazol werd bij de behandeling het meest ingezet (8 x), gevolgd door een penicillinepreparaat (5 x). Een patiënt overleed aan de gevolgen van de infectie. Bij een patiënt (v) werd na een half jaar een relaps gezien. Gerapporteerde problemen betroffen m.n. identificatie en bepaling van de gevoeligheid.
Op basis van de (voorlopige) Europese gegevens blijkt Nocardiosis een zeldzaam voorkomende infectie te zijn, echter een onderrapportage of een niet herkennen van de aandoening wordt vermoed.

D07
Aanpassing van het antibiotica-doseringsregime op basis van pharmacokinetische en pharmacodynamische eigenschappen bij patiënten met ernstige infecties

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De effectiviteit van een behandeling met antibiotica wordt voor een groot deel bepaald door pharmacokinetische (Pk) en pharmacodynamische (Pd) eigenschappen van het middel, zoals AUC/MRC-ratio, duur antibioticumserumconcentratie > MIC en topspiegel/MIC-ratio. Van deze grootheden kan gebruik worden gemaakt bij het optimaliseren van de behandeling. Dit is met name van belang bij patiënten met ernstige infecties. De Pk en Pd kan bij deze patiënten zodanig afwijken van de norm, dat doseringsaanpassingen nodig zijn.Bij een beademde ICU-patient met een gestoorde Pk en een luchtweginfectie met een multipele resistente *P. aeruginosa* werden op basis van bovenstaande uitgangspunten de doseringsschema's van de toegediende antibiotica met succes aangepast.

E01

Acute onset of type 1 diabetes mellitus caused by severe echo virus type 9 infection: investigation of putative pathogenic pathways

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Enteroviruses are blamed for causing type 1 diabetes mellitus, either directly by invasion of pancreatic islets, or indirectly by triggering onset of autoimmunity. We did analyse the role of an ECHO virus type 9 (EV9) which was isolated from a six weeks old girl, coincident with acute onset of diabetes. The girl, who was healthy before, presented with gastro-enteritis, complicated by meningo-encephalitis and hepatitis but without signs of pancreatitis or pre-existing diabetes (normal values for serum amylase and HbA1c). A day after admission she appeared completely insulin dependent. HLA typing revealed alleles HLA DQ8 and DR4 with high risk for diabetes.

The EV9 did not grow in human b-cell cultures from different donors. The viral protein 2C contained the PEVKEK sequence that shares homology with glutamate decarboxylase (GAD65). However, neither lymphocytes nor serum did react with GAD65 or the homologous peptides. Antibody against islet cell antigens (ICA) was repeatedly absent and cellular reactivity was found only against islet antigen IA-2 and against enterovirus antigens. Hence, there was no evidence for EV9 infection of the pancreas, nor for pre-existing autoimmunity against ICA. Therefore, the acute loss of b-cells during infection with EV9 must have been mediated by indirect mechanisms, possibly bystander killing through circulating cytokines.

E02

Influenza B virus in seals

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Influenza B virus is a pathogen found exclusively in humans. Its origin and possible reservoir in nature are not known. We here show the isolation of influenza B virus from a naturally infected harbour seal (Phoca vitulina).

The infected seal and a second seal, both of which had been admitted to a seal rehabilitation centre in the Netherlands, seroconverted to influenza B virus. Sequence analyses of the haemagglutinin (HA) and non-structural (NS) genes as well as serology, indicated that influenza virus is closely related to strains that have circulated in humans four to five years earlier. Retrospective analyses of sera collected from 971 seals stranded at the Dutch coast showed a prevalence of antibodies to influenza B virus in approximately two percent of the animals admitted after 1995, and in none before 1995. This is the first report on natural influenza B virus infection emerging in a non-human species. The data document that influenza B virus can be maintained in seals. This reservoir, harbouring influenza B viruses that have circulated in the past, may pose a direct threat to humans in the future.

E03

Interactions *in vivo* between the proteins of infectious bursal disease virus: capsid protein VP3 interacts with the RNA dependent RNA polymerase, VP1

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Little information is known about the intermolecular interactions between the viral proteins of infectious bursal disease virus (IBDV). By using the yeast two-hybrid system, which allows the detection of protein-protein interactions *in vivo*, all possible interactions were tested by fusing the viral proteins to the LexA DNA-binding domain and the B42 transactivation domain. A heterologous interaction between VP1 and VP3, and homologous interactions of pVP2, VP3, VP5 and possibly VP1, were found by co-expression of the fusion proteins in *Saccharomyces cerevisiae*. The presence of the VP1-VP3 complex in IBDV-infected cells was confirmed by co-immunoprecipitation studies. Kinetic analyses showed that the complex of VP1 and VP3 is formed in the cytoplasm and eventually is released into the cell-culture medium, indicating that VP1-VP3 complexes are present in mature virions. In IBDV-infected cells, VP1 was present in two forms of 90 kDa and 95 kDa. Whereas VP3 initially interacted with both the 90 kDa and 95 kDa proteins, later it interacted exclusively with the 95 kDa protein both in infected cells and in the culture supernatant. These results suggest that the VP1-VP3 complex is involved in replication and packaging of the IBDV genome.

E04

Yellow fever virus as an expression vector for the hepatitis C virus envelope proteins

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Hepatitis C virus (HCV) is the major causative agent of non-A, non-B hepatitis. Infections with this enveloped, positive-stranded RNA virus are found throughout the world. The infections are usually chronic and are associated with the development of cirrhosis and hepatocellular carcinoma. Despite the rapid progress in our understanding of the structure and expression of the HCV genome, the study of this virus is still seriously hampered by the lack of an efficient cell culture system. We aim to circumvent this problem by constructing chimeras of HCV and the related yellow fever virus. These chimeric viruses express the HCV structural proteins for the assembly of the viral nucleocapsid and envelope, but are depending on the non-structural proteins and replication signals of the related yellow fever virus (YFV) for their RNA amplification.

Several full-length HCV/YFV chimeric cDNAs, in which the YFV structural genes were exchanged for their HCV counterparts, were constructed. RNA transcribed from these plasmids was electroporated into BHK-21 cells. These cells were analyzed for viral RNA synthesis and protein expression. Efficient expression and processing and a correct sub-cellular localization of the HCV envelope proteins was observed in the transfected cells. Immunoprecipitation with a conformation sensitive monoclonal antibody indicated that proper maturation, folding and oligomerization of the HCV glycoproteins

occurred in this expression system. The HCV/YFV chimeric transcripts could be rescued from cells co-electroporated with YFV-17D RNA, which opens interesting possibilities for HCV vaccine development. Further studies of these chimeras, including the analysis of the potential production of HCV-like particles, are in progress.

E05
Disease association, transmission and natural history of HHV8/KSHV infection in the Amsterdam Cohort Studies on HIV infection and AIDS

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Background: The unequal distribution of Kaposi's Sarcoma (KS) among HIV-1 transmission groups suggests that AIDS-KS is caused by a sexually transmitted infection. Human herpesvirus 8 (HHV-8) has since been isolated and detected in all AIDS-KS biopsies. The following three issues have been examined in two prospective studies on HIV infection and AIDS; *(i)* the criteria for disease causation, *(ii)* the mode of transmission and *(iii)* the relationship between seroconversion to HHV8 antigens and primary infection.
Methods: *(i)* The Amsterdam Cohort Studies on HIV infection and AIDS started in 1984 for homosexual men and in 1985 for injecting drug users. Serum samples from 1,459 homosexual men and 1,167 drug users were tested for antibodies to recombinant HHV8 lytic-phase capsid (ORF65) and latent phase nuclear (ORF73) antigen using an Enzyme Immuno Assay (EIA) format. Individuals were retrospectively identified as HHV8-positive, HHV8-negative or HHV8 seroconverter. Incidence and prevalence data were calculated and hazards ratios were estimated. *(ii)* Risk factors for HHV8 seropositivity were assessed at study entry. Recent sexual behaviour and HHV8 seroconversion was examined in a nested case-control study. *(iii)* Antibody levels were compared using serum/cut-off (S/C) ratios and the impact of HIV investigated. HHV8 DNA in serum was detected by nested PCR.
Results: *(i)* The incidence of HHV8 seroconversion among drug users was 0.7 per 100 person-years based on 31 seroconversions, whereas an incidence of 3.6 was found among homosexual men based on 215 seroconversions. The hazard ratio for Kaposi's sarcoma was 3.15 (95% CI: 1.89-5.25) in HIV-infected individuals if HHV8 antibodies were present either at enrolment or at HIV seroconversion. In HIV-infected persons who later seroconverted to HHV8, Kaposi's sarcoma developed more rapidly: hazard ratio of 5.04 (95% CI: 2.94-8.64), an additional risk of 1.60 (95% CI: 1.01-2.53; P=0.04). *(ii)* HHV8 seroprevalence at study entry was 20.9% (305/1458), was highest among those with positive HIV status, no steady partner, Southern European or Latin American nationality, and increased with older age and higher number of sexual partners. Independent predictors for HHV8 seroconversion included; oro-genital insertive sex (OR: 5.95, 95% CI: 2.88-12.29) or oro-genital receptive sex (OR: 4.29, 95% CI: 2.11-8.71) with more than five partners in the past six months, older age (OR: 2.89, 95% CI: 1.13-7.34, when older than 45 years) and preceding HIV infection (OR: 2.47, 95% CI: 1.53-3.99). *(iii)* In HIV-infected, but not in uninfected, individuals, seroconversion to ORF73/LANA precedes that to ORF65/vp19. Antibody levels to both ORF65- and ORF73-encoded antigens were higher in HIV-infected than in HIV-uninfected men, and among HIV-seropositives, antibody levels to ORF65/vp19 rose even higher with declining $CD4^+$ cell counts and peaked with KS development. In 10.3% of HHV8 seroconversions, transient serum viraemia could be demonstrated before or at seroconversion.
Conclusions: *(i)* The distribution of HHV8 antibodies confirms a causal role for HHV8 in AIDS-KS. *(ii)* There is strong evidence that HHV8 is transmitted by oro-genital sex among homosexual men. *(iii)* HIV infection boosts HHV8 antibody levels. Serum viraemia is short-lived. These HHV8 seroconversions are consistent with primary infection.

E06
Receptor interaction and membrane fusion activity of alphaviruses

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Alphaviruses, such as Semliki Forest virus (SFV) and Sindbis virus (SIN), are enveloped positive-strand RNA viruses. SFV and SIN infect their host cells via a membrane fusion reaction, through which the viral genome gains access to the cell cytosol. It is well established that the cell entry mechanism of SFV involves uptake of virus particles by receptor mediated endocytosis and subsequent fusion of the viral envelope with the membrane of the endosomal cell compartment, induced by the acidic pH within the lumen of the endosome. For SIN, there has been controversy about the route of cell entry, several published observations suggesting that this virus may fuse directly with the plasma membrane. In a model system, both SFV and SIN fuse efficiently with liposomes, fusion occurring on the time scale of seconds. The kinetics of these fusion processes can be followed conveniently on the basis of dilution of fluorescent pyrene-phospholipids from biosynthetically labeled virions into the target liposomes. For both SFV and SIN, fusion is strictly dependent on a mildly acidic pH (~ pH 5.0), strongly suggesting that that not only SFV but also SIN enters cells through receptor-mediated endocytosis and fusion from within acidic endosomes. In the virus-liposome fusion process, the liposomes do not contain a protein or carbohydrate receptor, virus-receptor interaction is not a mechanistic requirement for the membrane fusion activity of SFV and SIN. Furthermore, similar fusion characteristics were observed with SIN variants adapted to different extents to the glycoaminoglycan receptor heparan sulfate, indicating that the initial virus-receptor interaction does not influence the subsequent fusion process. On the other hand, fusion does require the presence of cholesterol and sphingolipids in the liposomes. Cholesterol is primarily involved in low-pH-dependent binding, whereas sphingolipid or a complex of sphingolipid and cholesterol is required for the fusion process itself. At low pH, the E2/E1

heterodimeric envelope glycoproteins of SFV and SIN dissociate with formation of a trypsin-resistant E1 trimer. The E2/E1 heterodimer dissociation is necessary for the induction of fusion of SFV or SIN with the liposomal membrane, fusion being inhibited when the viral spike protein consists of a p62/E1 heterodimer, in which p62 is the uncleaved precursor protein of E2, stabilizing the heterodimer.

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E07
The complete DNA sequence of the genome of rat cytomegalovirus

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We have determined the complete genome sequence of the Maastricht strain of rat cytomegalovirus (RCMV). The RCMV genome has a length of 229,896 basepairs (bp), and is arranged as a single sequence flanked by 504-bp terminal direct repeats. RCMV was found to have counterparts of all but one of the open reading frames (ORFs) that are conserved between murine CMV (MCMV) and human CMV (HCMV). Like HCMV, RCMV lacks homologs of the glycoprotein genes belonging to the MCMV m02 gene family. However, RCMV contains 15 ORFs with homology to members of the MCMV m145 glycoprotein gene family, which may function in viral evasion from the host's immune system. A tandem array of four m145-like ORFs (r70.2, r70.3, r70.4 and r70.5) was identified at a nonconserved position within the RCMV genome, between ORFs R70 and R72. Four RCMV ORFs are predicted to encode homologs of host proteins; R33 and R78 both putatively encode G protein-coupled receptors, whereas r144 and r131 encode homologs of major histocompatibility class I heavy chains and CC chemokines, respectively. An intriguing feature of the RCMV genome is the presence of an ORF, r127, with similarity to the rep gene of parvoviruses as well as the U94 ORF of humal herpesvirus type 6A (HHV-6A) and HHV-6B. Counterparts of these ORFs, which might play a vital role in viral replication, are not present in the other sequenced herpesviruses.
Despite several unique characteristics, we conclude that the genome of RCMV is highly similar, both in gene content as well as overall structure, to that of MCMV as well as HCMV.

F01
Molecular physiology of Arc-dependent regulation of catabolism in *Escherichia coli*

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As an enteric bacterium *Escherichia coli* hardly ever experiences fully aerobic conditions. In contrast, it is frequently subjected to fluctuating semi-aerobic conditions. Therefore, the organism benefits from a high flexibility to adapt its metabolic machinery, to make optimal use of the available oxygen.
The regulation of the synthesis of key enzymes in aerobic/anaerobic catabolism is under control of a complex network that includes the ArcAB two-component-regulatory system, responding to variations in oxygen availability. However, molecular oxygen is not a direct signal, triggering activation of that system. The molecular nature of the signal that does trigger the ArcAB phosphorylation cascade has not been resolved yet.
In order to assess the physiological role of the ArcAB system, in part through identification of this signal, we analyzed the effect of oxygen supply to steady state chemostat cultures of wild type *E. coli* and of a mutant that lacked the ArcA regulator. Distribution of catabolic fluxes over parallel pathways, changes in redox state (as reflected by the NADH/NAD ratio), ArcAB-dependent gene expression and relative activity of the Arc system (as reflected by ArcA phosphorylation levels and analyzed by a reporter construct) were investigated through the entire microaerobic range, from fully aerobic to fully anaerobic conditions.

F02
Bio-imprinting of the epoxide hydrolase from *Rhodotorula glutinis*

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Epoxide hydrolases are present in all kind of life forms, ranging from bacteria to mammals. These enzymes hydrolyze carcinogenic epoxides to less harmful diols. Enantiopure epoxides are important building blocks in the pharmaceutical and agrotechnical industry. Many production processes lead to unwanted racemic mixtures unless expensive and environmentally hazardous catalysts are used. Epoxide hydrolases are versatile enzymes to be used as biocatalyst as a friendly way to produce enantiopure epoxides without the use of i.e. heavy metal-based catalysts. The yeast *Rhodotorula glutinis* contains an enantioselective epoxide hydrolase that converts a wide range of epoxides. This enzyme is partly purified. Unfortunately, the stability and enantioselectivity decreased during storage. To improve selectivity and stability, the effects of imprinting and/or polymerization of EH were analyzed. Before polymerization, the enzyme is kept in an active and enantioselective conformation by incubating the enzyme with imprinting molecules, which can be substrates or products. When the enzyme is subsequently polymerized, the forced conformation is locked in the polymer. This imprinted polymer is more stable and enantioselective compared to the free enzyme.

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F03
Are ectomycorrhizal fungi of importance for wet Alder carr forests?

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Ectomycorrhizal fungi usually live with their host trees in balanced, intimate associations from which fungi and trees derive benefit. The major functions that are contributed to ectomycorrhizal fungi are uptake of water and nutrients of which nitrogen is the most important. This has mainly been shown for ectomycorrhizal fungi associated with tree species growing in dry and nitrogen-poor soils. However, our knowledge about the diversity and functional role of

ectomycorrhizal fungi in wet forests is limited. In a project that was started in 1999, wet Alder carr forests were taken under study. Diversity of ectomycorrhizal fungi is relatively high in wet Alder carr forests which is remarkable considering that sufficient water and nutrients, particularly nitrogen, are available for the Alder trees. The first step in this project is to determine the diversity of the ectomycorrhizal community in wet Alder carr forests in a peatland and in a river valley. Sporocarp surveys are carried out to describe the above-ground ectomycorrhizal community, and PCR-based techniques are used to identify the below-ground ectomycorrhizal community. In addition, abiotic factors such as chemical composition of soil moisture are determined. Preliminary results on the ectomycorrhizal diversity in the wet Alder carr forests will be presented.

F04
Substrate binding and selectivity of the 2-hydroxy-carboxylate transporter family

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Secondary transporters from the 2-HCT family transport compounds containing a 2-hydroxycarboxylate (2-HC) motif, HO-CR2-COO^-. CitS of *Klebsiella pneumoniae* transports citrate in symport with Na^+ ions and functions in anaerobic citrate degradation. The citrate transporter CitP of the *Leuconostoc mesenteroides* and the malate transporter MleP of *Lactococcus lactis* function as precursor/product exchangers that catalyze the uptake of a substrate into the cell coupled to the exit of a metabolic endproduct. The $citrate^{2-}$/$lactate^-$ and $malate^{2-}$/$lactate^-$ exchange catalyzed by CitP and MleP, respectively, is electrogenic and plays a role in metabolic energy generation and in low pH resistance of the lactic acid bacteria. The ability of the precursor/product exchangers to transport different substrates with related chemical structure but different charge implies specific requirements for the substrate binding site of the transporters. The aim of my PhD study was to identify the interactions between substrate and protein providing affinity and specificity and to identify amino acid residues on the proteins that interact with the substrates.
The strategy was to first characterize the different transporters with respect to substrate affinity and specificity, and then to use the kinetic characteristics to analyze site directed mutants and chimeras to identify the substrate binding site. The ability to exchange two substrates with related structure and different charge was associated with a high specficity for the 2-hydroxy-carboxylate motif and a low specificity for the two R-groups. CitP and MleP transported next to their physiological substrate a wide range of 2-hydroxycarboxylates with R-group ranging from hydrogen atoms to a phenyl group. In contrast, the Na^+ symporter CitS was highly specific for citrate. Based on the affinity and translocation properties of the exchangers with different substrates a model of the binding site of CitP and MleP was made. For both transporters the model contained an interaction with the hydroxyl and carboxylate of the substrate motif which is essential for binding. An additional electrostatic interaction with one of the R groups results in high affinity binding when the R group contains a carboxylate group and low affinity when the R group is neutral. This interaction is also responsible for the highly stereoselective binding of dicarboxylates. Differences in substrate specificity of CitP and MleP were due to differences in maximal rates rather than affinity.
Analysis of chimeras of CitP and MleP revealed transporter specific interactions between amphipatic helix XI and the R-groups of the substrates, suggesting that this part of the protein is involved in the binding pocket. Subsequently, conserved residues in this region were mutagenized to find the interactions with the substrates, as proposed in the model of the binding site. Arg425 was found to be the residue responsible for the high affinity binding of the carboxylate containing R-group. Transport activity in Arg425Cys and Arg425Lys mutants could be inhibited by cysteine and lysine specific reagents. Protection of labeling was observed in the presence of substrates, which provides further evidence for the localization of Arg425 in the binding site.

F05
Peroxisome degradation in the yeast *Hansenula polymorpha*

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Peroxisomes are cell organelles that are found in virtually all eukaryotic cells. They are involved in a great diversity of metabolic processes, ranging from photorespiration in plants, cholesterol metabolism in man to the metabolism of certain carbon and nitrogen sources (e.g. methanol, fatty acids, primary amines) in yeasts. In the yeast *Hansenula polymorpha* peroxisomes rapidly develop upon transfer of glucose-grown cells to methanol-containing media. Under these conditions the organelles harbour enzymes involved in the initial steps of methanol-metabolism. The addition of glucose to methanol-grown cells results in the opposite process, namely selective peroxisome degradation. This process includes three distinct steps: *(i)* sequestration of the organelle to be degraded by membrane layers, *(ii)* fusion of the sequestered compartment with the vacuole and *(iii)* degradation of the organelle by vacuolar enzymes. We have isolated *H. polymorpha* mutants that are defective in selective peroxisome degradation (pdd mutants) and cloned the corresponding genes (PDD). These studies revealed that some PDD genes are also required for other cellular processes (e.g. non-selective autophagy, vacuolar protein sorting, endocytosis), whereas others are specifically involved in selective peroxisome degradation.

F06
Influence of oxygen partial pressure (pO_2) and salinity on the community shift of the ammonia oxidizing bacteria in the river Schelde

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The river Schelde is an eutrophic estuarine system. Parallel to the increase of oxygen and salinity and the decrease of the ammonia concentration a shift of the dominant ammonia-oxidizing bacteria was observed. Molecular analysis showed that the freshwater part was dominated by *Nitrosomonas* sequences, which are closely related to *Nitrosomonas urea*. In the brackish part sequences of a novel group of *Nitrosomonas*-like bacteria were found.[1] Continuous and batch cultures were used to investigate the reason for the

population shift. Water from the freshwater part was incubated under three different conditions: low and high pO_2, both without salt addition, and high pO_2 with salt addition. Ammonia consumption in all chemostats started at once. The chemostat that was incubated at low pO_2 showed lower ammonia consumption than the other two chemostats, indicating that ammonia oxidation was oxygen-limited. Batch incubations showed higher ammonia oxidation rates in the absence of salt. Molecular analysis resulted in a clear difference between the presence and absence of salt, indicating that salt is the factor which causes the population shift of the ammonia-oxidizing bacteria.

1. De Bie et al., Limnology & Oceanography submitted

F07
Combination of partial nitrification and anaerobic ammonium oxidation for the removal of ammonia from concentrated wastewater

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A combination of two novel systems for the removal of ammonia from wastewater is currently investigated in Delft. The SHARON process is ideally suited to convert ammonia into nitrite in waste streams with high ammonia 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 plants for the treatment of sludge digester effluents.
The ANAMMOX process is a novel process in which ammonia and nitrite are directly converted into dinitrogen gas, with hydrazine as an important intermediate. The dominant bacterium in the Anammox bioreactors was recently identified on the basis of 16 S rRNA analysis, and grouped deep inside the Order *Planctomycetales*. The key physiological parameters of the Anammox bacteria have been determined. The combination of the Sharon and Anammox processes remains a challenge for future application in the removal of ammonia from wastewater.

G01
Een tropische verrassing in een Hollandse neonaat

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Een zwangere vrouw van 31 jaar, sinds 1997 bekend met colitis ulcerosa (CU) en sinds zes weken bekend met placenta praevia, werd in mei 1999, bij 32 weken zwangerschap, opgenomen wegens ernstig vaginaal bloedverlies, waarvoor keizersnede werd verricht. Er werd een meisje geboren van 1850 gram met Apgar-scores van 2, 8 en 9 na respectievelijk 1, 5 en 10 minuten. De tweede levensdag werd het kind verdacht van sepis en pneumonie. Een X-thorax toonde een infiltraat rechts. Er werd gestart met i.v. amoxicilline en gentamicine. De volgende dagen traden verslechtering en problemen met de beademing op. Op de X-thorax werden bilateraal multipele abcessen gezien. Uit bloed- en sputumkweek werd een *Burkholderia pseudomallei* geïsoleerd. Bij navraag bleek de moeder tijdens de zwangerschap drie weken op vakantie in Thailand te zijn geweest, met aldaar een exacerbatie van de CU waarvoor zij 20 mg/dg prednison kreeg. Het kind werd verder behandeld met ceftazidime 150 mg/kg/dg gedurende acht weken, waarbij geleidelijk herstel optrad, gevolgd door zes weken Augmentin® p.o. Bij de moeder werden twee weken postpartum keel, urine, faeces en cervix op *B. pseudomallei* gekweekt. Uit de cervix werd *B. pseudomallei* geïsoleerd met een PFGE-patroon overeenkomstig aan dat van de isolaten bij het kind. Serologisch onderzoek op anti-*B. pseudomallei* antistoffen bij moeder en vader was positief bij de moeder. Bij revisie van het histologisch onderzoek van de placenta werden micro-abcessen gevonden.
Concluderend was er sprake van melioidose tijdens de zwangerschap, gevolgd door een neonatale melioidose. Intra-uteriene transmissie van *B. pseudomallei* is waarschijnlijk opgetreden vanuit de placenta.

G02
Ovine *Chlamydia psittaci:* een belangrijke oorzaak van abortus bij schapen en een gevaarlijke ziekteverwekker voor zwangeren

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Infecties met *Chlamydia psittaci* worden meestal geassocieerd met vogels. Echter ook zoogdieren kunnen een bron zijn, met name schapen. In Nederland is *C. psittaci* de tweede oorzaak van abortus bij schapen. Hoewel deze stammen minder virulent zijn dan vogelstammen, zijn ernstige septische beelden beschreven bij zwangeren, hetgeen waarschijnlijk samenhangt met het tropisme van ovine stammen voor trofoblastcellen. Recent zagen wij een dergelijke infectie bij een 26 weken zwangere vrouw. Zij was werkzaam op een schapenboerderij en had geholpen bij het lammeren. Een toename van het aantal doodgeboren lammeren was opgevallen. Daarnaast waren er schapen met verschijnselen van meningitis (listeriose?).
De vrouw presenteerde zich met koorts, buikpijn en hoofdpijn. In verband met verdenking op een acute buik werd een laparotomie verricht waarbij geen afwijkingen werden gezien. De volgende ochtend ontwikkelde ze het beeld van een septische shock en een ernstige ARDS waarvoor intubatie met beademing in buikligging en ondersteuning met inotropica noodzakelijk waren. Na enkele uren beviel ze van een doodgeboren kind. In eerste instantie werd gedacht aan listeriose. Ondanks vijf dagen behandeling met Augmentin en ciprofloxacin behield ze aanhoudende hoge koorts. Na toevoeging van doxycycline normaliseerde de temperatuur binnen twee dagen en kon ze worden gedetubeerd. Bloedonderzoek liet een seroconversie voor *Chlamydia*-antistoffen zien (CBR en IgG, IgA en IgM antistoffen in een commerciële EIA). De placenta vertoonde een kenmerkende ernstige intervillitis en was positief voor *Chlamydia*-antigenen (immuno-histochemie) en *C. psittaci* DNA (PCR).

G03

Evaluation of the *Helicobacter pylori*-Specific-Antigen (HpSA) test to assess *Helicobacter pylori* infection in children

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Helicobacter pylori infection is the major cause of gastritis and duodenal ulcer disease. Most *H. pylori* infections are acquired in childhood. Histology and culture of gastric biopsy specimen is the 'gold standard' to assess *H. pylori* infection in adults. In children gastroscopy is too distressing. Recently, the HpSA test, measuring *H. pylori* antigens in the faeces of *H. pylori* infected patients, proofed to be accurate in adults. The aim of this study was to evaluate the HpSA test for the assessment of *H. pylori* infection in children. Eighty-one children (median age: 9 years, range 1-18.5 years), referred for upper GI endoscopy, were eligible to enter this study. Patients were excluded if they had used bismuth, antibiotics or a proton pump inhibitor in the month prior to endoscopy. Gastric biopsy specimens were taken for the assessment of *H. pylori* by culture and histopathology. *H. pylori* infection was considered if histopathology or culture of one of the biopsy specimens was positive. Patients were considered *H. pylori* negative if both histopathology and culture were negative. A stool sample of each child was obtained within two days before or after gastroscopy and stored at -20 °C until analysis. *H. pylori* antigens in the patient's faeces sample were assessed by Premier Platinum HpSA® ELISA (Meridian Diagnostics Inc, Cincinnati, OH) according to the manufacturer's protocol. Of the 81 children, 27 were colonised by *H. pylori* and 51 were *H. pylori* negative. The sensitivity and specificity of the HpSA test were 100% (27/27) and 93% (50/54), respectively. Positive predicting value and negative predicting value were 87% and 100%, respectively. In conclusion: The non-invasive HpSA faeces test is a reliable test to assess *H. pylori* infection in children.

G04

De Triple Faeces Test (TFT) en ELISA bij de laboratoriumdiagnose van Giardiasis; hoe meerdere wegen naar Rome kunnen leiden

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Doel: Het vergelijken van de sensitiviteit en specificiteit van drie commercieel verkrijgbare ELISA-tests voor de detectie van *Giardia lamblia* met die van de Triple Faeces Test (TFT), een nieuwe, recent geïntroduceerde microscopische detectiemethode.
Design: Prospectieve studie.
Methoden: Onderzoek werd verricht bij 171 achtereenvolgende patiënten uit de routinepraktijk van het AMC. Microscopisch onderzoek werd uitgevoerd met de Triple Faeces Test, waarbij faeces van drie achtereenvolgende dagen microscopisch werd onderzocht met een combinatie van een permanente kleuring en een concentratietechniek. Voor de immunochemie werd elk monster getest met drie ELISA's: Prospect Giardia Alexon, Giardia test Techlab en Giardia Trinity Biotech. Analyse van de ELISA's en TFT werden onafhankelijk van elkaar uitgevoerd in respectievelijk Haarlem en Amsterdam.
Resultaten: *G. lamblia* werd microscopisch aangetoond in 9 van de 171 TFT's (5,3%). Bij één van deze 9 patiënten werd in alledrie de monsters van de TFT de parasiet gedetecteerd. Met de Techlab en Trinity ELISA-tests werden respectievelijk 26 en 51 patiënten positief bevonden, bij 7 was ook de microscopie positief. Bij de Prospect Giardia-test werd bij 10 van de 171 TFT's een positief resultaat gevonden, bij 8 werd de diagnose microscopisch bevestigd.
Conclusies: Twee van de drie ELISA's gaven een hoog percentage vals-positieve uitslagen. De Alexon ELISA had een hoge sensitiviteit en specificiteit die goed correleerde met die van de TFT.

G05

Serological screening for antibody response to the CagA virulence factor of *Helicobacter pylori:* validation of an ELISA based test

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The clinical outcome of *Helicobacter pylori* infection is related to the presence of the cagA virulence factor. Patients with peptic ulcer disease (PUD) are more likely to be infected with cagA positive *H. pylori* strains. The cagA gene encodes for a highly immunogenic protein (CagA). Antibodies against CagA can be used to predict the presence of the cagA gene. Cover et al. described a technique, using a recombinant antigen (Orovax), to detect these antibodies (Cover TL, J Clin Microbiol 1995;33(6):1496-1500). However, this test was validated in a small Belgian population. To assess the accuracy of this test in a Dutch population, biopsy specimens and sera of 142 patients undergoing gastroduodenoscopy were collected. *H. pylori* status was determined by histology and/or culture. Patients were considered *H. pylori* infected if histology or culture were positive. The presence of the cagA gene was determined by a PCR based LiPA procedure. Sera were tested by the technique described by Cover for the detection of CagA antibodies. Using the optimal cut-off (OD > 0,3 = positive), sensitivity and specificity were 75.5% (95% CI 61.72 - 86.24) and 97.8% (95% CI 92.12 - 99.73), respectively. In our population, the technique of Cover et al. combines a high specificity with a rather low sensitivity. This test is useful in detecting the presence of CagA antibodies. A negative test however can not exclude an infection with a cagA positive strain.

G06

No evidence for multiple strain infections in patients carrying both metronidazole susceptible and resistant *Helicobacter pylori*

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Susceptibility testing for metronidazole in *H. pylori* sometimes reveals the presence of both susceptible and resistant bacteria

within one clinical isolate. This phenomenon could be explained by the presence of multiple strains infecting one patient. To investigate this hypothesis, biopsy specimens of nine patients known to harbour both susceptible and resistant *H. pylori* were cultured under standard conditions. After three days of incubation ten colonies of the primary culture of each biopsy specimen were randomly selected and sub-cultured. The offspring of each single colony was tested for metronidazole susceptibility (E-test) and genotyped by Restriction Fragment Length Polymorphism (RFLP) of the ureC gene and Random Amplified Polymorphic DNA (RAPD) of the entire genome. According to the RAPD and the RFLP profiles, eight biopsy specimens contained one single strain and one biopsy specimen contained two different strains. In the multiple strain harboring isolate, susceptible as well as resistant colonies were found in both strains. The presence of metronidazole susceptible and metronidazole resistant bacteria in the same clinical isolate more likely reflects mutations in a single strain rather than a multiple strain infection.

G07
Evaluation of three peptide-based EIA assays for the diagnosis of tubal factor infertility caused by *Chlamydia trachomatis*

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In the present study, we evaluated three peptide-based EIA assays (*C. trachomatis* EIA, Labsystems, SeroCT, Savyon and pELISA, Medac, Germany), intended for the routine serological diagnosis of Ct infections. All tests were evaluated in four different study populations. The prevalence of CT-specific IgG and IgA antibodies in 443 blood donors was 5-12% and was negatively correlated with age, as expected. No significant titer rise was observed when using consecutive serum samples from 22 patients with pneumonia caused by *Chlamydia pneumoniae*. From 324 patients with active CT infection as observed by PCR, 68-75% were positive for Ct-specific IgG. Furthermore, all tests detected more IgG antibodies in patients with tubal factor infertility (n=85) than in patients with nontubal factor infertility (n=49). In conclusion, both tests detected speciesspecific antibodies, which were strongly correlated to active infection. Furthermore, these peptide-based EIA tests may be useful in the serodiagnosis of tubal factor infertility caused by *C. trachomatis*.

H04
Immuunevasie en HPV

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Human Papillomaviruses (HPV) infect skin and mucosa. In the basal epithelial layers HPV can persist for years, sometimes promoting malignant transformation. This is best illustrated by the role that HPV play in the development of cervical cancer, the second cause of cancer-related death in females worldwide. Furthermore, HPV are implicated in the development of genital warts, laryngeal papapilloma's and, possibly, non-melanoma skin cancer.

Knowledge about HPV-specific immunity is far from complete. To clear the infection, adequate T-cell activity is probably essential. Although the number of studies that have looked into this matter is rather low, a pattern emerges showing that only a proportion of patients have detectable HPV-specific T-cells. Whether T-cell presence coincides with disease regression is still a matter of debate. In general it seems as if HPV infections, at least for some period, remain unaffected by T cells in a substantial part of the infected population. Apart from technical imperfections to detect HPV-specific T-cells, this could mean that HPV-specific CTL are either not induced, tolerized or incompetent. Several factors can be thought of to explain these possibilities.

H05
Epstein-Barr-virus en immuunevasie: een onlosmakelijke eenheid

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Epstein-Barr-virus (EBV), een gamma-herpesvirus, persisteert levenslang in >90% van de wereldbevolking. Primaire infectie op kinderleeftijd verloopt meestal ongemerkt, maar uitgestelde besmetting kan in 50% van de gevallen leiden tot een lymphoproliferative aandoening die bekend staat als IM (Infectious Mononucleosis), Kissing Disease of Ziekte van Pfeiffer. Cruciaal in de levenslange persistentie van EBV is infectie en transformatie van de B-lymfocyt. Onlangs is EBV door de IARC gekenmerkt als 'group-1 human carcinogen', vanwege de sterke (causale) associatie met het ontstaan van diverse tumoren van zowel lymfoïde als epitheliale oorsprong.

EBV-persistentie kenmerkt zich door een levenslang dynamisch evenwicht tussen virus (DNA)replicatie en actieve controle door het immuunsysteem, waarbij EBV zich in lymfoïde cellen schuil houdt, voornamelijk in (memory-) B-cellen. Verstoring van dit evenwicht kan leiden tot virusreplicatie, reïnfectie en transformatie van nieuwe B-cellen, maar ook tot proliferatie van pre-maligne gastheercellen, uiteindelijk resulterend in tumorvorming.

Tijdens de normale 'latente' persistentie is de EBV-dragende gastheercel vrijwel onzichtbaar voor het immuunsysteem, vanwege beperkte transcriptionele activiteit en actieve beïnvloeding van antigeenpresentatie.

EBV is transcriptioneel actief in diverse tumoren, waarbij functionele immuunevasie, direct of indirect, een belangrijk aspect vormt. In de loop van de evolutie heeft EBV diverse eigenschappen verworven, waarmee het op subtiele wijze kan ontsnappen aan immuuneliminatie.

Directe immuunmodulatie door EBV lijkt voornamelijk een rol te spelen tijdens lytische virusreplicatie (e.g. vIL10, vBcl2), terwijl een combinatie van directe en indirecte effecten bijdraagt aan de virale oncogenese. Het essentiële eiwit EBNA1 ontsnapt aan cytotoxische T-cel (CTL) 'surveillance' herkenning door direct te interfereren met het proteasomale proces, maar is een dominant doelwit van de humorale respons. Het oncogen LMP1 wordt zowel door T-cellen als antilichamen nauwelijks als lichaamsvreemd herkend. Onlangs vonden

wij dat gezuiverd LMP1 in staat is een directe functionele anergie te veroorzaken in reactieve T-lymfocyten, mogelijk een cruciale eigenschap waarmee EBV+-tumorcellen kunnen ontsnappen aan eliminatie door het afweersysteem.
In de lezing zullen diverse aspecten van EBV-gemedieerde immuunevasie worden besproken, in het licht van normale persistentie en tumor immuun-escape.

K01
An outbreak of measles in a primary school in the Netherlands, June 1999

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Background: In the Netherlands, about 2-3% of the Dutch population refuses vaccination on religious grounds. In June 1999 we investigated an outbreak of measles in children of an Orthodox Reformed primary school and in their household contacts, to study the spread among this population.
Methods: We mailed questionnaires and visited at home, in order to administer questionnaires and to collect blood and throatswabs.
Preliminary results: From 251 out of 390 school children, questionnaires and vaccination status were collected. Biological samples were acquired from 85 persons of 27 families. We observed 123 confirmed and 98 suspected cases of which 97 and 42 attended the school. The attack rate for vaccinated schoolchildren was 0% and for unvaccinated schoolchildren it was associated with the number of previous epidemics encountered; it decreased from 88% in the 4-6-year-olds to approximately 10% in the 11-12-year-olds. The attack rate among schoolchildren without vaccination and with a history of measles was 95%. The complication rate was 14%; 7% developed otitis media; 4% pneumonia and one child (0.5%) encephalitis. Six non-clinical cases with a history of measles had a positive laboratory-based measles diagnosis.
Conclusions: Despite high national vaccine coverage (94-96%), measles outbreaks still occur due to clustering of unvaccinated religious groups. Both this clustering and the observed subclinical reinfections are critical issues that may complicate measles elimination.

K02
MRSA in nursing homes in the Netherlands 1989-1998

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Several recent MRSA-outbreaks in nursing homes in the Netherlands were seized as an opportunity to study the prevalence of MRSA in nursing homes from 1989 to 1998. Data from eight regional public health laboratories in the Netherlands suggested that the prevalence of MRSA in nursing homes in 1998 was higher than in preceding years (1989-1997). Apart from a number of sporadic cases, two MRSA-outbreaks were detected in two nursing homes, both concerning a unique phage type. One of these outbreaks was preceded by an MRSA-outbreak involving the same phage type in the nearby hospital. Although findings on the basis of these selected samples from a limited number of laboratories call for careful interpretation, there is yet some ground for alarm. The clear 1998 rise in the frequency of MRSA in nursing homes points at a possible problem. Spread of these MRSA strains may well give rise to colonisation outside nursing homes. Hence, there is sufficient reason to repeat the 1992-1993 study investigating the prevalence of MRSA in nursing homes. The present study, starting early 2000, will pay special attention to the possible role of nursing homes as a reservoir of MRSA hospital infections.

K03
MRP and EF protect pigs against challenge with virulent *Streptococcus suis* serotype 2 strains

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Streptococcus suis infections are a common cause of meningitis, arthritis, endocarditis and septicaemia in young pigs. At present, 35 serotypes of *S. suis* are described. Serotyping of 411 *S. suis* strains, isolated from diseased pigs in seven European countries, revealed that serotype 2 was most frequently isolated. However, in Belgium, Germany and the Netherlands serotype 9 was most prevalent. Virulence can differ within and among *S. suis* serotypes. Earlier we showed that MRP and EF proteins were markers of virulent serotype 1 and 2 strains. MRP and EF are both expressed *in vivo* and are highly immunogenic. For vaccine development, pigs were vaccinated with affinity chromatography purified MRP and EF and challenged with a virulent *S. suis* serotype 2 strains. The results showed that a vaccine with both MRP and EF conferred an almost complete protection while pigs vaccinated with either MRP or EF were less protected. Application of a vaccine based on MRP and EF may contribute in efforts to control *S. suis* infections.

K04
Antibodies to capsular polysaccharide of *Haemophilus influenzae* type b (Hib) in the Dutch population a few years after introduction of vaccination

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Objective: To assess antibody levels to the capsular polysaccharide of *Haemophilus influenzae* type b (HibCPS) in the Dutch population, in cohorts born after the introduction of routine vaccination with Hib tetanus-conjugate vaccine in April 1993 and in those born before.
Methods: Sera and questionnaires were collected from an age-stratified sample of 380 persons (0-79 years) from each of 40 municipalities, sampled proportional to their size. 7.864 (52%) serum samples were analysed for HibCPS antibodies by an ELISA.
Results: The seroprevalence ≥ 0.15 µg/ml and GMT for 20-24- to 75-79-year-olds decreased from 94% to 84%, and 1.5 to 0.7 µg/ml respectively. The seroprevalence ≥ 0.15 µg/ml in fully vaccinated children decreased from 99% (≥ 1.0 µg/ml 96%) within two months after the fourth vaccination to 83% (72%) after 27-29 months. The GMT declined from 8.7 to 0.8 µg/ml during this period.

Those children vaccinated although born before introduction of routine vaccination had higher seroprevalence (63%) than age-matched unvaccinated children (41%). Visiting a day care centre was positively associated with the presence of HibCPS anitbodies. **Conclusion:** The minimally protective HibCPS level after Hib-conjugate vaccination has not yet been ascertained. Therefore, the future consequences of the decline in antibody levels after vaccination on the – currently very high – effectiveness of Hib-vaccination are unclear.

K05

Pertussis serodiagnosis with single serum and estimation of frequency of *Bordetella pertussis* infection in the population

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Objective: To investigate whether high levels of IgG antibodies against pertussis toxin (IgG-PT) in a single serum sample are indicative for actual or recent pertussis. These data also provide insight into the frequency of *Bordetella pertussis* infection in the population.
Methods: IgG-PT measured in ELISA was analyzed in 7,756 population-based sera, in paired sera of 3,491 patients with more than fourfold increase of IgG-PT and in sequential sera of 57 pertussis patients (median follow-up 1.4 years).
Results: IgG-PT levels were ≥ 100 U/ml in less than 1% of population-based sera, but in 80% of second serum samples of patients with more than fourfold IgG-PT-increase. Regression analysis of follow-up sera predicted that a level of > 100 U/ml persists 4.5 months after disease onset. The proportion in the population with IgG-PT of ≥ 5 U/ml increases from 4 to 15 years of age and is stable afterwards.
Discussion: IgG-PT levels ≥ 100 U/ml are diagnostic of recent or actual infection with *B. pertussis*. Such levels have high sensitivity and specificity. They are reached in most pertussis patients within four weeks after disease onset and persist only temporarily. The IgG-PT distribution in the population suggests that *B. pertussis* infections occur frequently.

K06

Prevalence and determinants of fecal colonization with vancomycin-resistant enterococci in hospitalized patients in the Netherlands

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We determined the prevalence and determinants of vancomycin resistant enterococci (VRE) fecal carriage in Dutch Intensive Care (IC)-, Hematology-Oncology (HO)- and hemodialysis patients between 1995-1998. Rectal swabs obtained from 1,112 patients were cultured by selective enrichment. Resistance genotypes were determined by PCR and pulsed field gel electrophoresis (PFGE). We studied possible determinants (sex, age and log-transformed length of prior hospital stay) of VRE colonization with a logistic regression analysis model. 614 of the 1,112 patients (55%) were colonized with vancomycin sensitive enterococci (VSE) and 15 patients (1.4%) carried VRE. No increase in VRE colonization was observed. All *E. faecium* and one *E. faecalis* carried the *vanA* gene; the other three *E. faecalis* strains harbored the *vanB* gene. PFGE revealed that three *vanB* VRE isolated from patients hospitalized in one single ICU, were related, suggesting nosocomial transmission. VRE colonization in Dutch hospitals is an infrequent phenomenon. Although nosocomial spread occurs, most observed cases were unrelated, which suggests VRE acquisition from outside the hospital. Prolonged hospital stay, age and sex proved unrelated to VRE colonization.

K07

Succesvolle bestrijding van een epidemie met vancomycineresistente *Enterococcus faecium* op een hematologische afdeling

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In de periode november 1998 – mei 1999 ontwikkelden drie patiënten op de afdeling hematologie van het AZVU sepsis met een vancomycineresistente *Enterococcus faecium* (VRE). De drie isolaten werden getypeerd met behulp van Amplified Fragment Length Polymorphism (AFLP) en bleken genetisch identiek. In juni 1999 toonde een screening van alle op de afdeling aanwezige patiënten aan dat inmiddels 14 patiënten gekoloniseerd waren met een VRE-stam van hetzelfde type. Alle stammen waren positief in een VanA PCR. De gekoloniseerde patiënten werden in standaardisolatie verpleegd (zonder gebruik van neus/mondmasker), de overige patiënten werden in barrière verpleegd. Alle hematologische patiënten werden tweemaal per week gescreend op VRE-dragerschap middels anuskweken. In augustus bleek een patiënt, na aanvankelijk tweemaal een negatieve kweek gehad te hebben, toch gekoloniseerd te zijn met een VRE van het epidemische type. Deze patiënt was in de voorgaande periode meerdere malen opgenomen geweest op de afdeling hematologie. In de daaropvolgende dagen bleken vijf nieuwe patiënten besmet. Dit was aanleiding voor strengere isolatiemaatregelen: alle patiënten die tussen november 1998 en augustus 1999 opgenomen waren geweest op de afdeling hematologie werden als VRE-dragers beschouwd (ongeacht de uitslag van de screeningskweken) en in isolatie verpleegd. Nieuwe patiënten werden niet geïsoleerd. Daarnaast werd het antibioticumbeleid aangepast: het gebruik van tweede- en derde-generatie cefalosporines werd volledig gestaakt. Sinds deze matregelen zijn doorgevoerd zijn geen nieuwe patiënten meer gekoloniseerd geraakt met de epidemische VRE-stam.

K08

European Antimicrobial Resistance Surveillance System: *Streptococcus pneumoniae* susceptibility test results

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Background: The European Antimicrobial Resistance Surveillance System (EARSS) is a network of national

surveillance systems. Susceptibility test results of *Streptococcus pneumoniae* in blood- and CSF isolates are being collected since the last quarter of 1998.

Methods: Patient- and isolate information is collected through Isolate Record Forms that have been developed to enable standardized data processing. The laboratories interpret the test results according to national guidelines. The national coordinators collect resistance data from the different laboratories and send it in EARSS data exchange format (ASCII) to the central database quarterly. Susceptibility test results from 165 labs from 11 European countries were analysed. The proportion of oxacillin- (=penicillin-)resistant *S. pneumoniae* (PRSP) was calculated.

Results: The proportion of PRSP did not exceed 5% in Finland (8/213), Germany (4/150), Iceland (1/27), the Netherlands (8/352) and Sweden (25/1081). The proportion of PRSP in the UK was 8% (19/240), in Italy 14% (7/52), in Portugal 14% (5/37), in Ireland 21% (23/110), in Luxembourg 22% (2/9) and in Greece 52% (13/25), respectively.

Conclusion: These data show a north-south gradient in the proportion of invasive PRSP across Europe. In the northern European countries PRSP are not very prevalent, while southern European countries and Ireland report proportions ranging from 14% to 52%. EARSS will continue to monitor resistance in the EU, extending the set of pathogens under surveillance in the near future.

K09

Identification of eight *M. pneumoniae* P1 cytadhesin gene PCR-RFLP subtypes, and a new variable region in the P1 gene sequence

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Nucleotide sequence variations of the P1 cytadhesin gene of *M. pneumoniae* has been shown to divide clinical isolates of *M. pneumoniae* into two P1 types, type 1 and 2 (Su et al., 1990). The existence of two P1 types has been confirmed by application of PCR-RFLP on a large collection of *M. pneumoniae* isolates in Japan (Sasaki et al., 1996). In order to refine this typing method, we performed a modified PCR-RFLP analysis using an extended set of restriction enzymes, and were able to discriminate eight P1 subtypes among two reference strains and 21 *M. pneumoniae* clinical isolates. Based on the variation in the RFLP patterns, a selection from the clinical isolates was made to perform sequence analysis of their P1 genes. Two reference strains were also included. Primers ADH1-2 and ADH3-4 were used to amplify fragments of approximately 2,280 bp and 2,580 bp, respectively. The purified fragments were re-amplified and used for direct sequencing, using primer walking strategy. Several point mutations resulting in amino acid changes were detected in both the ADH1-2 fragments and the ADH3-4 fragments from the P1 type 1 and the P1 type 2 isolates. A major difference was detected in P1 type 1 strain M 4817, which showed a new variable sequence of approximately 600 bp. This variable region was distant from the previously reported regions known to differ between P1 type 1 and 2 genes, but showed a partial overlap with the variable region as recently reported by Kenri et al., which was observed in several P1 type 2 strains (Kenri 1999). Contrary to the latter region, which was found only among P1 type 2 strains, the variable region as detected by us, was found in a P1 type 1 strain. This suggests that recombination between sequences from outside the P1 gene and the P1 gene locus itself occurs in both P1 type 1 and P1 type 2 strains. The new sequence information will allow the development of a genotyping system for *M. pneumoniae* by direct sequencing and thus will expand the possibilities for detection of new P1 gene variants among clinical isolates of *M. pneumoniae*.

K10

Isoniazid resistance in *Mycobacterium tuberculosis*: the mutation on amino acid position 463 of the *katG* gene is not a causative factor

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Mycobacterium tuberculosis, the causative agent of tuberculosis, is the leading cause of death of infectious disease worldwide. The emergence of multidrug-resistant strains (resistant to at least isoniazid and rifampicin) has complicated the treatment of tuberculosis. Resistance to isoniazid (INH), one of the mainstays of treatment, occurs in 7% of cases in the Netherlands. In 50-70% of the resistant isolates INH-resistance is associated with mutations in the *katG* gene. We used a PCR/REA (Restriction Endonuclease Analysis) test to detect the two most predominant mutations in the *katG* gene associated with INH-resistance: at amino acid positions 315 and 463. In this study we performed the PCR-REA with *NciI* on 95 INH-sensitive and 123 INH-resistant (MIC ≥ 0.5 µg/ml) *M. tuberculosis* isolates recovered from patients in the Netherlands. We found that 32 (34%) of the 95 INH-sensitive isolates had the 463 mutation, similar as 33 (27%) of the 123 INH-resistant isolates. From these results we conclude that the mutation on amino acid position 463 in the *katG* gene of *M. tuberculosis* is irrelevant in INH resistance.

K11

Transfer of metronidazole resistance between two *Helicobacter* pylori strains

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Metronidazole (Mtz) resistance in *Helicobacter pylori* is caused by inactivation of a nitroreductase gene, *rdxA*, which converts Mtz to its active form. Inactivation of *rdxA* can occur either by spontaneous mutation or as a result from replacement of a Mtz^S *rdxA* allele with a Mtz^R allele from another strain. Here we present the first evidence for transfer of the *rdxA* gene in a patient whose *H. pylori* culture yielded both Mtz^S and Mtz^R colonies. Six Mtz^S and five Mtz^R (MIC > 8 µg/ml, E-test) colonies were isolated. Genetic fingerprinting by RAPD showed that the patient was infected with two different strains: genotype 1 (8/11 isolates) and 2 (3/11 isolates), but the distribution of resistance did not correspond to these genotypes (Table 1). The DNA sequence of a central part of the *rdxA* gene was determined for nine isolates. Three isolates of strain type 2 had *rdxA* allele A, whereas the eight isolates with strain type 1 contained either *rdxA* allele A or B. All Mtz^R

strains tested had *rdxA* allele A. Our results show transfer of the *rdxA* allele A during mixed infection, presumably from strain 2 to strain 1, and indicate that resistance genes are exchanged between *H. pylori* strains.

Table 1. Distribution of Mtz susceptibilities, strain genotypes and rdxA alleles

ISOLATE	1	2	3	4	5	6	7	8	9	10	11
Mtz susceptibility	S	S	S	S	S	S	R	R	R	R	R
strain genotype	1	1	1	2	1	2	1	1	2	1	1
rdxA allele	B	B	A	A	A	A	A	A	A	n.d.	n.d.

K12

Comparative analysis of four genotyping techniques for the *Campylobacter* species *C. jejuni* and *C. coli*

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Since traditionally used typing methods for *C. jejuni* and *C. coli* such as serotyping lack discriminatory power for epidemiological tracing of *Campylobacter* infections, genotyping techniques have been developed. *FlaA* typing, PFGE and in lesser extent ribotyping are now the most commonly used genotyping techniques. Recently AFLP has been developed and has shown great potential for epidemiological typing of *Campylobacters*. This study was performed to examine the value of these four genotyping techniques for epidemiological typing of *Campylobacters* by typing 50 poultry strains. Criteria were discriminatory power, ease of use, time etc. Furthermore, the effect of repetitive subculturing on fingerprints was also considered for *flaA* typing, PFGE, and AFLP. AFLP was the most discriminatory technique, closely followed by PFGE. No detectable changes in fingerprints were found before and after subculturing, indicating the reliability for long-term typing. Based on the discriminatory power, coupled with other criteria, we conclude that AFLP – on its own or preferably combined with other methods – is the preferred method for epidemiological typing of *Campylobacters*.

K13

Real-time quantitative PCR of *Bacteroides vulgatus* and *Escherichia coli* in mucosal samples from healthy humans and patients with IBD

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Introduction: The etiology of the inflammatory bowel diseases Crohn's disease (CD) and ulcerative colitis (CU) are still unknown. Chronic inflammation may be the result from a host immune respons to luminal microflora. Bacteroide species, especially *Bacteroides vulgatus*, significantly increase colitis and gastritis in transgenic rats, implicating a role of this organism in the pathogenesis of chronic intestinal inflammation. Among the numerous bacteria of the mucosa adherent intestinal flora, *Escherichia coli* has been thought to be involved in the pathogenesis of IBD. To study a quantitative role of *B. vulgatus* and *E. coli* we used a new molecular approach.
Methods: A specific primer/probe (TaqMan) combination was developed on the 16S-rDNA gene. Subsequently, real-time quantitative PCR analysis for *B. vulgatus* and for *E. coli* was carried out in an ABI-Prism 7,700 sequence detector. Sensitivity of both PCR amplifications was determined by viable counts after bacterial culture. Specificity was determined by PCR analysis on more than ten related mucosal bacterial species. Mucosal sigmoid biopsies from 15 apparently healthy persons, nine ulcerative colitis patients and ten Crohn's disease patients were screened for PCR inhibition, and quantitation was compared to known culture results.
Results: Both primer/probe combinations showed a high sensitivity and specificity. Inhibition on biopsies was variable, but quantification was in concordance with culture. The amount of *B. vulgatus* and *E. coli* in healthy persons and in patient samples was not significantly different.
Conclusions: The TaqMan PCR assay can be used to study the adherent intestinal flora in mucosal biopsies. For this assay no cultivation of bacteria is necessary. The first results indicate no significant difference in the amount of *B. vulgatus* and *E. coli* between IBD patients and healthy persons. In the near future, more biopsies will be included and a broad panel of primers and probes will be developed to quantitate different bacteria to determine a possible role of the luminal flora in IBD.

L01

Mitochondrial chaperonine Hsp60 encoding genes in anaerobic chytrids

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Anaerobic fungi from the digestive tracts of herbivores lack mitochondria, but they contain an organelle called hydrogenosome. The evolutionary origin of these hydrogenosomes is still an unsolved question. Ultrastructurally, the hydrogenosomes of anaerobic fungi resemble peroxisomes. Also the genes encoding a hydrogenosomal adenylate kinase of *Neocallimastix* sp. *L2* and *Piromyces* sp. *E2* might be indicative of a peroxisomal origin of these hydrogenosomes since they possess a putative C-terminal ('peroxisomal') targeting signal (SKL). However, phylogenetic analysis of these genes revealed that they are closely related to mitochondrial adenylate kinases. Since it has also been shown that additional hydrogenosomal proteins of mitochondrial descent but with a putative N-terminal ('mitochondrial') targeting signal exist, a chimeric origin of the chytrid's hydrogenosomes seems possible.
In a search for components of a potential mitochondrial-like importsystem we succeeded to identify genes encoding a 'mitochondrial' Hsp60, and several cytoplasmic- and ER-specific Hsp70 chaperonines. However, since we did not identify a 'mitochondrial' Hsp70 or a 'mitochondrial' Hsp10, the presence of a functional mitochondrial import machinery in anaerobic chytrids is unlikely.

L02
Studies on the bacterial protein-conducting pore: the SecYEG complex

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Preprotein destinated to be secreted across the inner membrane of *Escherichia coli* are targeted to the translocase by an N-terminal signal sequence. The *E. coli* translocase consists in its minimal form of three integral membrane proteins termed SecY, SecE and SecG, and a peripheral ATPase SecA. SecY and SecE form a stable stoichiometric complex in the cytoplasmic membrane that does not dissociate *in vivo*. SecG is not an essential component, but stimulates the *in vitro* translocase activity. SecA is a homodimeric protein that serves both as a receptor for precursor proteins and as an ATP-driven molecular motor during the translocation reaction. Precursor-stimulated cycles of ATP-binding and hydrolysis by SecA permit the stepwise movement of a translocating polypeptide chain across the membrane by a two-stroke reaction.
To facilitate the study of the structure, function and dynamics of the membrane domains of the bacterial translocase, a system was developed that allowed overproduction of the SecYEG complex to up to 40% of the total membrane protein. Membranes which contained high levels of the SecYEG complex ($SecYEG^+$) were tested for activity, and showed a large increase in both precursor protein stimulated ATPase activity of SecA and in the *in vitro* translocation of proOmpA. SecYEG complex purified from these membranes was reconstituted into proteoliposomes using a rapid dilution method, and was found to be highly active in both precursor protein stimulated SecA ATPase activity and the *in vitro* translocation of proOmpA. The reconstitution studies demonstrated that negatively charged phospholipids are essential for activity, while non-bilayer forming lipids stimulate protein translocation. The purified SecYEG present in detergent solution was further characterized. Solubilized SecYEG binds SecA with high affinity and support the AMP-PNP driven conformational change of SecA. Circulair dichroism measurements showed that the enzyme is largely α-helical. The quaternary structure of the SecYEG complex was analyzed by high-resolution negative stain electronmicroscopy, single particle alignment and averaging. Detergent solubilized SecYEG complex was found to be present mainly as a dimeric species with dimensions of approximately 8.5 by 6.5 nm. After incubation of SecYEG proteoliposomes with SecA and AMP-PNP, and re-purification of the SecYEG complex, a significant fraction of the SecYEG was found in a larger complex with a diameter of approximately 10.5 nm and a stain-filled indentation of about 5 nm. The size of these particles and determination of their molecular mass by scanning transmission electron microscopy indicate that these large particles represent tetramers of the SecYEG complex. These data suggest that the ATP binding induced conformational change of SecA allows the recruitment of SecYEG complexes to yield a stable tetramer of SecYEG that possibly forms the protein-conducting channel. More detailed information about the structure of the SecYEG complex was obtained from cysteine scanning mutagenesis, which revealed contacts between several helices of SecY and SecE.

L03
Phosphorylation state of HPr determines expression and activity of the lactose transport protein from *Streptococcus thermophilus*

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The phospho*enol*pyruvate: carbohydrate phosphotransferase system (PTS) catalyses the uptake and concomitant phosphorylation of a number of carbohydrates. The phosphoryl group is transferred from phospho*enol*pyruvate (PEP) via the general energy coupling proteins Enzyme I and HPr to the sugar-specific phosphoryl transfer protein/domain IIA; IIA~P transfers the phosphoryl group to the sugar-specific IIB protein/domain which phosphorylates the sugar that is translocated via the sugar-specific IIC protein/domain. Besides PEP/Enzyme I-mediated phosphorylation of a histidine residue [HPr(His~P)], the HPr protein of Gram-positive bacteria can also be phosphorylated on a serine by an ATP-dependent kinase. Amongst others, HPr(Ser-P) affects the expression of catabolite repression sensitive genes by activating the transcription factor CcpA.
The lactose transport protein (LacS) of *Streptococcus thermophilus* catalyses heterologous exchange of lactose for intracellularly formed galactose as well as proton motive force (Δp)-driven lactose uptake. Apart from the translocator domain, the LacS protein has a carboxyl-terminal domain that is homologous to IIA proteins of the PTS, and this IIA^{LacS} domain is phosphorylated by HPr(His~P). Lactose transport may not only be regulated by HPr at the level of the activity of the LacS protein, but also at the level expression as the *lac* promoter region comprises an catabolite responsive element. To address whether or not CcpA-HPr(Ser-P)-mediated catabolite control plays a role, the expression levels of LacS were determined under conditions that the cellular phosphorylation state of HPr differed greatly. It appears that HPr(Ser-P) is mainly present in the exponential phase of growth, whereas HPr(His~P) dominates in the stationairy phase. The transition from HPr(Ser-P) to HPr(His~P) parallels an increase in LacS level, a drop in lactose and an increase in galactose concentration in the growth medium. Since the K_m^{out} for lactose is rather high and higher than that for galactose, the lactose transport capacity will decrease as lactose decreases and galactose accumulates in the medium. Our data indicate that *S. thermophilus* compensates for the diminished transport capacity by synthesising more LacS. The increased synthesis reflects an increased transcription, which is affected by the decrease in HPr(ser-P) concentration, which in turn is elicited by a diminished glycolytic activity (HPr kinase activity) as a result of the diminished lactose uptake.
Since an increase in HPr(His~P) parallels the decrease in HPr(Ser-P), and HPr(His~P) phosphorylates LacS, the effect of this phosphorylation on the transport activity was determined next. Indeed, phosphorylation of the LacS protein stimulated the exchange activity. Kinetic analysis showed that the maximal velocity (V_{max}) of exchange transport was increased upon phosphorylation of LacS, whereas the V_{max} of the proton motive force (pmf)-driven lactose transport was not affected. In line with a range of kinetic studies, we propose that the phosphorylation affects the rate constants for the

reorientation of the ternary complex (LacS with bound lactose plus proton). This step is rate-determining for exchange but not for Δp-driven transport. Overall, the data indicate that when lactose uptake becomes limiting for growth, the transport capacity is increased by raising the LacS protein level (slow response) and activation of the LacS protein (rapid response).

L04
Development and evaluation of synthetic pneumococcal vaccines

W.T.M. Jansen

The scope of the thesis is the development of *(i)* minimal synthetic pneumococcal vaccines and *(ii)* an assay that can assess the efficacy of these vaccines. *Streptococcus pneumoniae* is an encapsulated pathogen that causes pneumonia, bacteremia, meningitis and otitis media. To overcome the thymus independency of the conventional 23-valent polysaccharide (PS) vaccine, clinical trials are nowadays conducted for multivalent PS-carrier protein conjugate vaccines. The 23-valent vaccine as well as the experimental conjugate vaccines are based on capsular PS, which are purified from bacteria. These vaccines have several drawbacks such as vaccine impurities, batch to batch variation and low immunogenicity for some serotypes, due to antigenic competition or carrier suppression. In addition, use of whole PS in conjugate vaccines might limit the T-cell dependent character of the vaccine. Our aim is to define minimal protective epitopes on PS, and apply this knowledge for the design of minimal, synthetic vaccines, either as a synthetic saccharide-protein conjugate vaccine or peptide mimotope vaccine. These vaccines are potentially pure and precisely defined, and have a T-cell dependent character. Moreover, synthetic saccharides or peptide mimotopes can be used to circumvent unwanted anti-PS antibody cross reactivities recently described in the pneumococcal ELISA.[1] The first strategy was to synthesize serotype 6B di-, tri- and tetrasaccharide conjugates and study their immunological and protective properties in mice and rabbits. In rabbits, the disaccharide conjugate, and in mice the tetrasaccharide conjugate, contained the minimal required epitope capable of inducing 6B specific, protective antibodies. Human pneumococcal vaccine antisera contained antibodies recognizing the synthetical oligosaccharides. Therefore, these synthetic conjugates have the potential to induce anti PS 6B antibodies in humans as well, which makes them candidates for a synthetic 6B conjugate vaccine.[2] For the second strategy, a set of anti-PS monoclonal antibodies (mAbs) were developed, and structurally and functionally characterized.[3] Three different peptide libraries (phages displaying either random peptides or single chain antibody fragments, or chips coated with random peptides) were screened with phagocytic serotype 17F mAbs and protective S3 mAbs. Peptides were identified that competed with the S3 mAbs for their PS-epitopes in inhibition ELISA, suggesting that these peptides mimic protective S3 epitopes.[4] This hypothesis is currently being tested in a mouse model. Since protection against pneumococci is mainly based on antibody and complement mediated phagocytosis, an *in vitro* assay, needed for the evaluation of experimental pneumococcal vaccines, should measure phagocytic capacity of the antibodies present in these vaccine sera. We have developed a serotype specific phagocytosis assay based on flow cytometry. This assay is easy to perform, highly standardized and it resembles the human defense system, since human PMN and human complement are used.[5] We have shown that Fcγ-receptor polymorphisms on PMN have a profound influence on the phagocytosis of pneumococci for both conjugate vaccine sera[6] and 23-valent polysaccharide vaccine sera[7]. Therefore, our phagocytosis assay has been standardized for the Fcγ-receptor allotype of the donor PMN. The laborious and time consuming classical killing phagocytosis assay (based on pneumococcal CFU counting) is considered to be the golden standard for the assessment of pneumococcal vaccine efficacy. Since our phagocytosis assay displays excellent correlations, in general, with the classical killing assay for sera obtained from adults[8] and infants[9] vaccinated with experimental conjugate vaccines, our phagocytosis assay has the potential to replace the classical killing assay for the evaluation of pneumococcal vaccines.

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8. W.T.M. Jansen et al. Comparison between Classical Killing Pneumococci Phagocytosis Assay and a Flow Cytometry Assay for the Assessment of the Phagocytic Capacity of Adult Pneumococcal Vaccination Sera. Submitted for publication.
9. M. Anttila, W.T.M. Jansen et al. Correlation between classical killing phagocytosis assay, a flow cytometric assay phagocytosis assay and a radiolabelled phagocytosis assay for infant conjugate antisera. Submitted for publication.

L05
Tat-dependent secretion of the phosphodiesterase PhoD of *Bacillus subtilis*

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Both prokaryotic and eukaryotic organisms are capable of transporting proteins from one compartment to another via the well-conserved Sec translocon. For this process, transported proteins have to be (partly) unfolded. In addition to the Sec translocon, bacterial and chloroplast thylakoidal membranes contain a second type of translocon that appears to be capable of transporting proteins in a folded conformation. In *Escherichia coli*, this so-called Tat translocon (for twin-arginine translocation) is essential for the export of proteins that bind complex redox cofactors in the cytoplasm. Signal peptides with a well-conserved twin-arginine motif (RR-signal peptides) direct such proteins to the Tat translocon. In *E. coli*, five genes are known to encode proteins involved in the Tat export pathway; four of these form an operon (*tatABCD*), whereas the *tatE* gene is monocistronic. Four of the gene products (*tatABCE*) are membrane-bound and are believed

to function in the Tat protein translocase in the plasma membrane. Thus far, it is not known whether the cytoplasmic TatD protein has a role in protein export. Homologues of these proteins can be found in nearly all eubacteria of which the genomes have been sequenced completely.
Interestingly, three *tatABE*-like genes, two *tatC*-like genes and one *tatD*-like gene were identified in *Bacillus subtilis*, showing that all putative Tat components of this organism are present, most of them even in multiple (paralogous) copies. Notably, each of the two *tatC* genes of *B. subtilis* was preceded by a *tatABE*-like gene. These observations are consistent with the identification of genes for (putative) exported proteins with RR-signal peptides in *B. subtilis*. We have been able to show that secretion of a certain reporter protein is dependent on *B. subtilis* Tat components. The effects of single and multiple mutations of tat-genes on secretion of this reporter protein will be reported and discussed.

Lo6
The role of *ram* cluster during morphological differentiation of *Streptomyces lividans*

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Streptomycetes undergo a complex process of morphological differentiation that normally results in sporulation. The complete lifecycle has three characteristic stages: *(i)* the formation of vegetative mycelium, *(ii)* the formation of aerial hyphae and *(iii)* the production of spores. Our efforts aim at the elucidation of the physiological role of the *ram* cluster during this process. The *ram* cluster (rapid aerial mycelium) was originally identified for its ability to stimulate the development when introduced as an extra copy into *S. lividans*. It contains genes encoding a serine threonine kinase (*ramC*), an ABC transporter (*ramAB*) and a small ORF (*ramS*) for which no homology to any of the known sequences in the database was found. Transcriptional analysis revealed that the transcription of *ramC, ramS* and *ramAB* is coupled and coincides with the onset of aerial hyphae formation. A complex mechanism was found to be involved in the regulation of the various levels of *ramAB, ramC* and *ramS* transcription. To determine the physiological role of this cluster of genes during morphogenesis, we have constructed *ramAB* gene disruption mutants, which showed to be impaired in the ability to form aerial hyphae and spores. However, when grown in close proximity to differentiating wild type strains, normal development of these mutants was observed. This phenomenon indicates the involvement of *ramAB* in the transport of an extracellular signal molecule or building block, essential for morphogenesis.

Lo7
Regulation of autotrophic growth by the chemoautotrophic bacterium *Xanthobacter flavus*

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The chemoautotrophic bacterium *Xanthobacter flavus* assimilates carbon dioxide via the Calvin-Benson-Bassham cycle which contains three unique enzymatic activities: RuBisCO, PRK and SBPase. Most Calvin cycle genes are encoded in three transcriptional units: the *cbb* and *gap-pgk* operons and the *tpi* gene. The LysR-type transcriptional regulator CbbR controls expression of the *cbb* and *gap-pgk* operons in response to the availability of carbon and energy sources. CbbR binds to three inverted repeats in the *cbb* promoter containing the CbbR consensus sequence TNA-$N_{7/8}$-TNA. The role of these inverted repeats has been analyzed with respect to DNA binding of CbbR and to transcriptional regulation of the *cbb* operon. DNA binding and bending by CbbR is affected by NADPH. *In vivo* experiments support the critical role of reducing equivalents in regulation of Calvin cycle genes.

Lo8
Predictive microbiology as a tool for the estimation of the functionality of a sausage starter culture

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Predictive models are commonly applied by food microbiologists for describing the behaviour of spoilage bacteria and food-borne pathogens in food stuffs. Literature on this subject is abundant and the application potential of such models in the food industry is powerful. However, research has essentially been concentrating on the outgrowth and inactivation of harmful bacteria. Modelling of the biokinetics of beneficial microorganisms represents therefore a whole new field of investigation. As an example, it may bring new light to the topic of bacteriocin-producing lactic acid bacteria. The potential of using such strains as novel, functional starter cultures in the food fermentation industry is currently a subject of discussion. Apparently, bacteriocin production is strongly reduced under food fermentation conditions, when compared to optimal *in vitro* laboratory experiments. The use of predictive microbiology may hence contribute to a better understanding of the positive and negative influences on the functionality of bacteriocin producers in a food matrix. As a case study, the relation between the conditions prevailing in a fermented sausage environment and the functionality of *Lactobacillus sakei* CTC 494, a potential bacteriocin-producing starter culture, is studied in detail.

Lo9
Molecular typing of *Legionella pneumophila*

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Legionella infection (legionellosis) can vary from a mild respiratory illness to an acute life-threatening pneumonia. Legionellosis is acquired by inhalation or aspiration of legionellae from a contaminated environmental source, which may be established by comparing the environmental and clinical strains. When detailed epidemiologic characterization is required, standardized molecular typing methods are indispensable for the subtyping of *Legionella pneumophila* serogroups. Therefore, a comparison was made between pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) for the genotyping of *L. pneumophila*, using standardized protocols (with minor modifications)

from the European Working Group on Legionella Infections (EWGLI). Our results show that the DNA fingerprint patterns derived from AFLP seem to be superior to the patterns derived from PFGE, in both speed and interpretability. Furthermore, we emphasize the importance of the standardization of data-analysis and -exchange using the latest software (BioNumerics). Studies are underway to determine both the intra- and inter-laboratory reproducibility for further standardization of these powerful subtyping methods, (to be) used by an increasing number of European countries.

L10
Mechanisms for respiratory oxidation of cytosolic NADH by *Saccharomyces cerevisiae* mitochondria

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The strong preference of *Saccharomyces cerevisiae* for fermentative growth is a complicating factor in biomass-directed applications of this yeast, as alcoholic fermentation leads to a lower biomass yield on sugars than respiration. One of the factors governing the switch between fermentation and respiration could be the redox balance in the different compartments of the cell. During respiratory glucose dissimilation, *S. cerevisiae* produces intramitochondrial NADH in the Krebs cycle and cytosolic NADH via glycolysis. The cytosolic NADH has to be reoxidized outside the mitochondria, because the mitochondrial inner membrane is impermeable to NADH. In *S. cerevisiae* the mitochondrial inner membrane contains two types of NADH dehydrogenase that couple oxidation of NADH to the mitochondrial respiratory chain. The catalytic site of the 'external' NADH dehydrogenase faces the intermembrane space and oxidises cytosolic NADH. In addition, an 'internal' NADH dehydrogenase faces the mitochondrial matrix and oxidises the intramitochondrial NADH. Another cytosolic-NADH oxidising system is the glycerol-3-phosphate shuttle consisting of soluble (Gpd1p or Gpd2p) and membrane-bound (Gut2p) glycerol-3-phosphate dehydrogenases. The physiological relevance of these two mechanisms for oxidation of cytosolic NADH was studied in chemostat cultures of wild-type *S. cerevisiae* and of mutants in which essential enzymes for either of these two mechanisms had been deleted. A possible involvement of alternative routes was investigated by using a mutant in which both mechanisms were disabled. Aerobic, glucose-limited chemostat cultures of a *gut2*Δ mutant exhibited fully respiratory growth at low specific growth rates. Alcoholic fermentation set in at the same specific growth rate as in wild-type cultures (0.3 h^{-1}). Apparently, the glycerol-3-phosphate shuttle is not essential for respiratory glucose dissimilation. An *nde1*Δ*nde2*Δ mutant already produced glycerol at specific growth rates of 0.10 h^{-1} and above, indicating a requirement for external NADH dehydrogenase to sustain fully respiratory growth. An *nde1*Δ*nde2*Δ*gut2*Δ mutant produced even higher amounts of glycerol at specific growth rates ranging from 0.05 h^{-1} to 0.15 h^{-1}. Apparently, even at a low glycolytic flux, alternative mechanisms could not fully replace the external NADH dehydrogenases and glycerol-3-phosphate shuttle. Yet, at low dilution rates the *nde1*Δ*nde2*Δ*gut2*Δ mutant did not produce ethanol. Since glycerol production could not account for all glycolytic NADH, another NADH-oxidizing system has to be present. The experimental data suggest two possible alternative mechanisms for reoxidizing cytosolic NADH: *(i)* cytosolic production of ethanol, followed by its intramitochondrial oxidation, and *(ii)* a redox shuttle linking cytosolic NADH oxidation to the internal NADH dehydrogenase. Which of these mechanisms is functional will have to be determined by further research.

L11
Taxonomic characterization of strains from a microbial endemism study of 3-chlorobenzoate mineralizers

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Dogma in microbial ecology has suggested that many bacteria are globally dispersed among various habitats, and are therefore cosmopolitan. In a study examining the role that biogeography plays in determining the spatial pattern of microbial diversity found in pristine soils, over 500 bacterial strains demonstrating the phenotype of 3-chlorobenzoate mineralization were characterized. Microbial populations from two boreal forest regions, northern Saskatchewan (five sites) and northwestern Russia (four sites) along with four Mediterranean woodland regions, California, southwestern Australia, the Cape region of South Africa (five sites in each) and central Chile (four sites) were compared. The isolates were initially characterized by RFLP analysis of SSU-rRNA genes and rep-PCR genomic fingerprinting. Several major SSU-rRNA fingerprint types were found and representative strains from these groups were chosen for further taxonomic characterization. Presumptive identification was obtained by sequence analysis of the SSU-rRNA genes. Phenotypic identification of the strains was performed using conventional biochemical tests and the commercial API and BIOLOG test panels and several databases. Genotypic and phenotypic identification results were in general concordance. Several *Burkholderia*-like strains appear to be novel taxa and represent possibly new species.

L12
The modified Embden-Meyerhof pathway of *Pyrococcus furiosus* and other hyperthermophilic Archaea

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During growth on poly- or disaccharides, *Pyrococcus furiosus* uses a novel type of glycolytic pathway involving two ADP-dependent kinases instead of the usual ATP-dependent kinases, and a novel tungsten-containing enzyme GAPOR in place of glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase. These enzymes are very interesting from both a biochemical and an evolutionary viewpoint.
In our laboratory we study the enzymology and bioenergetics of the glycolysis of *P. furiosus*. We characterized the

ADP-dependent glucokinase and phosphofructokinase both biochemically and genetically. The amino acid sequences of the kinases show significant homology and several conserved regions, and therefore it was suggested that they are phylogenetically related. Recently, ADP-dependent phosphofructokinase activity was detected in *Methanococcus jannaschii* and other *Methanococcus* species, indicating the presence of the modified Embden-Meyerhof pathway in these methanogenic Archaea. The *M. jannaschii* phosphofructokinase, which was purified and characterized, showed considerable similarity with the *P. furiosus* phosphofructokinase.

L13
Glycolytic Enzymes and Their Control in *Pyrococcus furiosus*

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Glucose conversion in hyperthermophilic Archaea differs from the canonical glycolytic pathways, involving novel enzymes and unique control mechanisms. In recent years, we have focused our attention on three novel glycolytic enzymes, *viz*. ADP-dependent glucokinase, ADP-dependent phosphofructokinase, and glyceraldehyde-3-phosphate oxidoreductase. These enzymes were first discovered in *Pyrococcus furiosus* but have now been found to be distributed among a variety of Archaea. These enzymes show unique functional and structural features. By using advanced search programs on available archaeal, bacterial and eucaryal sequence projects, we have recently completed the set of glycolytic enzymes from *P. furiosus*. In addition to the aforementioned novel types, some other glycolytic steps are catalyzed by enzymes that differ substantially from their canonical counterparts, *viz*. glucose-6-phosphate isomerase, fructose-1,6-bisphosphate aldolase and phosphoglyceromutase. With this complete data of glycolytic enzymes we are in the position to elucidate the regulation of the complete glycolytic pathway in *P. furiosus* both on transcriptional as protein level.

L14
Isolatie van genen coderend voor natief aardappelzetmeel afbrekende enzymen van *Bacillus firmus/lentus* en *Microbacterium* sp.

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Natief aardappelzetmeel bestaat uit amylose- en amylopectine-bevattende zetmeelkorrels die voor veel micro-organismen moeilijk af te breken zijn. Bij het voormalige NIKO-TNO zijn de twee bacteriestammen *Bacillus firmus/lentus* en *Microbacterium* sp. geïsoleerd, die natief aardappelzetmeel efficient kunnen afbreken en de afbraakprodukten kunnen gebruiken als substraat voor groei. *Bacillus firmus/lentus* bezit zeker zes extracellulaire enzymen die betrokken zijn bij de afbraak van zetmeel, waarvan vier ook natief zetmeel afbreken. In tegenstelling tot *Bacillus firmus/lentus* gebruikt de *Microbacterium*-stam maar twee enzymen voor de afbraak van natief zetmeel. Het doel van dit onderzoek is de verschillende genen in kaart te brengen die coderen voor de enzymen betrokken bij de afbraak van zetmeel. Om dergelijke genen te isoleren zijn genomische banken gemaakt van 3-12 Kb DNA-fragmenten in een expressievektor, waarna een screening volgde met behulp van cibacron-roodgekleurde-amylopectine agarplaten. Afbraak van amylopectine leidt tot het ontstaan van een ophelderingszone. Voor *Bacillus* zijn 23.000 kolonies gescreened, waarbij twaalf transformanten positief bleken te zijn; voor *Microbacterium* zijn 7.000 kolonies gescreened, waarvan twee kolonies in staat bleken om amylopectine af te breken.

M01
Regulation of iron uptake in *Helicobacter pylori* by the Fur protein

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Acquisition of iron is essential for bacterial survival and growth. Hence, iron-restriction by the human host is a non-specific host defence mechanism. The gastric pathogen *Helicobacter pylori* synthesizes haem- and lactoferrin-binding proteins upon iron restriction. Iron acquisition systems in Gram-negative bacteria are mostly regulated through the Ferric Uptake Regulator protein (Fur). Analysis of the genome sequence of *H. pylori* has shown the presence of a Fur homolog, and of six homologs of iron acquisition systems. At the molecular level neither function nor regulation of these genes has been described. Our aim was to determine the role of Fur in regulation of iron acquisition systems of *H. pylori*. A promoterless *lacZ* reporter gene was fused to the six *H. pylori* iron acquisition genes (*frpB* homologs Hp0876, Hp0916 and Hp1512 and *fecA* homologs Hp0686, Hp0807 and Hp1400). The effect of a mutation in the Fur gene was determined by comparing the expression of the reporter gene fusion in both strains after growth on plates supplemented with lysed horseblood, using a quantitative (galactosidase assay. The inactivation of Fur showed differential effects on the expression of the *fecA* and *frpB* homologs. Expression of Hp1512 and Hp1400 was not affected by the Fur mutation, whereas the very low expression of Hp0876 and Hp0807 in wildtype *H. pylori* was more than 20-fold increased in the *H. pylori* fur mutant. Hp0916 and Hp0686 were expressed at an intermediate level in wildtype *H. pylori*, but their expression was increased 3-fold in the *H. pylori* fur mutant. We demonstrate that *H. pylori* uses Fur to regulate genes involved in iron acquisition. *H. pylori* expresses a basal level of iron acquisition proteins, which can be increased when necessary. This is in contrast with many of the enteric bacteria which repress iron uptake upon increased availability of iron. Fur-regulation of iron acquisition provides *H. pylori* with the ability to adapt to varying levels of iron compounds in the gastric mucosa.

M02
Antimicrobial activity of single chain antibodies against *S. mutans* coupled to glucose oxydase

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Enzymes can be used as antimicrobials. A problem however is their low specificity and low substantivity. Antibodies on the contrary are highly specific and have a relatively high

substantivity, e.g. in the oral cavity. However, their toxic properties are in general limited. Covalent coupling of toxic compounds to antibodies could combine the positive effects of these molecules and result in novel 'Magic bullets'. For application in oral care products chemical coupling seems not a feasible option in view of safety and cost aspects. An alternative approach is the production of fusion proteins combining the binding domain of an antibody with a protein having bactericidal capacity. Recently, it has been found that binding domains derived from llama 'heavy chain' antibodies (VHH fragments) are particularly suited for such an approach. Llamas produce active antibody molecules that are composed of heavy chain dimers only. The advantage of these Llama-heavy-chain antibodies is that by using molecular biological techniques, fusion proteins can be made with bactericidal capacities.
A llamas were immunized with *S. mutans* strain HG982. B-lymphocytes were isolated and via RT-PCR cDNA fragments encoding for the VHH fragments were obtained. A glucose-oxidase-gene (Gox) was linked to the VHH gene and cloned into the yeast *Saccharomyces cerevisiae*.
The antibodies, tested for specificity against *S. mutans* and *S. sanguis* strains in an ELISA, showed a high specificity for *S. mutans* strains.
The antibodies were tested for their antimicrobial activity in a system that contained glucose, lactoperoxidase and potassiumiodide. *S. mutans* strains were killed (> 99.99% kill using 10^8 bacteria/ml) within 15 minutes, while *S. sanguis* was not affected. Based on these results, we conclude that specific single-chain antibodies, linked to Gox and in the presence of glucose, lactoperoxidase and iodide, are able to selectively kill *S. mutans* cells.

M03
Characterization of transcriptional phase variation in a type III restriction modification system of *Helicobacter pylori*

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The on- and off-switching of the expression of virulence factors (phase variation) plays an important role in the pathogenesis of many bacterial infections. Here, we report for the first time transcriptional on- and off-switching of gene expression in *H. pylori*. A library with random genomic transcriptional *lacZ* fusions in *H. pylori* 1061 was screened for mutants showing blue, white and sectored colonies on X-Gal. X-Gal is converted into a blue product by β-galactosidase, the expression product of the *lacZ* gene. The presence of sectored colonies indicate a fusion of the *lacZ* to a gene that displays transcriptional phase variation. Sequence analysis of one mutant with sectored colonies showed that the lacZ gene was fused to a putative type III methylase gene *(mod)*. 13 out of 483 colonies switched from on (blue) to off (white) (2.7%), while 12 out of 1,603 colonies switched from off to on (0.75%). RNA spot blot hybridization and RT-PCR demonstrated that only blue colonies and not white colonies transcribed the *mod* gene. An open reading frame encoding a putative type III restriction enzyme gene *(res)*, was located immediately upstream of *mod*, and contained a polynucleotide C-tract. C-tracts direct translational phase variation in various bacterial systems. No relationship was found between the number of cytosines in the C-tract of *res* and the switching of *mod*, indicating that the C-tract does not affect *mod* transcription. In *H. pylori*, a putative type III methylase gene displays phase variation at the transcriptional level. It is known that methylation of promoter sequences can affect the transcription of bacterial virulence factors. We propose a specific role for *mod* and related restriction-modification systems in *H. pylori* in the regulation of the expression of virulence genes.

M04
Genetic polymorphism in the *agr* locus of *Staphylococcus aureus* strains

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The synthesis of virulence factors in *S. aureus* is controlled by a regulatory system, the *agr* locus. The extracellular inducer of this system is a modified peptide, encoded by *agrD* of which the structure varies among the various *S. aureus* strains. The *agr*-PCR RFLP patterns of unique *S. aureus* strains (n=192) were analyzed. Distinct patterns (n=12) were sequenced, to count to the three *agr*-groups described before. Two major *agr* types occurred among the meticillin-resistant *S. aureus* (MRSA) strains, and the prevalence of each type was related to the geographical origin. Amplicons were sequenced and analyzed for homologies. The intragroup sequence similarity ranged from 96 to 100% and varied from 75 to 80% between *agr*-groups. Divergent sequence variability among the different genes of the *agr* locus was observed. The conserved *agrD* octapeptide sequence within each *agr*-group confirmed the results of former studies, indicating the importance of the *AgrD* structure with respect to the ligand-receptor specificity for *agr* activation. Conceivable effect of *agr*-polymorphism on bacterial interference was measured among strains derived from persistent and intermittent carriers. Considering the definition of nasal *S. aureus* carriership, it may be concluded that the simultaneous presence of *S. aureus* strains harboring different *agr*-groups seems to exclude competitive colonization conflicts between strains.

M05
Helicobacter pylori genes needed for growth under acidic conditions typical of the gastric mucosa

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The ability to grow under the acidic conditions that are characteristic of the human gastric mucosa (pH 5) ought to be important for the ability of *Helicobacter pylori* (Hp) to colonize and persist in this special niche. We found that urease activity is not needed for the growth at pH 5, unlike the resistance to severe acid shock. To identify genes needed for mild acid resistance a library of 1,200 independent mutants was screened for mutants unable to grow at pH 5. Ten different acid-sensitive mutants were recovered. All grew normally at pH 7, had normal urease activity, and were normally resistant

to brief exposure to pH 3.5. Plasmid rescue and DNA sequencing were used to identify the disrupted 'mild acid resistance genes'. They were divided into four functional groups: energy metabolism, DNA repair, trans-membrane transport and function unknown. One gene, although present in the genome of strain 26695, was absent from that of J99, even though these two strains have equivalent acid resistance. This indicates redundancy in pathways for this important attribute. The plethora of genes contributing to the mild acid resistance of Hp and the apparent redundancy reinforce our view of Hp as an organism uniquely adapted to the acidic environment of the gastric mucosa. This vision is currently being tested with appropriate small animal models.

M06
Selection of recombinant antibodies specific for pathogenic *Streptococcus suis* by subtractive phage display

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A semi-synthetic antibody phage display library was used to select recombinant antibodies directed against surface components of a pathogenic strain of *S. suis* serotype 2 and against EF, a protein known to be exclusively associated with pathogenic *S. suis* serotype 2 strains. Three unique monoclonal phage antibodies directed against conformational epitopes of surface protein components of *S. suis* were selected. In addition, three different monoclonal phage antibodies were isolated that recognized EF. To isolate antibody fragments that recognize epitopes specific for a pathogenic *S. suis* serotype 2 strain, compared to a non-pathogenic serotype 2 strain, we applied a subtractive selection procedure. With this procedure only one unique phage antibody was found, and it was shown to be directed against EF. This demonstrates the selectivity of the applied procedure. It also demonstrates that EF is a major immunogenic component determining the difference between pathogenic and non-pathogenic strains of *S. suis* serotype 2.

M07
A novel *Salmonella typhimurium* protein involved in resistance against macrophages oxidative stress

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Aerobically growing micro-organisms have evolved elaborate genetic systems to detoxify reactive oxygen species such as superoxide that are formed as byproducts of normal metabolism. In *Escherichia coli*, several of such systems have been identified such as the *sox-R/S* and *mar-A* regulon that respond to oxidative stress by activation of several genes involved in a protective response. Superoxide also serves as a microbical effector mechanism of the macrophage, and is produced after activation of the NADPH oxidase. To study the role of oxidative stress regulons in the resistance of bacteria to oxidative killing by macrophages, *E. coli* may not be an optimal choice since *E. coli* are rapidly killed after phagocytosis. In this respect, *Salmonella typhimurium*, a homologous bacterium, seems more relevant because it is able to survive and even replicate inside macrophages. By random chemical mutagenesis, we obtained *S. typhimurium* mutants that are resistant against oxidative stress. By *in vivo* cloning, the gene that conferred this phenotype was isolated. The gene, designated *rox-A* (resistance against oxidative stress), was located about 12 minutes of the *S. typhimurium* chromosome and not present in *E. coli*. Sequence homology with Sox-S and Mar-A suggested that Rox-A is a transcriptional activator in an oxidative stress regulon. This was confirmed by bandshift assays showing that Rox-A is a DNA binding protein. In conclusion, *S. typhimurium* has an additional oxidative stress regulon, compared to *E. coli,* that may be relevant to withstand the antimicrobial action of phagocytic cells.

M08
Identification of *Helicobacter mustelae* virulence factors by screening of a random insertional mutant library

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Background: *Helicobacter mustelae* infection of ferrets has been associated with gastritis, duodenal ulcer disease and gastric cancer. Although experimental *Helicobacter pylori* infections have been developed, natural infection has only been described in humans and non-human primates. Therefore infection of ferrets with *H. mustelae* is recognised as an important natural animal model of *H. pylori* infection in humans. The aim of this study was to develop a method for generating insertion mutants in *H. mustelae* with a view to identifying genes important in a natural *Helicobacter* infection.
Methods: *H. mustelae* chromosomal DNA was digested with *Cla*I and self-ligated to create circular DNA. This DNA was then re-digested with *Bgl* II and recircularized by ligation with the *aphA-3* kanamycin resistance cassette. The resultant *aphA-3* containing circles were then naturally transformed into *H. mustelae* strain NCTC 12032. Successful introduction gives rise to homologous cross-over with corresponding genes in the chromosome, resulting in disruption of a gene and conferring kanamycin resistance. Mutants were selected on blood agar plates containing kanamycin.
Results: Natural transformation of *H. mustelae* with the constructs resulted in 500 kanamycin resistant transformants per µg DNA. This indicates that *H. mustelae* is naturally competent for transformation with DNA. A Southern blot with 12 randomly selected transformants probed with the kanamycin cassette showed that the constructs integrated into different sites in the *H. mustelae* chromosome, suggesting random integration. An initial mutant library of 500 mutants was created and screened for the absence of virulence factors. Screening for urease activity using Christensens broth has revealed two urease deficient mutants.
Conclusion: In this study we describe a method for generating a mutant library in *H. mustelae*. The ability to screen for *H. mustelae* mutants attenuated in virulence both *in vitro* and *in vivo* represents an important opportunity to investigate the pathogenesis of gastric *Helicobacter* species in their natural hosts.

M09

Expression analysis by NASBA of the *Chlamydia trachomatis* virulence genes Hsp60, Hsp70 and CT-MIP in symptomatically versus asymptomatically infected patients

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We investigated whether there is a difference in constitutive expression profiles for the virulence genes Hsp60, Hsp70 and CT-MIP between serovar E strains of *Chlamydia trachomatis* (CT) with a symptomatic or an asymptomatic course of infection. Primers for the three targets were selected and evaluated to detect only CT RNA of all CT serovars without detecting 13 other potential urogenital pathogens, human homologs or other *Chlamydia* species. The RNA amplification system NASBA showed, using run-off transcript of the cloned virulence genes, a sensitivity of 10-100 molecules for all three targets. Since the expression profiles might be CT-cell cycle-dependent, eight different time points in the CT bimorphic lifecycle were analyzed in an *in vitro* cell culture system. Six strains (3 symptomatic and 3 asymptomatic) were analysed. For the three virulence genes analysed, no expression differences between symptomatic and asymptomatic strains were found. Further studies could focus on possible host immune influences on the transcription levels and the clinical course of infection. Finally, the expression of these virulence genes might be regulated on the translation level rather than on the transcription level.

M10

Identification of *comH:* a novel, *Helicobacter pylori*-specific transformation gene

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Helicobacter pylori is naturally competent for transformation, which means that it can take up extracellular DNA and incorporate it in its genome. To identify genes that are essential for transformation, we screened a random mutant library of *H. pylori* for transformation-deficient mutants by natural transformation with chromosomal DNA of a clarithromycin resistant strain (ClaR DNA). This screen resulted in the identification of one mutant that was impaired in transformation. In this mutant the ORF HP1527 was disrupted. Site-directed mutants in HP1527 were constructed in strain 1061 and strain 26695 by insertion of the *aphA-3* kanamycin resistance cassette. Like the original library mutant, these mutants were not competent for uptake of DNA nor for uptake of plasmids. Complementation of HP1527 *in trans* restored the normal transformation frequency, which indicates that it is a gene essential for transformation. Therefore, we propose the name *comH* (**com**petence of **H***elicobacter*) for gene HP1527. No homologs of this gene are present in any other sequenced bacterial species, including many transformable species, which indicates that the competence system of *H. pylori* has unique properties.

M11

Recombinant H-TC, a thrombocidin-derived peptide antibiotic

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Pathogenic microorganisms are increasingly gaining resistance against commonly used antibiotics. Possible alternative antimicrobials may be derived from microbicidal proteins such as thrombocidins (TCs). TCs are microbicidal proteins with extensive homology to CXC-chemokines, stored in and released from human blood platelets. Recombinant H-TC (rH-TC), derived of TC-1, had a potent, broad-range microbicidal activity against Gram-positive and Gram-negative bacteria, and was fungicidal for *Cryptococcus neoformans,* but not for *Candida* species. Microbicidal concentrations generally were between 0.5 and 2 µM. rH-TC activity was salt and serum sensitive, but was independent of pH. Bacterial susceptibility to rH-TC was independent of metabolic activity, independent of the presence of anionic phospholipids in the cytoplasmic membrane of an *Escherichia coli* test strain, moderately dependent on the presence of a membrane potential and was severely decreased at low temperature. These results indicate that electrostatic interactions between peptide and bacterial surface are decisive for cidal activity. rH-TC had detectable hemolytic activity at high concentrations. In contrast to natural TCs, rH-TC had no neutrophil chemotactic activity, and may have potential for further development towards a clinically applicable antimicrobial agent.

M12

Helicobacter pylori gene expression induced upon interaction with gastric epithelial cells

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Helicobacter pylori, a gram-negative spiral-shaped microorganism, colonizes the gastric mucosa of about 50% of the human world population. *H. pylori* is the major cause of chronic gastritis and is also an important etiological factor in the pathogenesis of peptic ulcer disease, gastric carcinoma and gastric lymphoma. The natural niche of *H. pylori* is the stomach, where the bacterium has to be adapted to extreme conditions like low pH and low iron availability. *H. pylori* colonizes the gastric mucosa by adhering to the mucous epithelial cells. To study *H. pylori* gene expression in its natural niche, and in association with the gastric epithelium, a random cat reporter gene fusion library in *H. pylori* was constructed. Differences in expression of the cat reporter gene between *H. pylori* grown in close association with gastric epithelial cells and *H. pylori* grown in the absence of epithelial cells will be determined.

M13
Impaired mobility of human neutrophils in response to cryptococcal mannoproteins

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Cryptococcal meningitis patients establish high levels of glucuronoxylomannan and mannoproteins (MP) in CSF and serum. These fungal antigens induce the production of proinflammatory cytokines TNFa, IL1b, IL6 and IL8. Despite the fact that cytokine levels are higher in CSF than plasma, CSF leukocyte-counts are remarkably low. Glucuronoxylomannan is held responsible for this inhibition of migration. Investigating the inhibition underlying mechanism, we surprisingly detected that culture filtrate from acapsular strains (a-CneF) prevented neutrophil migration in a trans-well assay even at lower concentrations than culture filtrate (CneF) from glucuronoxylomannan-producing strains. Here we show the following: *(i)* MP are responsible for the activity found in a-CneF. MP strongly inhibit neutrophil migration towards different chemoattractants (IL8, fMLP, PAF and C5a) in a trans-well assay. *(ii)* MP have intrinsic chemo-attractive activity, both in trans-well and under-agarose assays. *(iii)* MP strongly induce neutrophils to shed L-selectin, and cause increased surface expression of CD11b and CD18, molecules playing important roles in extravasation. These effects are cell-type specific: they are not observed on monocytes, where Lewis antigen CD15 is upregulated. *(iv)* Stimulation of neutrophils with MP4 strongly induces Ca-mobilization. These findings indicate that *Cryptococcus neoformans* produces a virulence factor that impairs chemotaxis towards infected sites.

M14
The neutrophile activating protein of *H. pylori* is a DNA binding protein essential for growth at pH 5

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It has been suggested that the neutrophil activating protein (NAP) of *Helicobacter pylori* is a DNA-binding protein that is essential for survival of acid shocks. In this study we determined the role of NAP in the growth of *H. pylori* at low pH. Isogenic NAP$^-$ mutants were created in *H. pylori* strains NCTC 11637 and 1061 by allelic replacement; mutants were tested for growth at pH 5. To measure the binding of NAP to DNA, a microwell plate was coated with either *H. Pylori* or *Klebsiella* sp. chromosomal DNA. Binding of purified recombinant NAP (rNAP) at various pH was determined by ELISA with a polyclonal antiserum raised against rNAP. In contrast to the parental strains, the NAP$^-$ mutants in both *H. pylori* strains were unable to grow at pH 5. The binding of NAP to *Klebsiella* sp. chromosomal DNA was identical to the binding to *H. pylori* DNA. Interestingly, the binding of NAP to chromosomal DNA was highly dependent on pH. Binding of NAP to DNA was maximal at pH 5, and decreased gradually with increasing pH, to reach the lowest level at pH 7, where almost no binding was observed. To investigate the putative function of NAP during growth at low pH we determined whether NAP is able to protect DNA from damage. Prelimenary results show that the NAP$^-$ mutant displayed a significant decrease in its survival after exposure to UV light compared to the parental strain. From this study we conclude that NAP is a DNA binding protein which is essential for the growth of *H. pylori* low pH. Binding of NAP to DNA is highly dependent on pH, but is not restricted to DNA from *H. pylori*. The function of NAP seems to be the protection of DNA. As the binding of NAP to chromosomal DNA is optimal at pH 5 and loss of NAP is lethal for *H. pylori* under acidic circumstances, we propose that the function of NAP is to protect the bacterium from lethal DNA damage induced by low pH.

N03
Risks involved in the veterinary use of antibiotics

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The widespread and non-selective use as well as the incorrect use of antiparasitic drugs speed up the development of resistance of pathogens. The threat posed by resistant strains and the concern that resistance is leading to increased losses and treatment costs are by no means new. The potential for the development of antibacterial resistance of zoonotic pathogens and their transfer to man is a matter of special concern. Therefore, prudent use of antibiotics in animals and, equally important, effective surveillance of antibiotic resistance are necessary. Antibiotics should be 'prescription only' medication for curative and rational preventive use. A code of practice (GVP) on when and how drugs should be prescribed was introduced in 1990. The selective and correct use of drugs should be targeted on the basis of an accurate diagnosis of the condition, its pathophysiology, the antibiotic sensitivity of the pathogen involved, the bioavailability and pharmacokinetic properties of drugs and knowledge of potential side effects. Equally important are management strategies to prevent outbreaks of infectious diseases such as: a closed farming system, an 'all in-all out' procedure, the proper use of vaccins, desinfection of pens, control of rodents and other vermin, strict separation between sections, a good housing system, and food and water of good quality.

N05
Health risks of foodborne pathogens

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Foodborne infections and microbial intoxications are a serious health problem in the Netherlands, causing an estimated one half to one and a half million illnesses per year. The causes of foodborne illness include bacteria, viruses and parasites, and the symptoms range from mild gastro-enteritis to life-threatening syndromes. Products from animal origin play an important role as they are often contaminated with foodborne pathogens and enter the homes in the raw state. Improvement of the situation is actively pursued by introducing integrated control measures throughout the production chain, from the farm to the consumer.
Processed foods are less frequently incriminated in foodborne diseases. However, there are trends towards less heavy processing to produce so-called minimally processed foods. In these products with a limited shelf life, selected pathogens may survive and may show an adaptive response to the food

environment, eventually allowing growth to occur. This scenario will be highlighted with a number of recent outbreaks of foodborne disease.
Characteristically this type of outbreaks is not always easily recognized, as single cases often seem unrelated according to location and/or time of occurrence. That is why so-called early-warning surveillance systems are now being put in place. In these systems, modern molecular and other typing methods are used to detect identical isolates from patients distributed over a wide geographical area. Combining these data with food anamnesis of patients allows the early detection of the incriminated food product as well as its producer.

N11
Beschrijving van gevallen van gastro-enteritis waarvoor de huisarts wordt geconsulteerd

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Van 1996 tot 1999 is onderzoek uitgevoerd onder patiënten in de huisartsenpraktijk naar de incidentie van gastro-enteritis en een breed panel van veroorzakende micro-organismen. Alle patiënten die de huisarts consulteren met gastro-enteritis werden geturfd. Daarnaast werden consulterende patiënten met en zonder gastro-enteritis gevraagd deel te nemen aan een patiënt-controle-onderzoek, door middel van het invullen van een vragenlijst en het insturen van faecesmonsters. De incidentie van gastro-enteritis waarvoor een huisarts werd geconsulteerd was 79,7 per 10.000 persoonjaren. In totaal werd in 37,5% van de monsters van patiënten en in 9,8% van controles een pathogeen aangetoond. *Campylobacter* werd het meest frequent pathogeen (10% van patiënten), gevolgd door *Giardia lamblia* (5%), rotavirus (5%), Norwalk-like virussen (5%) en *Salmonella* (4%). In Nederland consulteren jaarlijks circa 128.000 personen hun huisarts voor gastro-enteritis. Dit is iets minder dan in een vergelijkbare studie in 1992-1993. Een pathogeen kon worden aangetoond in bijna 40% van de patiënten (bacteriën 16%, virussen 15%, parasieten 8%).

R01
Aspergillose bij vogels in de collectie van Diergaarde Blijdorp

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Aspergillose is in de vogelcollectie van Diergaarde Blijdorp één van de belangrijkste bevindingen bij postmortaal onderzoek. In de periode 1995-1999 is bij 51 vogels (7,9%) van de 644 voor sectie aangeboden dode vogels een schimmelinfectie veroorzaakt door een *Aspergillus* sp. aangetroffen.
Tijdens deze presentatie wordt ingegaan op de transmissie, predispositie, pathogenese, pathologie, diagnose, behandeling en preventie van *Aspergillus*-infecties bij vogels.
Bij deze punten komt steeds de vraag terug of er sprake is van een verminderde weerstand dan wel een verhoogde infectiedruk.

R02
Comparison of phagocytosis of black yeasts by human neutrophils

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Phylogenetically closely related members of the ascomycetal black yeast show highly divergent pathogenicity. Therefore, we compared phagocytosis, and evoked oxidative burst and killing of *Exophiala bergeri, E. castellanii, E. dermatitidis* (wild type and mel-), *E. jeanselmei, E. mesophila, E. spinifera, Hortaea werneckii* and *Phaeoannellomyces elegans* by human granulocytes, using flow cytometry in seven independent experiments. *Candida albicans* and *Saccharomyces cerevisiae* were included as controls. Phagocytosis and evoked oxidative burst showed a similar extent for all fungi studied. Phagocytosis could be blocked by about 50% when granulocytes were preincubated with mAbs CD11b/18 in case of *E. dermatitidis*, indicating involvement of the iC3b-receptor Mac-1.

R03
Eumycetoma due to *Madurella mycetomatis:* current laboratory experience

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Madurella mycetomatis is the commonest cause of eumycetoma in Sudan and other countries in tropical Africa. Currently, the early diagnosis of mycetoma can be difficult. In an attempt to improve the diagnosis of mycetoma we have developed specific oligonucleotide primers based on the sequence of the internal transcribed spacer (ITS) region in the operon encoding the genes for the fungal ribosomal RNA. The ITS regions were amplified using universal primers, sequenced, and then two sets of species-specific primers were designed that specifically amplify parts of the ITS and the 5.8S rDNA gene. The new primers were tested for specificity using DNA isolated from human mycetoma lesions and DNA extracted from cultures of *M. mycetomatis* reference strains and related fungi as well as human DNA. To study the genetic variability of the the ITS regions of *M. mycetomatis,* ITS amplicons were obtained from 25 different clinical isolates and subjected to restriction fragment length polymorphism (RFLP) analysis using *Cfo*I, *Hae*III, *Msp*I, *Sau3*AI, *Rsa*I and *Spe*I restriction enzymes. RFLP analysis of the ITS region did not reveal even a single difference, indicating the homogeneity of the tested isolates.

R04
NASBA-based detection of *Candida* spp. in blood cultures

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Introduction: *Candida* spp. have become increasingly important causes of infection in immunocompromised patients. Different *Candida* spp. show differences in resistance against antimycotic drugs. Therefore, a rapid detection and identification is essential. Many of the automated blood culture systems are inadequate. We investigated whether Nucleic Acid Sequence-Based Amplification (NASBA) can be used to improve the detection rate of *Candida* spp. after a culture step.
Methods & Results: RNA was extracted from 1 ml samples of BacT/Alert blood-culture bottles. For the NASBA-assay, general yeast/fungi primers directed against the 18S rRNA and species-specific biotinylated probes were used. We tested culture-positive as well as -negative blood cultures from ten patients with a proven candidaemia and compared the results of BacT/Alert monitoring with the results of NASBA-based detection of yeast RNA. In 34% of the 92 blood cultures tested, *Candida*-RNA was detected with the NASBA, whereas only 21% was positive in the BacT/Alert monitoring system. All BacT/Alert-positive cultures were also positive in the NASBA-assay.
Conclusion: Using NASBA, we were able to improve the detection rate of *Candida* spp. in blood cultures compared to BacT/Alert monitoring alone.

R05
A new look at *Acremonium* nail infection

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More than 20 years ago, Nardo Zaias pointed out that superficial white onychomycosis caused by *Acremonium*-like species was common. He called the causative agent *Cephalosporium roseogriseum*. Isolates sent to CBS, however, were all identified as *Acremonium potronii* by Dr. W. Gams. This corrected identification was mentioned only in notes for A. potronii in Gams' monograph, *'Cephalosporium*-artige Schimmelpilze (Hyphomycetes)' (Gustav Fischer Verlag, Stuttgart, 1971) and remained little known. Recently, we observed that if small patches of superficial white onychomycosis were critically studied, *Acremonium* infection was surprisingly common. Conventional laboratory mycology often failed to recognize these infections, in part because direct microscopy was often false-negative for fungal elements. With unusually intensive searching in scraped material, however, heavily colonized nail fragments could be found. The counting and statistical analysis of positive inocula helped to clarify significant cases: for *Acremonium,* if 5 out of 15 planted inoculum pieces from nail (nail flakes from scraping) were positive for consistent growth, there was a more than 90% chance of significant infection. Cases of distal-lateral subungual onychomycosis were also found. Species other than *A. potronii* were often involved.

R06
'Rotterdamse' schimmels

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Een overzicht wordt gegeven van de in 1999 geisoleerde schimmels op het Regionaal Medisch Microbiologisch Laboratorium van het Zuiderziekenhuis te Rotterdam. De nadruk ligt hierbij op isolaten die werden doorgestuurd naar het CBS voor nadere determinatie: welke isolaten werden doorgestuurd, en waarom? Enkele van de meer zeldzame isolaten zullen uitgebreider worden besproken, waaronder *Lecythophora* spp.

R07
Evaluation of different media for the growth of filamentous fungi with a microdilution kinetic model

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Different media have been used to culture filamentous fungi. RPMI1640 buffered with MOPS has been chosen by NCCLS for the antifungal susceptibility testing of these fungi. In this study four different media antibiotic medium 3 (AM3), yeast nitrogen base medium (YNB), liquid sabauraud SAB, RPMI1640 and RPMI1640 with 2% glucose were evaluated for the growth of filamentous fungi in order to compare the growth kinetics between the media.
A kinetic micodilution model was used for the evaluation of the growth of five strains of *Aspergillus fumigatus* and *Scedosporium prolificans* in different media. 100 µl of twofold concentrated media where inoculated with 100 µl of conidia suspensions in water containing 0.02% Tween 80. The final inoculum was adjusted to 1-5 x 10^4 CFU/ml. The fungi were incubated in flatbottom wells at 37 °C for 99 hours and the optical density (OD) of each well was measured every 15 minutes at 405 nm. The changes of OD during the time were represented graphically. The maximal growth and the maximal rate of growth with the corresponding time points as well as the time when the first growth was detected for each medium were calculated. Each strain was tested in triplicate, and the average values for each medium and for each species were used for the comparisons.
With YNB medium the germination was delayed for two hours in case of *Aspergillus* strains and for eight hours for *Scedoporium* strains compared with other media. Although the time of maximal growth was between 90-99 hours for both species, the maximal growth as well as the maximal rate of growth in RPMI (with or without glucose) was two to four times less than the other media for *Aspergillus* strains, with SAB giving the highest growth, followed by YNB. For *Scedosporium* strains the SAB provided the highest growth followed by AM3 despite the fact that YNB was the poorest medium providing growth less than in RPMI.
These results show that the common used medium for the antifungal susceptibility testing of filamentous fungi, RPMI1640 buffered with MOPS, does not provide the highest fungal growth, which may be obtained with different media for each species.

R08
Aspergillus in tabak

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Immuungecompromitteerde patienten hebben een verhoogd risico op invasieve aspergillose. Inhalatie van aerogene sporen is de belangrijkste transmissieroute voor infectie. Roken van sigaretten zou een risicofactor zijn, maar er is weinig bekend over het voorkomen van *Aspergillus* in tabak. In dit onderzoek zijn 11 Nederlandse sigaretten- en shagmerken en 1 Tanzaniaanse onderzocht op het voorkomen van *Aspergillus*.
Methode: Aan de tabak werd 26 ml 0,2% Tween 20 toegevoegd. Na vijf uur schuddden werd 10 ml gecentrifugeerd en in 0,5 ml gedestilleerd water opgelost. Dit concentraat werd uitgeplaat op Sabouraud-dextrose-agar (SDA). Aan de overgebleven tabakssuspensie werd vloeibaar Sabouraud-medium toegevoegd. Van ieder sigarettenmerk werden zes sigaretten gekweekt. Alle handelingen vonden plaats in een Flow-kast.
Resultaten: De kweekresultaten per sigarettenmerk waren: Barclay: 2 van de 6 sigaretten bevatten *Aspergillus* spp. (1 sigaret bevatte *A. fumigatus*); Caballero: 6/6 (1); Camel: 4/6 (4); Camel light: 3/6 (3); Drum: 5/6 (5); Gauloise: 4/6 (1); Gladstone: 1/6 (1); Marlboro: 3/6 (3); Marlboro light: 2/6 (0); Samson: 2/6 (2); Sportsman: 5/6 (4); Stuyvesant: 5/6 (2). Overige schimmels die aangetroffen werden: *Rhizopus* spp. (6x), *Acremonium* spp. (2), *Microascus* spp. (2), *Penicillium* spp. (2), *Mucor* spp. (1), *Scedosporium prolificans* (1), *Ascomyceet* (5), ongedetermineerde filamenteuze schimmel (4). Per sigaret (700 mg) werden tussen 4 en 70 CFU's gekweekt.
Conclusie: Viabele sporen van verschillende schimmels waaronder *A. fumigatus* zijn aanwezig in sigarettentabak. Of deze bij het roken kunnen worden ingeademd wordt nader onderzocht.

POSTERS

P01
The occurrence of *Helicobacter pylori*-negative peptic ulcer disease (PUD): trends over a period of eight years

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In some patients with PUD, neither *Helicobacter pylori* nor NSAIDs can be incriminated (idiopathic PUD). It has been suggested that idiopathic PUD is more common than previously assumed and that the decrease in prevalence of *H. pylori* infection may unmask this condition. To investigate the frequency of *H. pylori*-negative PUD we studied all endoscopy reports with the diagnoses gastric ulcer (GU) and/or duodenal ulcer (DU) over an eight year period (1991-1998; n=451: GU=164, DU=264, GU+DU=23). Patients were considered *H. pylori* infected if any test – culture, histology or rapid urease test – was positive. Hospital files of all *H. pylori*-negative patients were examined for possible NSAID use. In 405 patients tested for *H. pylori* infection, 340 patients were *H. pylori*-positive (84.0%; GU=114, DU=206, GU+UD=20) and 65 patients were *H. pylori*-negative (16.0%; GU=34, DU=19, GU+DU=2), of whom 27 (6.7%; GU=15, DU=12) used NSAIDs and 38 (9.4%; GU=19, DU=17) did not. Timetrends were calculated by linear regression analysis. The occurence of *H. pylori*-negative PUD with NSAID use increased over the past eight years while the prevalence of idiopathic PUD did not (p=0.004 and p=0.238 respectively). The prevalence of *H. pylori*-negative PUD was increasing, due to an increase of NSAID associated ulcera. The prevalence of idiopathic PUD remained the same over our study period: 9.4%.

P02
The production of the bacteriocin amylovorin is not widespread among strains of the *Lactobacillus acidophilus* group

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Bacteriocin production is one of the properties responsible for the antibacterial activity of lactic acid bacteria against closely related species and possibly food spoilers and pathogens. Almost all members of the *Lactobacillus acidophilus* group produce bacteriocins. Lactobin A and amylovorin L471 are two bacteriocins produced by *Lactobacillus amylovorus* LMG P-13139 and *L. amylovorus* DCE 471, respectively. A 110-bp fragment of the structural lactobin A gene was obtained and sequenced using degenerate primers based on the lactobin A amino acid sequence. With this PCR fragment as a probe, a 3.6-kb *Hind*III chromosomal DNA fragment of the size expected from hybridization experiments was isolated. Partial sequencing of this fragment revealed the nucleotide sequence of the structural gene of lactobin A. Subsequently, the structural bacteriocin genes of lactobin A and amylovorin L471 were compared by PCR amplification by using specific primers based on the nucleotide sequence of the lactobin A structural gene. Sequencing of the amplified fragments revealed that both structural genes were identical.
Among 38 strains of the *L. acidophilus* DNA homology group, another six *L. amylovorus* strains were inhibitory towards *L. delbrueckii* subsp. *bulgaricus* LMG 6901^T, the strain that was most sensitive to amylovorin L471. However, the lactobin A or amylovorin L471 structural genes could not be detected by stringent PCR amplification conditions,

indicating that the inhibitory substances were slightly different. Bacteriocinogenic material could be isolated from these strains using a novel and simple isolation protocol.

P03
Changes of the microbial flora of patients during hospitalization

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The aim of the study was to establish which species and body sites can be used to monitor transmission of microorganisms among patients during their admission to standard clinical departments. Twenty patients from the departments of internal medicine, infectious diseases and neurology were included. Swabs from nose, pharynx, sternal skin, perineum and rectum were taken within 24 hours and after 5-7 days of admission and inoculated onto bloodagar, CLED and Rainbow® Agar UTI plates. After incubation, colonies with aspects of *Enterobacteriaceae,* enterococci, *Staphylococcus aureus,* and non-fermenting Gramnegative bacteria were identified to species by phenotypic tests. Strain identification of isolates of *Escherichia coli, Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. was done by RAPD typing. In total, 485 colony variants were obtained and identified to 24 species. *E. coli* was the predominant species followed by *S. aureus* and *Enterococcus faecalis*. During admission the total number of *Enterobacteriaceae* decreased, while the number of enterococci increased significantly. The 208 *E. coli* isolates recovered were allocated to 57 RAPD types (strains). Perineum and rectum were significantly more colonized with these strains than nose and pharynx. Three possible cases of *E. coli* strain transmission were found. For *Klebsiella*, *Proteus* and *Pseudomonas* spp. most isolates were found in perineum and rectum without evidence of transmission. It is concluded that *E. coli* is a good candidate for monitoring transmission events with perineum or rectum as sampling sites.

P04
Identification of *Campylobacter* species with AFLP fingerprinting

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The fluorescent amplified fragment length polymorphism fingerprinting (AFLP) method was tested for its ability to differentiate species of *Campylobacter.* A protocol for AFLP analysis of *C. jejuni* and *C. coli* has been developed previously and appeared to be highly discriminatory for typing of *C. jejuni* strains and to discriminate both species. For analysis the restriction enzymes *Hind*III and *Hha*I were used in combination with one selective A nucleotide at the 3' end of both primers. Bandingpatterns were analyzed with the pearson correlation coefficient and UPGMA cluster analysis. To establish if AFLP analysis was able to discriminate between different *Campylobacter* species, characterized strains consisting of 5 *C. jejuni,* 5 *C. coli,* 5 *C. jejuni* ssp. doylei, 3 *C. fetus* ssp. *fetus*, 3 *C. fetus* ssp. *venerealis,* 5 C. *upsaliensis,* 6 *C. hyointestinalis* ssp. *hyointestinalis,* 2 *C. hyointestinalis* ssp. *lawsonii,* 3 *C. sputorum* and 20 *C. lari* were studied. All species formed separate clusters. The subspecies were differentiated from the corresponding species but remained related. Strains of *C. lari* were separated in four distinct clusters, indicating high diversity within this species. The analysis of 30 blindly submitted strains confirmed that AFLP analysis can be used for identification of *Campylobacter* species.

P05
Resistance to third generation cephalosporins remains low in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in the Netherlands

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In ongoing surveillance of routine samples in seven Dutch laboratories (Arnhem, Enschede, Haarlem, Heerlen, Leeuwarden, Nijmegen and Tilburg), resistance to third generation cephalosporins (cefotaxime or ceftazidime) was studied in 65,000 *Escherichia coli* and 11,000 *Klebsiella pneumoniae* hospital isolates. Resistance to these compounds remained low; from 0.2% in 1989 to 0.7% in 1999 in *E. coli* and from 1.1% in 1989 to 1.6% in 1999 in *K. pneumoniae*. When the resistant strains were studied in more detail, it became clear that these strains were often multi-resistant: Next to high resistance against co-trimoxazole (30-40%), especially *K. pneumoniae* was also frequently resistant against gentamicin (> 20%). Most alarming however, was the increasing resistance against ciprofloxacin. Especially in *K. pneumoniae* fluoroquinolone resistance increased from 0% (1989-1996) up to 20% in 1999. We did not find any resistance against carbapenems (imipenem). We conclude that the production of beta-lactamases in *K. pneumoniae* and *E. coli* is still at a low level and that most infections with these pathogens can still be treated with the help of (third generation) cephalosporins. Nevertheless, there are indications that, when an occasional outbreak with cephalosporin resistant *K. pneumoniae* occurs, treatment opportunities become more limited.

P06
Carbapenemresistente *Citrobacter freundii*-stammen

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Bij een 65-jarige patiënt die langdurig werd beademd ten gevolge van complicaties van een biliaire pancreatitis, werden over een periode van twee maanden vijf *Citrobacter freundii*-stammen geïsoleerd uit buikvocht. De eerste drie stammen (I, II en III) bleken carbapenem-gevoelig (MIC ≤ 0,25) te zijn en de laatste twee (IV en V) waren carbapenem-ongevoelig (MIC > 16). Middels Pulsed-Field Gel Electrophoresis (PFGE) werden de stammen II, III, IV en V getypeerd. Hierbij werd gebruik gemaakt van het restrictie-enzym XbaI. De bandenpatronen van de gevoelige en ongevoelige stammen verschilden slechts twee banden van elkaar, hetgeen inhoudt dat de stammen aan elkaar gerelateerd zijn. De verandering is mogelijk toe te schrijven aan het ongevoelig worden van de stammen.
Resistentie van *C. freundii* voor carbapenem is een enkele

maal beschreven en kan onstaan door afwezigheid van het 40- en 42-kda-eiwit.[1] Om dit aan te tonen werd van de stammen een SDS-page uitgevoerd. Hieruit bleek dat bij de gevoelige stammen het 40-kda-eiwit aanwezig is, en dat bij de ongevoelige stammen dit eiwit ontbreekt.

1. Mainardi J, et al. Carbapenem Resistance in a Clinical Isolate of *Citrobacter freundii*. AAC, 1997; 41: 2352-4.

P07
Evaluation of the VIDAS Campylobacter Assay for direct detection of *Campylobacter* in broiler caecal material

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The VIDAS CAM Assay (bioMérieux sa, Marcy-L'Etoile, France) is an automated qualitative enzyme-linked fluorescent immunoassay for a rapid (<2h) and specific detection of *Campylobacter* in food samples, after enrichment. A pilot study was carried out to evaluate the VIDAS CAM Assay for direct detection of *Campylobacter* in 115 samples of broiler caecal material, without enrichment. *Campylobacter* was detected by conventional plating on CCDA in all 45 fresh *Campylobacter*-positive samples, but failed in 14 out of 34 frozen samples. VIDAS CAM correctly detected *Campylobacter* in all 34 frozen samples and in all-but-one of the 45 fresh samples. Overall, 1 (0.9%) false-negative and 10 (9%) false-positive results were observed with the VIDAS CAM Assay. The results from this pilot study indicate that the VIDAS CAM Assay can be used for direct detection of *Campylobacter* in fresh as well as stored caecal material. Optimization of the VIDAS caecal sample preparation protocol is currently carried out to reduce the number of false-positive results. The adjusted protocol will be validated in a second evaluation study, and results will be presented.

P08
Application of obligately alkaliphilic sulfur-oxidizing bacteria for the removal of H_2S from gas streams

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Several environmental problems are caused by SO_2, H_2S and methylated sulfur compounds. A successful process for the removal of H_2S from gas streams is the production of elemental sulfur from H_2S by sulfur-oxidizing bacteria in the Thiopaq® process. In this system, H_2S is absorbed in a scrubber unit and subsequently oxidized biologically (at neutral pH) to elemental sulfur. A point for major improvement of this process is the enhancement of the absorption efficiency of H_2S in the scrubber (by elevating the pH). The aim of our research is to realize such innovations by the use of halophilic obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria. In our laboratory, various chemolithoautotrophic sulfur-oxidizing bacteria have been isolated from soda lakes of Siberia (Russia) and Kenya. These alkaliphilic chemolithoautotrophs grow and oxidize sulfur compounds at conditions with pH up to 11 and salt concentrations up to 4 M Na^+, making them attractive for biotechnological sulfide removal at high pH. Based on 16S rRNA analyses, the new isolates have been placed into the genera *Thioalcalomicrobium* and *Thioalcalovibrio*. In this paper the preliminary results of the characterization of these new bacteria are discussed with respect to the potential use for sulfur production from H_2S-containing gas streams at high pH.

P09
Vancomycine p.o. voor het opheffen van MRSA-dragerschap

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Verschillende locale en orale anti-microbiële middelen worden gebruikt voor het opheffen van MRSA-dragerschap. Tijdens een MRSA-epidemie in een ziekenhuis werden de gekoloniseerde medewerkers en patiënten behandeld met een 5-daagse kuur: vancomycine p.o. (4 dd 250 mg), locale mupirocine neuszalf en betadineshampoo. De effectiviteit van dit beleid (ziekenhuis A) werd vergeleken met die van het beleid in een ander ziekenhuis in de regio (ziekenhuis B) waarbij er geen vancomycine werd gegeven. Middels een open enquête werden de bijwerkingen en de verdraagbaarheid van de gebruikte middelen gescoord.
De follow-up-periode was 165 dagen in ziekenhuis A en 222 dagen in ziekenhuis B. MRSA-dragerschap was opgeheven in alle personen in ziekenhuis A (35/35) en in 87% (20/23) in ziekenhuis B. Het gemiddelde aantal voorgeschreven kuren per persoon was 1,6 in ziekenhuis A versus 2,1 in ziekenhuis B (p-value=0,03). Van de personen die vancomycine p.o. (n=35) en rifampicine plus co-trimoxazol (n=12) hebben gebruikt, kreeg respectievelijk 74% en 58% bijwerkingen. De bijwerkingen werden in het algemeen niet als ernstig ervaren. Eén persoon had forse klachten van onaangename smaak, mondontsteking en veranderd defecatiepatroon.
De resultaten van deze studie suggereren dat vancomycine p.o. een effectief middel is voor het opheffen van MRSA-dragerschap. Hiertegen moet een hoger percentage bijwerkingen worden afgewogen.

P10
Modulating the distribution of fluxes among respiration and fermentation by overexpression of *HAP4* in *Saccharomyces cerevisiae*

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High biomass yields on sugar and low rates of byproduct formation are beneficial for biomass-directed applications of the yeast *Saccharomyces cerevisiae,* such as the production of heterologous proteins. At high glucose concentrations or at high growth rates *S. cerevisiae* has a strong tendency towards alcoholic fermentation, even under fully aerobic conditions. Aerobic fermentation results in a decreased biomass yield on glucose and in an increased formation of byproducts such as ethanol and CO_2. Controlling the distribution of sugar over fermentation and respiration via genetic engineering could therefore improve biomass-directed processes. *HAP4*

is a positive transcriptional regulator of genes associated with respiration. Overexpression of *HAP4* has previously been shown to increase the biomass yield on glucose of *S. cerevisiae* Dl1 in aerobic batch cultures. In this study the effects of *HAP4* overexpression are investigated in *S. cerevisiae* CEN.PK 113-7D under a variety of growth conditions in batch and chemostat cultures.
The dilution rate at which fermentation started in aerobic glucose-limited chemostat cultures of the *HAP4* overexpressing strain was approximately 10% higher than in the isogenic wild type. When a pulse of 50 mM glucose was given to a glucose-limited culture, the *HAP4*-overexpressing strain exhibited alcoholic fermentation, but all ethanol was consumed one hour earlier than in the wild-type culture. In nitrogen-limited chemostat cultures the biomass yield on glucose increased more than twofold. In batch cultures no effect of *HAP4* overexpression in CEN.PK was observed, but the yield of the prototrophic wild type CEN.PK was comparable to that of the auxotrophic Dl1 strain overexpressing *HAP4*.

P11
Natural selection for motile *Campylobacters:* deletion and duplication of flagellin sequences

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Campylobacter jejuni is an enteropathogen for humans, but it is a commensal for chickens. In both hosts the flagella and motility are important colonization factors. The flagellin gene is duplicated in *Campylobacter*, but only one flagellin gene, *flaA*, is sufficient for motility. We found that, during colonization of the chicken intestine for several weeks, a non-motile *flaA* mutant of *C. jejuni* regained its motility and colonization capacity. This was due to major rearrangements within its flagellin locus; deletions as well as duplications were found. In contrast, *in vitro* motile revertants isolated after several weeks from liquid culture showed different flagellin DNA rearrangements than after reversion in the chicken. It seems that the circumstances in the chicken's intestines apply a different selection pressure on the flagellin locus than *in vitro* conditions. The possible molecular mechanism that caused these rearrangements will be discussed.

P12
Oxidation of cytosolic NADH by *Saccharomyces cerevisiae* mitochondria

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During dissimilation of sugars via respiration by eukaryotic cells, glycolysis leads to NAD^+ reduction in the cytosol. As the mitochondrial inner membrane is impermeable to NADH, respiratory growth requires continuous reoxidation of this cofactor in the cytosol. The mitochondrial inner membrane of the yeast *Saccharomyces cerevisiae* contains two types of NADH dehydrogenase that couple the oxidation of NADH to the mitochondrial respiratory chain. The catalytic site of the 'external' NADH dehydrogenase faces the inter-membrane space and oxidises the cytosolic NADH. In addition an 'internal' NADH dehydrogenase faces the mitochondrial matrix and oxidises the intramitochondrial NADH.
Other cytosolic NADH oxidising systems have been proposed in addition to the 'external' NADH dehydrogenase; a glycerol-3-phosphate shuttle and an ethanol-acetaldehyde shuttle. The glycerol-3-phosphate dehydrogenase has been shown to function in ethanol-grown cells, but which of these systems function during aerobic growth on glucose and which is the most important one is not clear. Growth studies with defined mutants in aerobic glucose-limited chemostat cultures indicated that the 'external' NADH dehydrogenase is the most important system. In its absence the glycerol-3-phosphate shuttle could partly substitute for this system. When both these systems were deleted a third mechanism was still capable of oxidising cytosolic NADH.

P13
Competition in denitrifying mixed cultures

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Emission of nitrous oxide has been observed in complex microbial denitrifying communities. In this study, defined mixed cultures of *Alcaligenes faecalis* and *Ralstonia picketii* G9 were used. *A. faecalis* is not able to reduce nitrate, but can denitrify nitrite to nitrous oxide and dinitrogen gas. *R. picketii* G9 can convert nitrate to nitrite, and is able to grow on nitrous oxide. In acetate-limited mixed cultures provided with excess nitrate, both bacteria were able to coexist in an about 1:1 ratio. When the culture was switched to nitrite, *A. faecalis* became dominant, and *R. picketii* washed out, probably due to the higher affinity of *A. faecalis* for acetate. However, when the culture was switched back to nitrate, the 1:1 ratio was re-established. Nitrous oxide emission in the nitrate cultures was always very low.
Addition of biomass from a denitrifying pilot plant to the nitrate culture resulted in a dramatic change of the composition in the bacterial community. The *R. picketii* and *A. faecalis* cells were gradually replaced by another tandem of bacteria. Isolation and phylogenetic analysis of these new denitrifying communities members is presently in progress.

P14
Automated immunomagnetic separation and ELISA for high-capacity detection of *Salmonella* in porcine matrices

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The EiaFoss method combines immunomagnetic separation and ELISA to detect pathogenic microorganisms in enrichment cultures of food or environmental samples. We compared the EiaFoss with a reference method (BPW, RV, BGA) for the ability to detect *Salmonella* in 83 porcine samples, including liver, kidney, spleen, lymph nodes, muscle, and blood from pigs that were challenged with *Salmonella typhimurium*. All enrichments of the EiaFoss-protocol were confirmed by isolation in RV and BGA. Salmonella was isolated from 17 samples. *S. typhimurium* (challenge strain) and *S. anatum*

(natural contaminant) were isolated. The reference method, EiaFoss and confirmed EiaFoss-cultures detected *Salmonella* in 11 (65%), 10 (59%) and 12 (71%) samples, respectively. The reference method and EiaFoss performed equally. The agreement was not very good ($\kappa = 0.42$), but the occurence of true positive and negative samples, instead of false, could not be ruled out. The EiaFoss and confirmed EiaFoss-cultures performed equally, with good agreement ($\kappa = 0.77$). Sensitivity, specificity and predictive positive and negative values of EiaFoss were 0.83, 1.0, 1.0 and 0.92, respectively.

P15
Caffeic acid affects the survival of Shiga-toxin producing *Escherichia coli* O157 (STEC O157) in synthetic apple juice

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Several outbreaks of STEC O157-infections in North-America have been linked to unpasteurized apple juice. All of these outbreaks occurred in October and November, while most STEC O157-infections usually occur in the summer. Interestingly, *E. coli* is found only in apple juice produced from apples which were harvested between mid-October and mid-November. Apparently, the presence of a certain intrinsic factor influences the presence of *E. coli* in apple juice. While literature data indicate that pH and °Brix could be ruled out, caffeic acid (CA) might be this factor, since its concentration in apples decreases from 1.3 g/kg in June to < 0.1 g/kg in October. We studied the effect of CA on the survival of STEC O157 in a synthetic medium resembling apple juice (SAJ). In SAJ without CA, STEC O157 survived well, but in SAJ containing CA (0.2 to 1.0 g/L) the population size significantly dropped. The sensitivity of STEC O157 to CA was influenced by the growth phase of the culture, the pH of SAJ and the temperature. We conclude that the concentration of CA in apples may explain the season-dependent presence of *E. coli* in apple juice, and corresponding outbreaks of STEC O157-infections in North-America.

P16
High-capacity detection of Shigatoxin-producing *Escherichia coli* O157 (STEC O157) in bovine faeces

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The presently used methods to detect STEC O157 need four to five days before a result is obtained. The capacity of the methods is about 80 samples per week for one technician. To enable large-scale surveys on the presence of STEC O157 in cattle, an easy to perform detection method which allows the analysis of high numbers of samples was evaluated. This method (EiaFoss™) combines automated immunomagnetic separation with ELISA, allows the analysis of more than 400 samples per week, and gives presumptive results within 24-30 h. The use of ECn for the enrichment resulted in many false-positive responses, and was therefore replaced by a two-step protocol consisting of pre-enrichment in BPW, followed by selective enrichment in ECn with cefixime and tellurite (ECctn). The new protocol allowed the detection of less than 10 CFU in 0.1 g bovine faeces, while the number of false-positive responses was significantly reduced. Preliminary results indicated that EiaFoss is a sensitive, specific and efficient method for the detection of STEC O157 in bovine faeces, given that a selective enrichment protocol is used. A validation of this method is currently carried out at our laboratory.

P17
Completely autotrophic ammonia removal over nitrite

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Usually ammonia-rich waste water is treated using a combined nitrification/denitrification process. In this process, autotrophic nitrifying bacteria oxidize ammonia via nitrite to nitrate, using oxygen. The formed nitrate is subsequently converted by heterotrophic denitrifying bacteria, that need a electron donor (i.e. carbon source) for this reduction. In the concept we present here, no heterotrophic bacteria are involved. The new process is carried out by two types of autotrophic bacteria. Oxygen-limited *Nitrosomonas*-like bacteria oxidize part of the ammonia to nitrite, while planctomycete-like bacteria convert the formed nitrite to dinitrogen gas using ammonia as electron donor. The dynamics of the community during start-up of the process were monitored with fluorescent *in situ* hybridization and activity measurements. In the activity tests, no heterotrophic denitrification or autotrophic nitrite-oxidation potential were ever detected. In contrast to many traditional treatment systems, no hazardous nitrous or nitric oxide gasses were produced. This new autotrophic process has been named the CANON (Completely Autotrophic Nitrogen removal Over Nitrite) process.

P18
Modelling the effects of (green) antifungals, droplet size distribution and temperature on mould outgrowth in water-in-oil emulsions

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Prevention of fungal spoilage is a key microbiological issue for the shelf life of fat spreads. Our aim was to assess and model the scope of (natural) antimicrobials for extending shelf life of spreads (water-in-oil emulsions). First, an emulsion test system had to be set up. In such a system proof of principle can be obtained that antifungals are also effective in an environment of spreads. Production conditions were established to make 60% fat spreads with reproducible droplet size distributions. The mould vulnerabilities ranged from 1-20 weeks. The system allowed feasibility testing of lytic enzymes (Novozym 234) and LMW compounds against *Penicillium roqueforti*, a key-spoilage mould. The action of Novozym 234, carvacrol, undecanol and dihydrocarveol was benchmarked against sorbate and preservative-free controls under ambient and chilled conditions. Novozym 234 was ineffective to prevent outgrowth of *P. roqueforti*. Carvacrol, undecanol and dihydrocarveol had limited effects on shelf life extension compared to sorbate. Fungal growth boundaries of (un-)preserved spreads were modelled. The emulsion droplet size distribution (DSD) was captured in a new parameter

DSD-I (I= Influence). DSD-I is a move away from the mean droplet diameter $D_{3,3}$ as a sole quantitative droplet-size distribution parameter to traditionally classify the mould susceptibility of emulsions. DSD-I is a combination of available water droplets and surface area to initiate and sustain fungal outgrowth. Predictive models were developed for the effects of DSD-I, KSO and temperature on the shelf life of emulsions. The DSD-I and the predictive models can be used in future spread expert systems and quantitative risk analysis.

P19
Understanding the synergistic action of nisin, ultra-high pressure (UHP) and low temperature against bacteria

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Mild preservation systems generally depend on combined action. Insight in a synergistic system based on antimicrobial peptides (nisin, histatin-5, MB21), UHP, and low T against Gram-positive and -negative microorganisms may assist in the further development of food preservation. Nisin with UHP showed synergistic effects against *Lactobacillus plantarum* and *Escherichia coli* at reduced temperatures (< 15 °C). The effect was substantially lower at 25 °C. Pasteurization was achieved for *L. plantarum* at 10 °C with 0.5 µg/ml nisin at 150 MPa. Pasteurization was also obtained for *E. coli* at 10 °C with 2 µg/ml nisin at 150 MPa. Combining nisin, UHP and lowered temperature allowed considerable reduction in intensity of UHP treatments. The enhanced synergistic kill with UHP at reduced temperatures was also observed for the synthetic peptides MB21 and histatin-5. The cytoplasm membrane played a key role in nisin and UHP susceptibility. Increases in fatty acid saturation and lysylphosphatidyl membrane content of *L. plantarum* were correlated with increased UHP respectively nisin susceptibility. A mode of action was postulated for the synergistic action of nisin, reduced temperature and UHP. The first step, the binding of nisin and other peptides to the membrane surface, will reduce the fluidity and increase the efficacy of an UHP treatment. The next step, the pore formation by peptides, may not be required to get enhanced kill in combination with UHP at low T. The hypothesis was tested using mutant nisin molecules. Mutant nisin with a reduced affinity for the headgroups was, however, not less synergistic, than mutants with a perturbed pore formation.

P20
Ontwikkeling van ceftazidime resistentie van *Pseudomonas aeruginosa* in een patiënt met COPD

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Een 63-jarige man bekend met COPD met emfyseem en bronchiëctasieën is sinds vier jaar chronisch gekoloniseerd met *Pseudomonas aeruginosa* in het sputum. Onlangs werd hij opgenomen met een sepsis, waarvan de bron terug was te voeren op een infectieuze exacerbatie van de chronische longaandoening. Patiënt werd initieel behandeld met ceftazidime en tobramycine. Tijdens opname werd uit sputum en bloed *P. aeruginosa* geïsoleerd. De stam uit sputum was gevoelig voor ceftazidime (MIC = 1 mg/l), die uit bloed was ongevoelig (MIC > 16 mg/l). Van patiënt was vaker *P. aeruginosa* gekweekt uit sputa, en deze stammen (in totaal negen) waren opgeslagen bij -80 °C. Twee stammen hiervan bleken ongevoelig voor ceftazidime. Alle geïsoleerde stammen (oude en nieuwe) werden middels Pulsed-Field Gel Electrophoresis (PFGE) getypeerd. Hierbij werd gebruik gemaakt van het restrictie-enzym SpeI. De bandenpatronen van de gevoelige en ongevoelige stammen verschilden slechts twee banden van elkaar, hetgeen inhoudt dat de stammen aan elkaar gerelateerd zijn. De gesignaleerde kleine verandering verklaart mogelijk de ongevoeligheid voor ceftazidime.

P21
Ultrastructure of *Brocadia anammoxidans*, an autotrophic planctomycete that oxidises ammonium anaerobically

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We have investigated the ultrastructure of *Brocadia anammoxidans*, the planctomycete responsible for anaerobic ammonium oxidation (the Anammox process). Using negative staining of whole cells and thin-sectioning after glutaraldehyde and cryofixation, we found this bacterium to have many features in common with previously investigated planctomycetes. Crateriform structures in the cell wall, extracellular appendages, reproduction by budding, the presence of internal compartments and a dense, fibrillar nucleoid were all consistent with observations made previously for *Gemmata obscuriglobus, Pirellula marina* and *Pirellula staleyi*.
Cells of *B. anammoxidans* were also found to have a new internal, membrane-bounded compartment. Based on immunogold labelling studies, we conclude that *B. anammoxidans* uses this compartment to oxidise ammonium anaerobically.

P22
Interactions of cyclodextrin glycosyltransferase (CGTase) from *Bacillus circulans* strain 251 with starch*

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Cyclodextrin glycosyltransferase (CGTase) belongs to the industrial important α-amylase family of glycosylases, which catalyze cleavage (or formation) of α-1,4 and α-1,6 glucosidic bonds in amylose and amylopectin, using identical catalytic residues and proceeding via a covalently linked

intermediate. CGTase produces cyclodextrins from starch by cleaving an α-1,4 bond after which the intermediate reacts with its own non-reducing end, resulting in the formation of a cyclic oligosaccharide consisting of six, seven or eight glucose residues (α-, β- or γ-cyclodextrins). The maltose dependent crystal structure of *Bacillus circulans* strain 251 CGTase shows that the protein has five domains (A-E), of which A-C have structural similarity with the three α-amylase domains. Domain E is a raw starch binding domain. Three maltose binding sites (MBS) were identified (two in the E-domain, one in the C-domain). Crystal soaking yielded structures with various oligosaccharides bound to the MBS sites and a maltononaose inhibitor in the active site, allowing identification of nine glucose binding subsites. Modification of hydrogen bonds or hydrophobic interactions in these subsites allowed rational construction of CGTase derivatives with modified cyclodextrin product specificity.

P23
European Antimicrobial Resistance Surveillance System: *Staphylococcus aureus* susceptibility test results

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Background: The European Antimicrobial Resistance Surveillance System (EARSS) is a network of national surveillance systems. Susceptibility test results of *Staphylococcus aureus* in blood isolates are being collected since the last quarter of 1998.
Methods: Patient- and isolate information is collected through Isolate Record Forms that have been developed to enable standardized data processing. The laboratories interpret the test results according to national guidelines. The national coordinators collect resistance data from the different laboratories and send it in EARSS data exchange format (ASCII) to the central database quarterly. Susceptibility test results from 160 labs from 11 European countries were analysed. The proportion of oxacillin- (= methicillin-)resistant *S. aureus* (MRSA) was calculated.
Results: The proportion of MRSA did not exceed 5% in Denmark (0/502), Finland (9/250), Iceland (0/21), the Netherlands (1/495) and Sweden (24/1615). The proportion of MRSA in Germany was 7% (24/331), in Luxembourg 13% (5/40), in Greece 36% (15/42), in Italy 41% (279/687), in Ireland 42% (127/306) and in Portugal 57% (47/83), respectively.
Conclusion: These data show a wide variation in the proportion of invasive MRSA across Europe. In the northern European countries MRSA is seldomly seen, while southern European countries and Ireland report proportions ranging from 36 to 57%. EARSS will continue to monitor resistance in the EU, extending the set of pathogens under surveillance in the near future.

P24
Vóórkomen van ESBL-positieve *Escherichia coli* en *Klebsiella* sp. in het LUMC: evaluatie van de Double Disk Synergy Test

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Extended-spectrum β-lactamases (ESBLs), verantwoordelijk voor resistentie tegen derde-generatie cefalosporinen en monobactams, komen wereldwijd steeds vaker voor.
Gezien het gebruik van derde-generatie cefalosporinen in het LUMC werd het wenselijk geacht om ESBL-positieve isolaten te kunnen detecteren. Om een indruk te krijgen van het vóórkomen van deze isolaten werden gedurende 1 maand alle *Escherichia coli* en *Klebsiella* sp.-isolaten gescreend op de aanwezigheid van ESBL, middels de Double Disk Synergy (DDS) Test. De positieve isolaten werden vervolgens ter confirmatie geanalyseerd door middel van PCR van SHV- of TEM-gen, pI, cefpodoximzone en β-lactamaseprofiel. Van de in totaal 210 isolaten, afkomstig van 126 patiënten, werden er 13 positief bevonden in de DDS-Test. Na confirmatie bleken er uiteindelijk drie werkelijk ESBL positief te zijn. De overige isolaten voldeden niet aan de criteria (PCR, pI, cefpodoximzone en -profiel) van ESBL. Conclusie: het gebruik van de DDS Test leidt tot overschatting van het vóórkomen van ESBL-positieve isolaten. De cefpodoximzone lijkt volgens deze studie een betere indicator voor het vóórkomen van ESBL.

P25
Urease expression in *Helicobacter pylori* is induced by nickel

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Urease is an important virulence factor of the gastric pathogen *Helicobacter pylori*. Active urease consistst of two subunits (UreA and UreB) and requires nickel as a cofactor. Although urease represents about 5% of the total bacterial protein content, and hence constitutes a heavy metabolic burden, the enzyme production is not regulated by environmental pH or urea concentration. We here report on the effect of medium supplementation with nickel or iron on the expression of *H. pylori* urease. Addition of $NiCl_2$ at non-toxic (< 1 mM) concentrations caused an approximately fivefold increase in expression of the UreA and UreB proteins in all tested *H. pylori* strains. Addition of $FeCl_3$ upto 500 μM did not affect UreAB expression, indicating that the effect was specific for nickel. The increase of UreAB expression was already maximal at 10 μM $NiCl_2$. We also tested the effect of nickel addition in *H. pylori* 1061 strains mutated in two metal-responsive regulators: the iron-responsive regulator Fur (HP1027) and the putative nickel-responsive regulator NikR (HP1338). Inactivation of NikR did not affect the nickel-dependent increase of UreAB expression. However, inactivation of Fur reduced the increase of UreAB expression to only twofold. In this study, we show that the high-level expression of urease subunits in normal growth media can even be increased at least fivefold by the addition of nickel to the growth medium. Such high levels of urease expression may be

necessary during transmission or colonization. As induction was significantly lower in a *H. pylori* fur mutant, it is likely that Fur is involved in urease expression. Whether this regulation is mediated directly through binding of Fur to the urease promoter, or indirectly through other Fur-regulated genes is currently under investigation.

P26
Campylobacter infection in neonate and puppy: a genotypically proven relationship

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A three-week old child was presented at the Children's hospital with fever. Five days before admission she started vomiting, produced mucoid stools and became lethargic and irritable. Sepsis evaluation was done and after cerebrospinal fluid appeared normal, treatment with amoxycillin-clavulanic acid and gentamicin was initiated. The clinical situation improved within 12 hours. After five days, the blood culture became positive for *Campylobacter*. The therapy was changed into erythromycin. The child was discharged from the hospital after ten days of hospitalisation. Six days prior to the hospitalisation of the child, a Labrador Retriever puppy arrived in the family. This dog has soft faeces from the day of arrival and a serious diarrhoea started six days after hospitalisation of the child. A faecal sample was found positive for *Campylobacter*. The *Campylobacter* isolates from the child (blood and faeces), the dog, and puppies from the same litter were genotyped by AFLP. All isolates showed the same genotype, so it can be concluded that the infection was transmitted from the puppy to the child. This represents to our knowledge the first bacteriological proven relationship between *Campylobacter jejuni* infection in child and dog within the same family.

P27
Effect of broiler's cecal volatile fatty acid concentrations on a glucose limited growing *Salmonella enteritidis*

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The cecal microflora of chickens can have a protective function against enteropathogenic bacteria like *Salmonella*. The mechanism behind this protection is unknown but could be ascribed to volatile fatty acids (vfa) produced by cecal microflora. In a previous study, average concentrations of vfa in ceca of 5, 8 and 15 day old broilers were determined. In this study, the effect of these concentrations on an anaerobic, glucose limited and pH controlled growing *Salmonella enteritidis* (SE) was studied in a semi-continuous culture. Day 5 vfa concentrations did not reduce O.D. of Salmonella during the first 24 hours after addition. Thereafter, the O.D. decreased until 72 hours (1.4 to 0.8) and subsequently it returned to an O.D. of 1.2 at 108 hours. Day 8 vfa concentrations reduced O.D. during the first 24 hours (1.3 to 0.2). Thereafter, a slight recovery of the O.D. was observed during the period up to 228 hours (0.2 to 0.4). O.D. followed the wash-out curve for 24 hours when day 15 vfa concentrations were used. Thereafter, the O.D. remained stable at 0.04 for 216 hours. These results demonstrate that cecal vfa concentrations can reduce biomass of SE growing under conditions mimicking chicken's ceca (i.e. glucose limitation, pH control, anaerobiosis).

P28
Surveillance of respiratory pathogens and influenza like illnesses in general practices in the Netherlands in winter 1998/1999

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Introduction: NIVEL is running a surveillance network of 43 sentinel general practice (GP) stations spread over the country, which covers 1% of the Dutch population. The incidence of influenza-like illnesses (ILI) is calculated weekly during the winter season by NIVEL from the data of the network. The system was extended by the RIVM with virus isolation and detection from nose/throat swabs obtained from patients with an acute respiratory infection (ARI), of whom about 70% are registered with ILI. The aim of the study was to provide insight in the etiology of ARI in the general population.
Materials and methods: A total of 405 nose/throat swabs were examined by virus isolation and PCR, using standard procedures. PCR was performed for respiratory syncytial virus, rhinovirus, enterovirus, coronavirus OC43 and 229E and *Mycoplasma pneumoniae*.
Results: In 58% of the samples at least one virus or bacterium was detected. 28% of the respiratory pathogens were recognized by PCR only. In 5% of the samples a double-infection was observed. Influenza viruses were detected most often (25%), followed by rhinoviruses (13%). Of the rhinoviruses, 77% was detected by PCR only, whereas 23% was detected by isolation and PCR. Registration of ILI and isolation of influenza viruses were in accordance with each other. However, in 26% of the patients registered with ILI other respiratory pathogens than influenza virus were detected (44% rhinovirus) and in 47% no pathogen was detected.
Conclusions: *(i)* Influenza viruses and rhinovirus were the predominant viruses detected in patients with ARI from a GP sentinel system. *(ii)* Application of the PCR improved the detection of respiratory pathogens in nose/throat swabs considerably. *(iii)* Registration of ILI by GP's was in accordance with isolation of influenza viruses in the laboratory.

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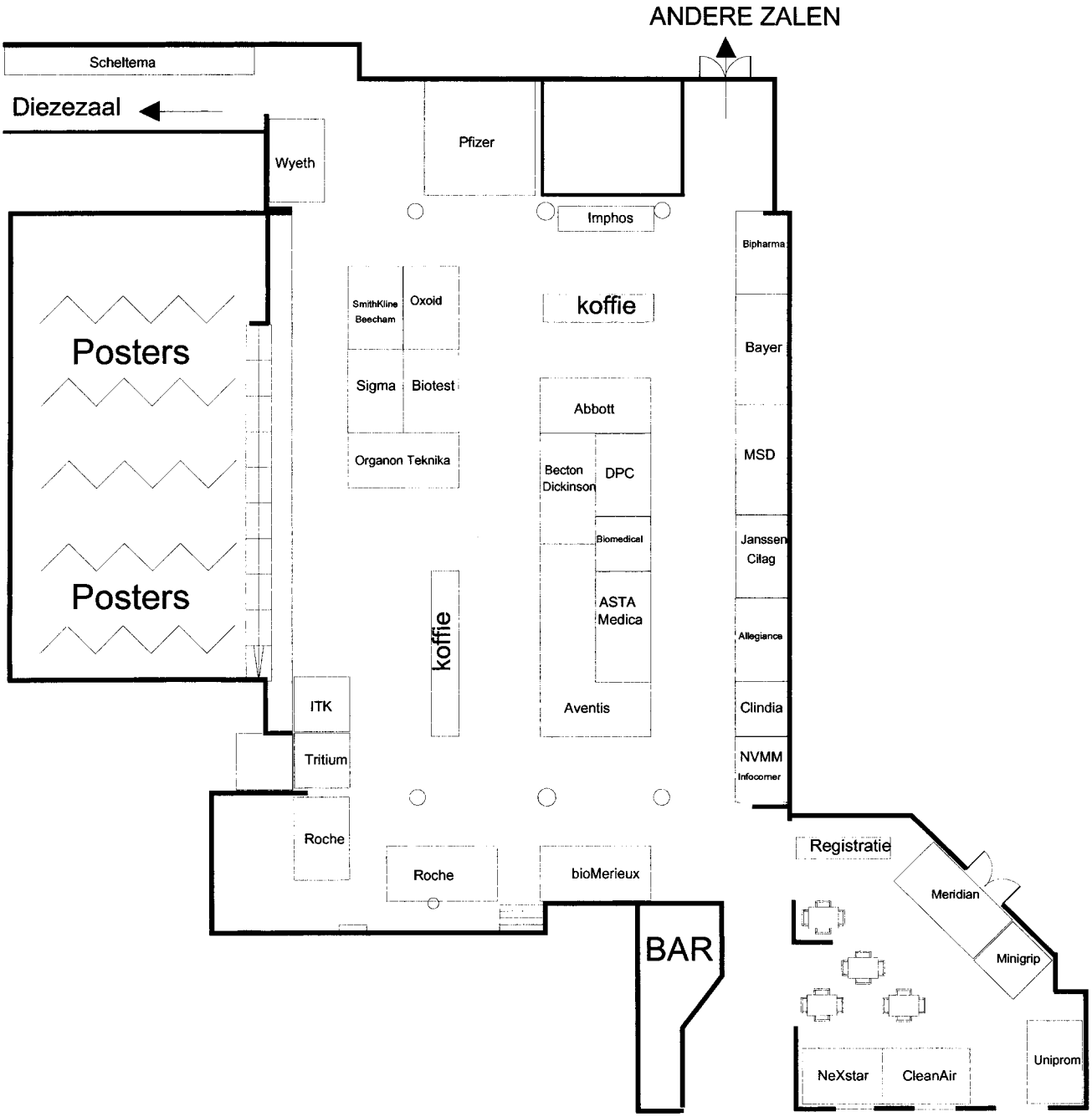
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