Challenges in diagnosis of Hepatitis E virus infections

Suzan D. Pas

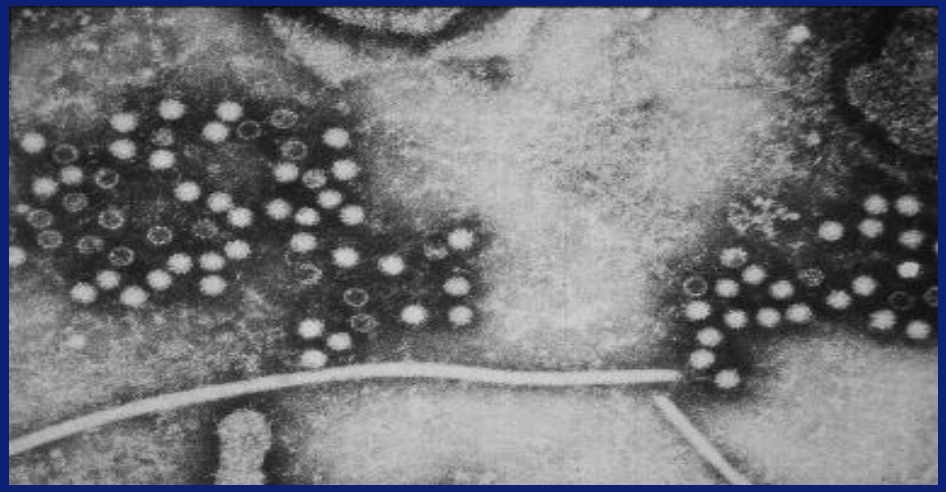
Afdeling Virologie

ErasmusMC Rotterdam

s.pas@erasmusmc.nl

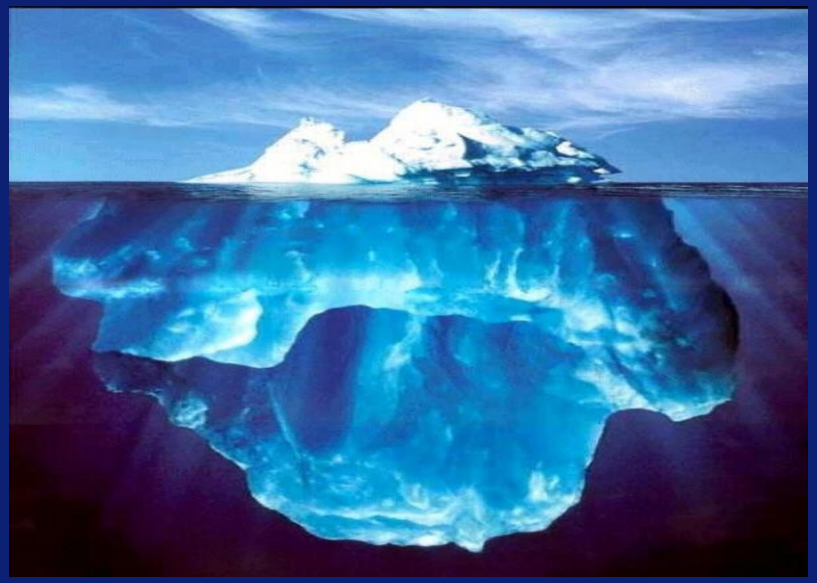
Hepatitis E Virus

- Hepeviridae family
- Non-eveloped virus
- Positive sense, single stranded RNA of ±7200 bp
- 27-34 nm in diameter



Erasmus MC

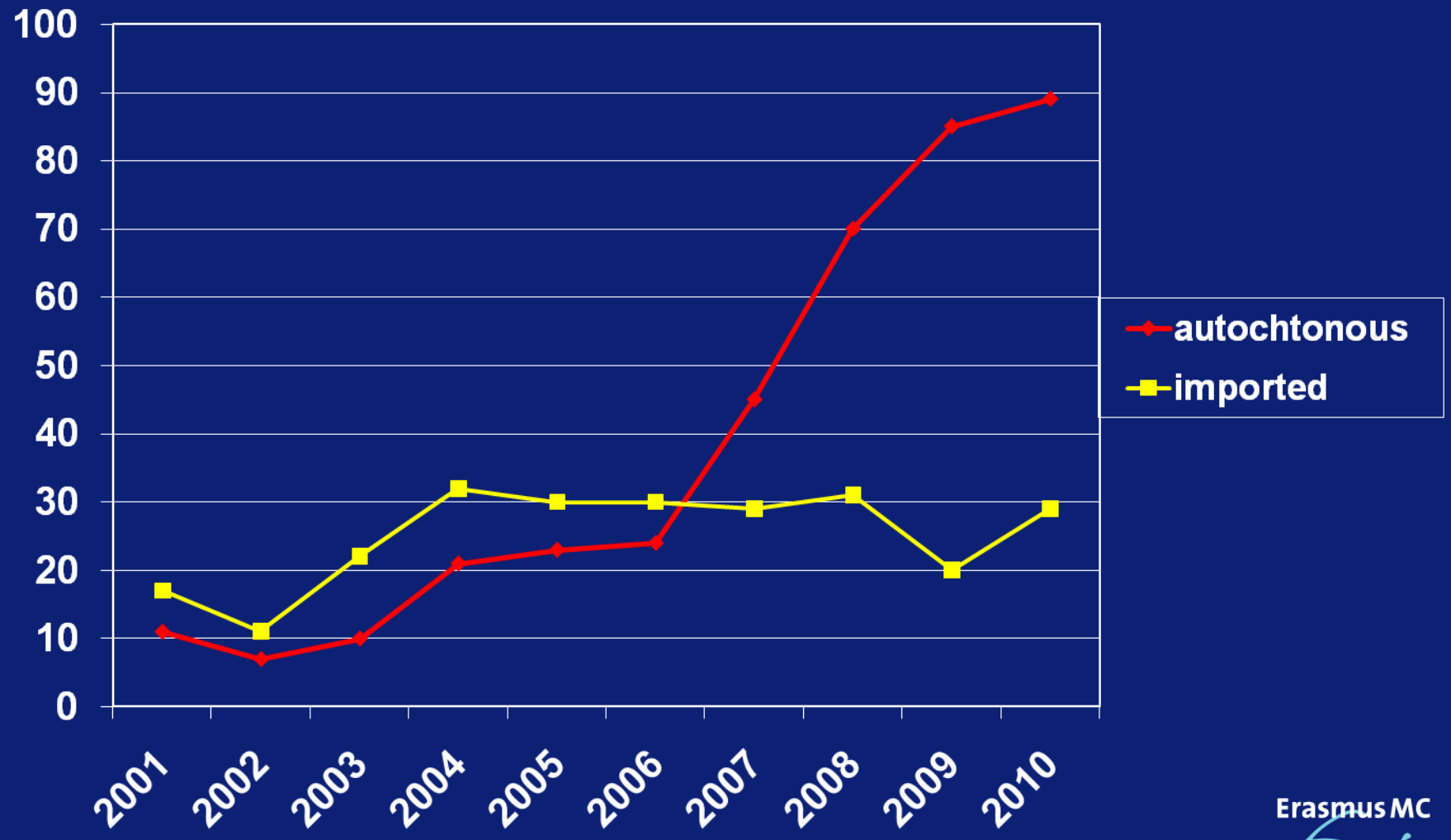
Clinical presentation of hepatitis E virus



- Fever
- Fatigue
- Loss of appetite
- Nausea
- Vomiting
- Abdominal pain
- Jaundice
- Dark urine
- Clay-colored stool
- The ratio of symptomatic to asymptomatic infection is reported to range from 1:2 to 1:13.
- Mortality: overall 1-4%, pregnant women 15-25%

Erasmus MC

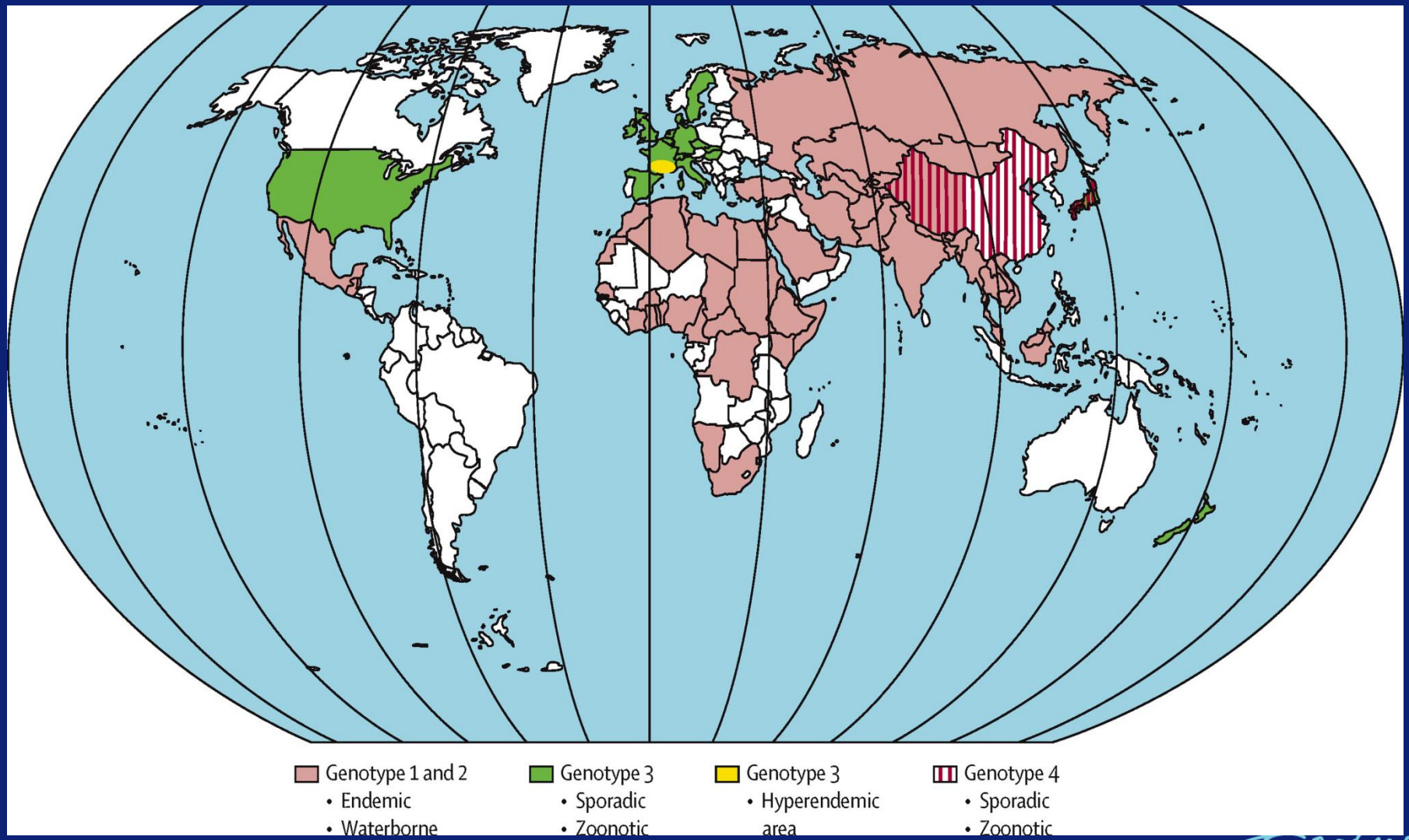
Number of reported HEV-cases in Germany



Source: Robert Koch Institute, Germany

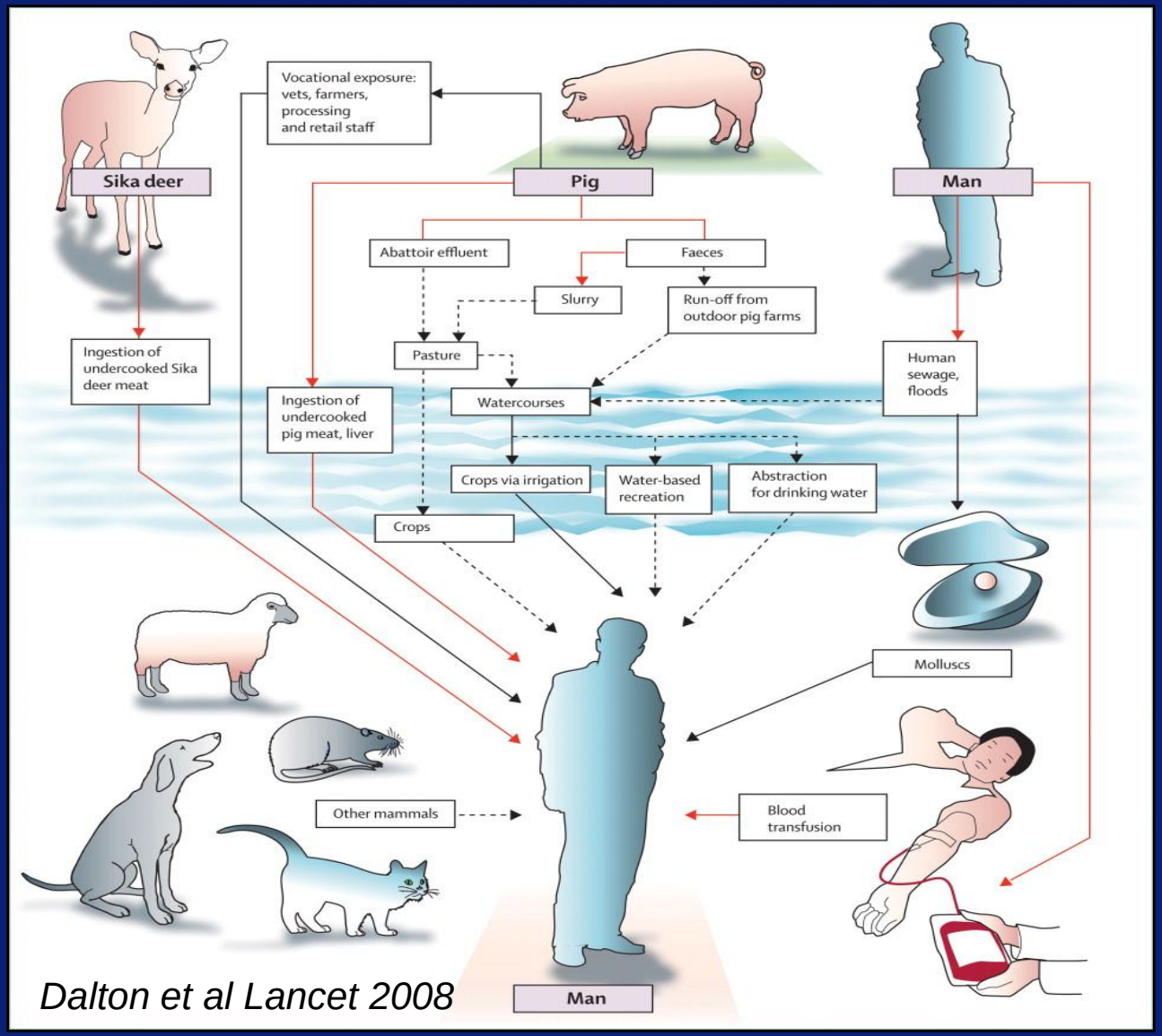
Erasmus MC

Global distribution of HEV genotypes



Kamar, Lancet 2012

HEV transmission in developed countries - zoonose



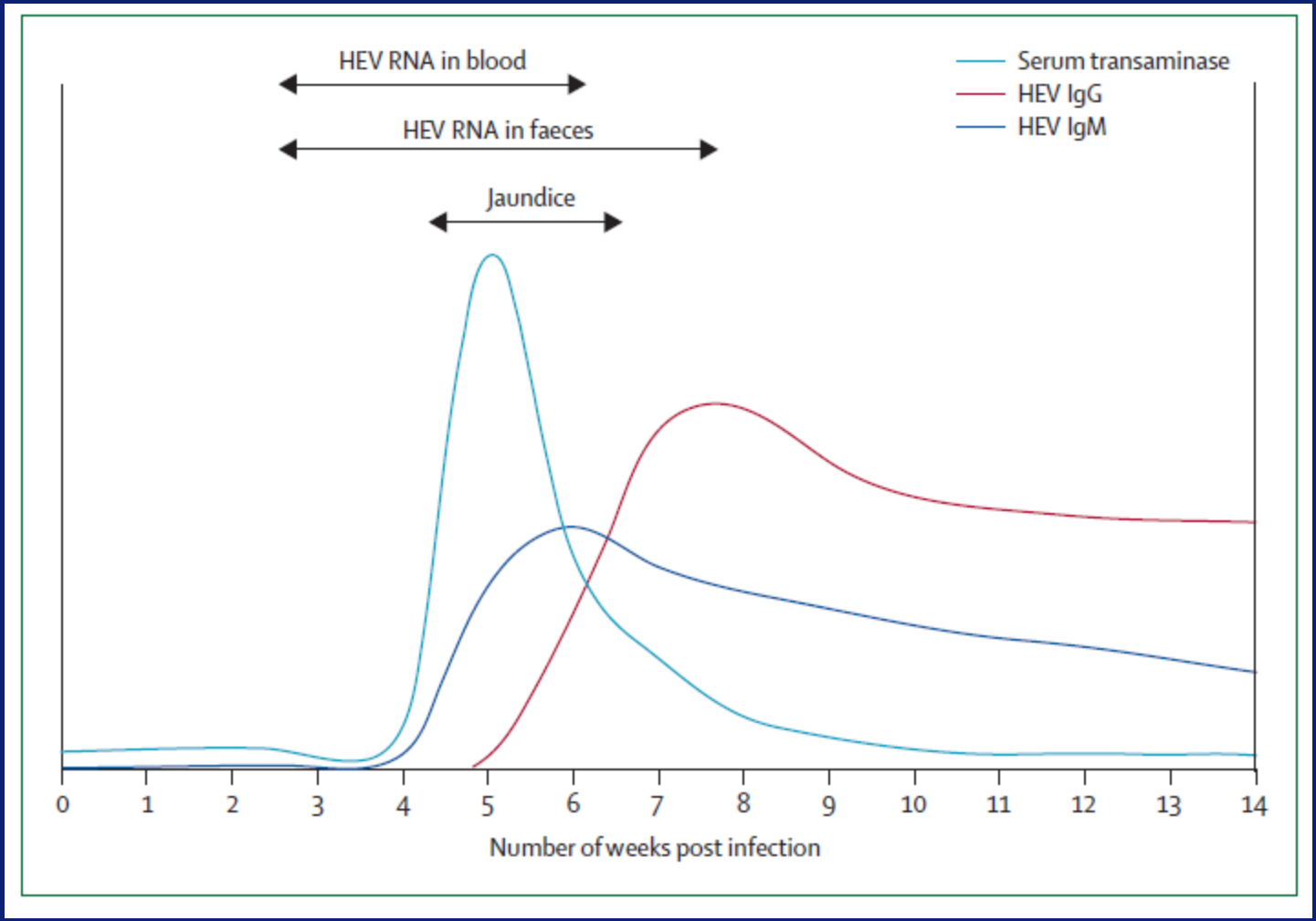
Dalton et al Lancet 2008

~55% of fecal tanks in Dutch pig farms HEV RNA positive
EID 2007

~ 6% of Dutch porcine livers HEV RNA positive.
J Food Prot. 2007

Erasmus MC

Course of HEV infection in the immunocompetent



Dalton et al, Lancet 2008

Erasmus MC

Chronic HEV in solid organ recipients

- Chronic HEV infection reported in the transplant setting
  - Persistent viraemia
  - Persistently raised transaminase activity
  - Histological features associated with chronic hepatitis
  - Evidence of rapid development of cirrhosis

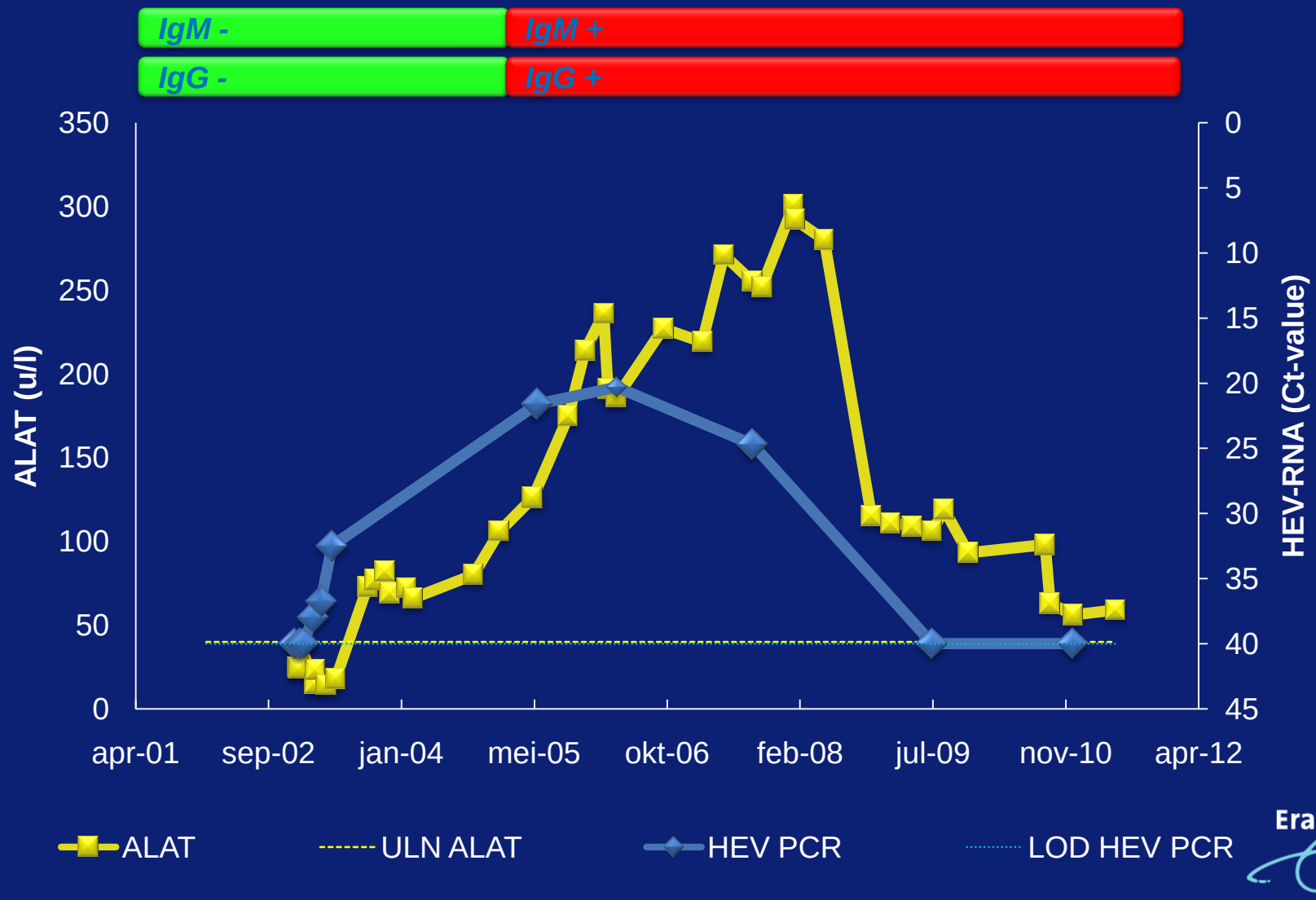
- Association with a more profound immunosuppression

Kamar, N Engl J Med 2008;358(8):811-7.

Haagsma, Liver Transplantation 2009;15(10):1225-8

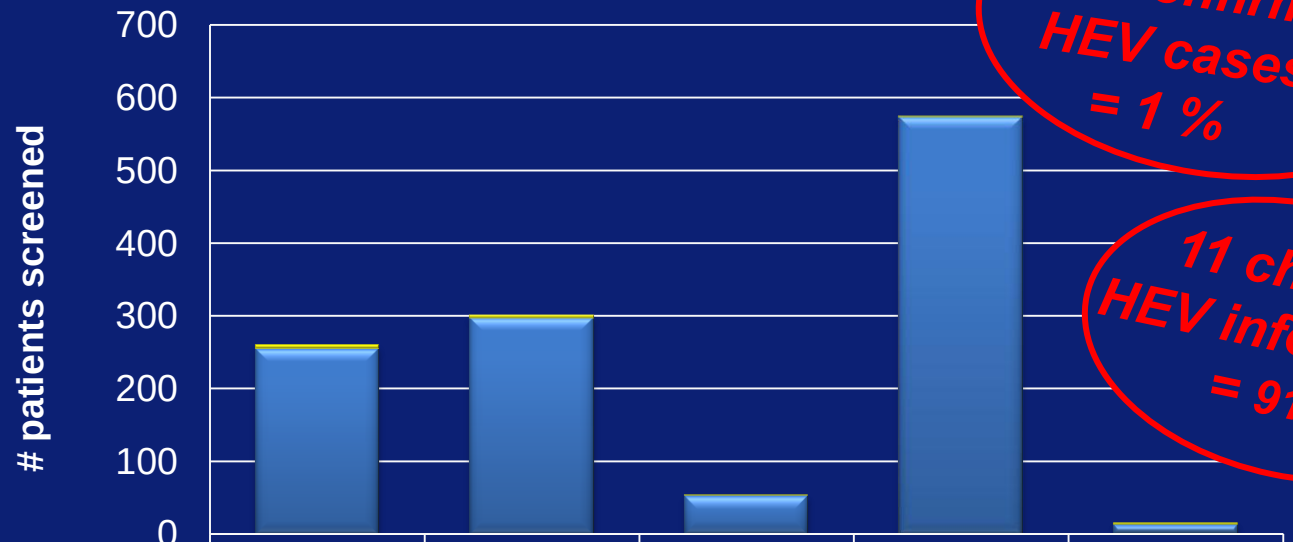
Erasmus MC

Chronic HEV infection misdiagnosed as Graft v Host



Erasmus MC

HEV in living adult SOT transplant recipients



12 confirmed HEV cases = 1 %

11 chronic HEV infections! = 91,6 %

Mixed Tx group:

confirmed HEV case: *1 NTX-LTX , 1 NTX-HTX*

no HEV infection: *8 NTX-LTX, 3 NTX-HTX, 1 NTX-LuTX*

Erasmus MC

Pas et al, EID 2012

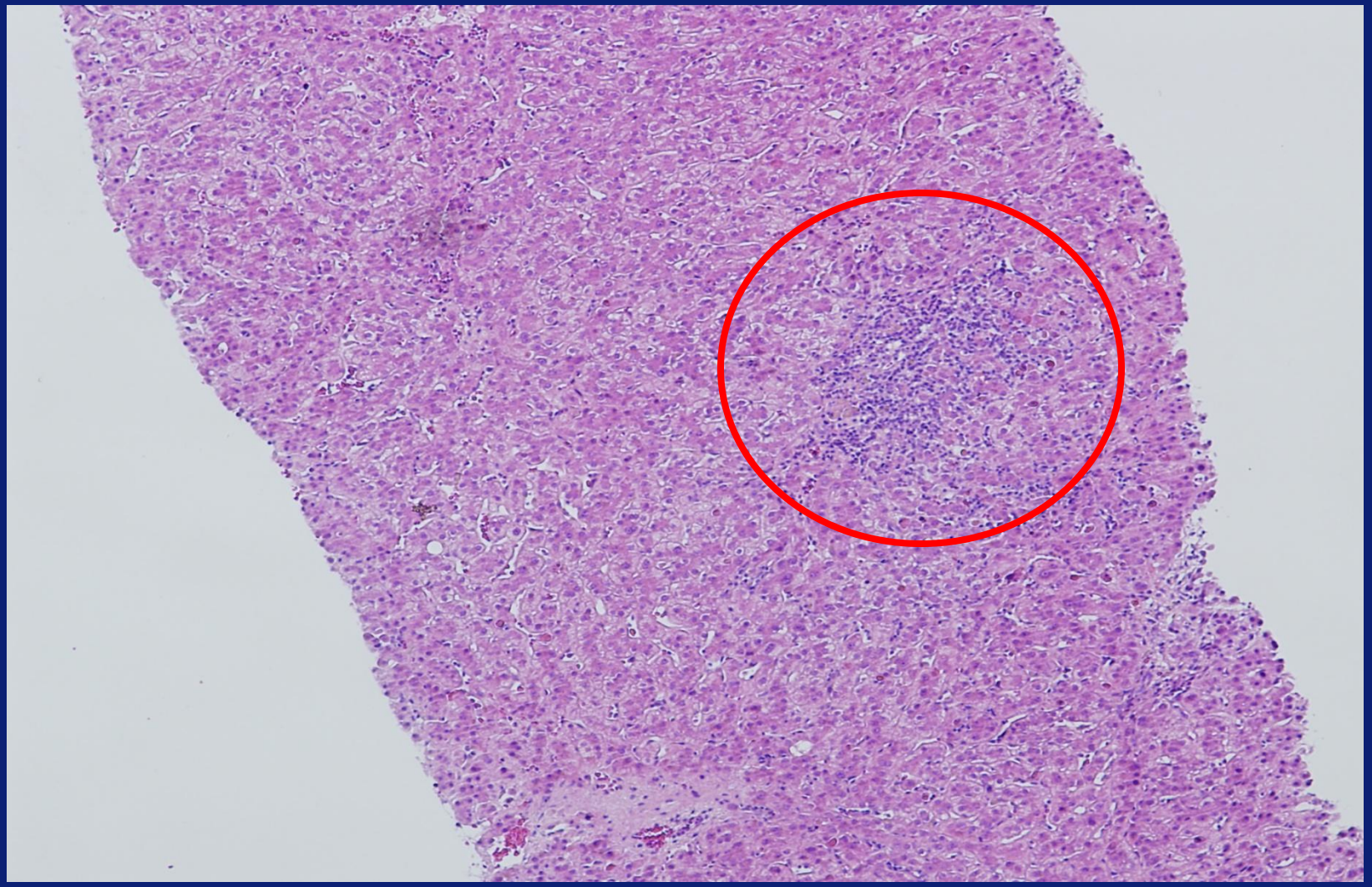
Current status of HEV diagnostics

* **Pathology** — **not specific**

  Invasive

Erasmus MC

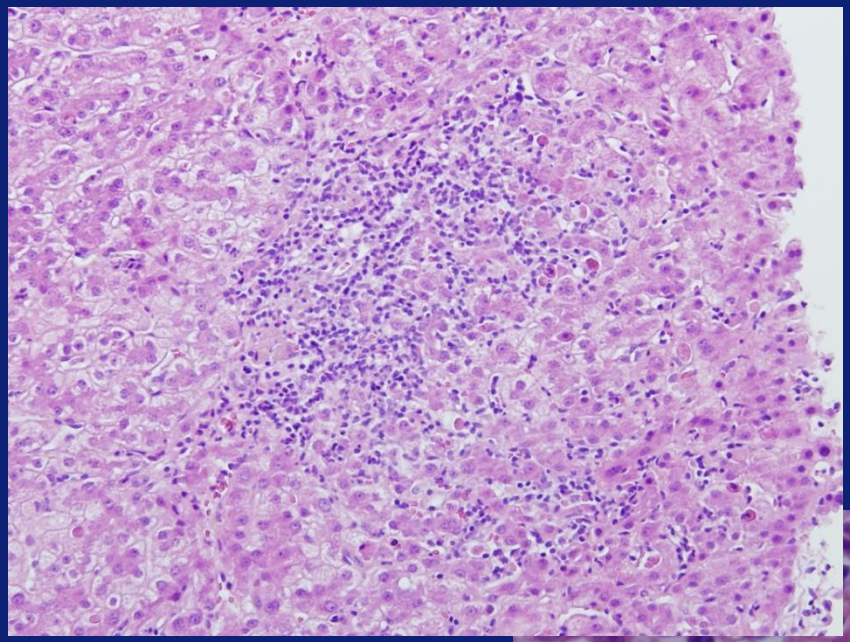
Current status of HEV diagnostics - Histopathology

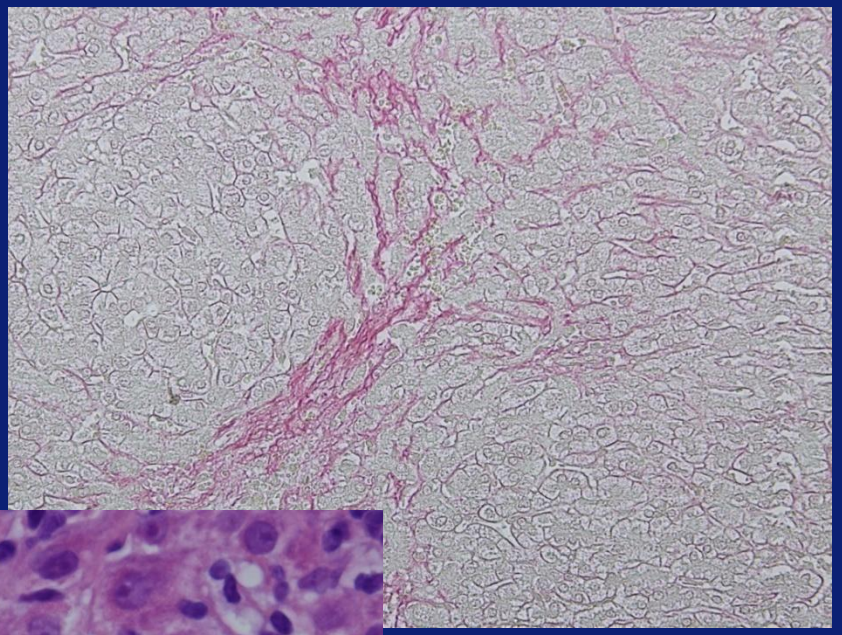


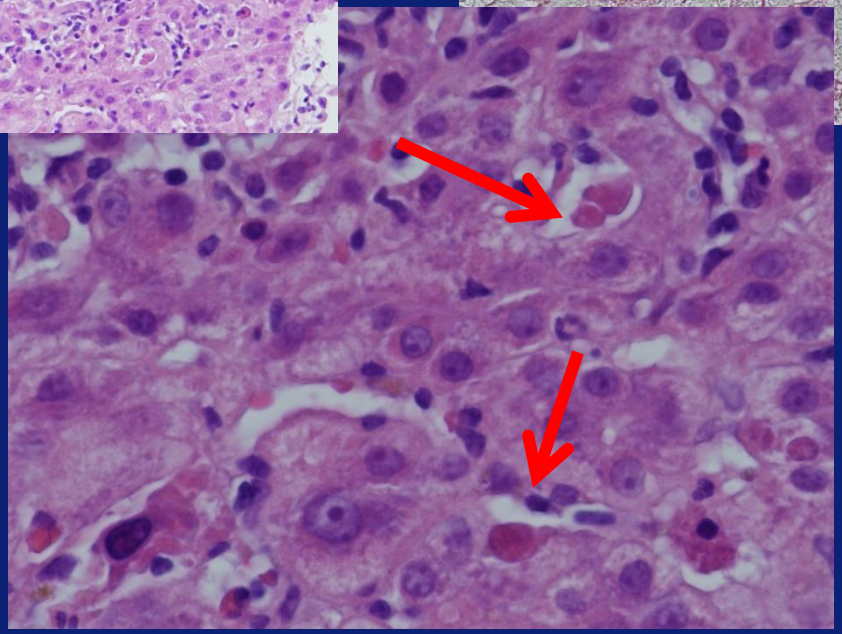
Liver biopsy overview

Erasmus MC

Current status of HEV diagnostics - Histopathology







Erasmus MC

Current status of HEV diagnostics

* Pathology not specific

  Invasive

* **Virus culture -** inefficient

Erasmus MC

Virus culture -HEV

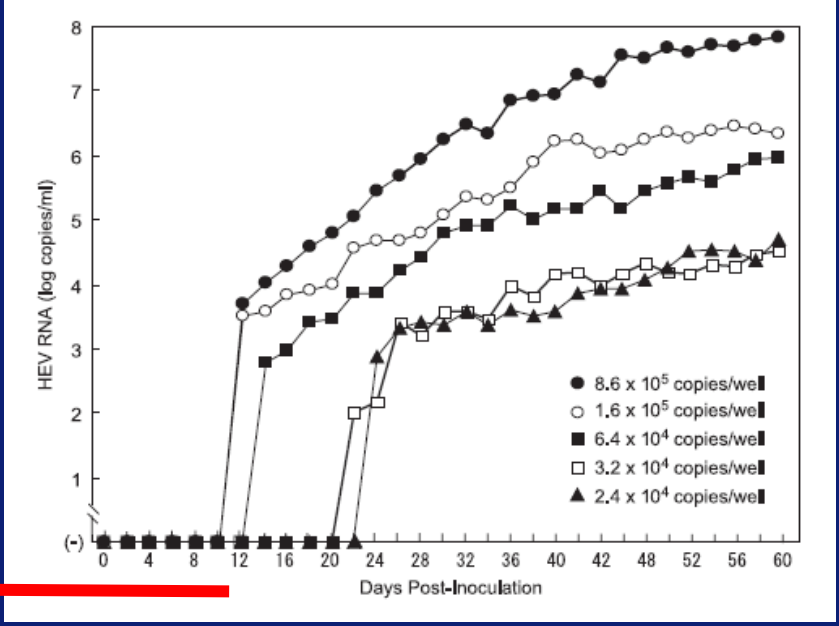
Rev. Med. Virol. 2011; **21**: 18–31.
Published online in Wiley Online Library
(wileyonlinelibrary.com)
DOI: 10.1002/rmv.678

cal Virology

Efficient cell culture systems for hepatitis E virus strains in feces and circulating blood

Hiroaki Okamoto*
Division of Virology, Department of Infection and Immunity, Jichi Medical University School of Medicine, Shimotsuke-Shi, Tochigi, Japan

PLC/PRF/5 (hepatocellular carcinoma) and A549 (lung cancer) cells



long incubation period, low sensitivity

Erasmus MC

Current status of HEV diagnostics

* Pathology not specific

  Invasive

* Virus culture - inefficient

* **HEV serology**

  - validation of commercial assay

  - conformational testing using blot

Erasmus MC

HEV serology - literature

A lot of in house assays described

CLINICAL AND VACCINE IMMUNOLOGY, May 2007, p. 562–568 Vol. 14, No. 5
1556-6811/07/$08.00+0 doi:10.1128/CVI.00231-06

Use of Serological Assays for Diagnosis of Hepatitis E Virus Genotype 1 and 3 Infections in a Setting of Low Endemicity▽

M. Herremans,* J. Bakker, E. Duizer, H. Vennema, and M. P. G. Koopmans

IgM and IgG *Genelabs, Mikrogen Recomblot*

Journal of Medical Virology 82:799–805 (2010)

A Comparison of Two Commercially Available Anti-HEV IgG Kits and a Re-Evaluation of Anti-HEV IgG Seroprevalence Data in Developed Countries

Richard Bendall,[1]* Vic Ellis,[1] Samreen Ijaz,[2] Rachel Ali,[3] and Harry Dalton[3]

IgG ; *Genelabs, Wantai, 4.5x higher seroprevalance with Wantai*

HEV serology - literature

Serologic Assays Specific to Immunoglobulin M Antibodies against Hepatitis E Virus: Pangenotypic Evaluation of Performances

Jan Drobeniuc,[1] Jihong Meng,[1,2] Gábor Reuter,[3] Tracy Greene-Montfort,[1] Natasha Khudyakova,[1] Zoya Dimitrova,[1] Saleem Kamili,[1] and Chong-Gee Teo[1]

CID 2010:51 (1 August) •

2 in-house assay, 4 commercial available assays

	Sens.(%)	Spec.(%)
Int. Immuno-Diagnostics	82	91,2
MP biomedicals (former Genelabs)	72	93
RPC Diagnostic Systems	98	95
Mikrogen	92	95,6

Sens. panel 50 samples, ***4 genotypes***
Spec. panel 229 samples

Erasmus MC

HEV Serology validation - included ELISAs

	Name	Company	Country	Genotype inclusivity	antigens
IgM/ IgG	HEV Elisa v3.0	MP diagnostics	Singapore	gt 1 en 2	mix of peptides from ORF2 and complete ORF3
	*recom*Well HEV IgM/IgG	Mikrogen Diagnostik	Germany	gt 1	synthetic, ORF2 en ORF3 (e.coli)
	*recom*Well HEV IgM/IgG ***new***	Mikrogen Diagnostik	Germany	gt 1 and 3	synthetic, ORF2 en ORF3 (e.coli)
	HEV IgM/IgG	DRG	Germany	gt 1 en 2	synthetic, ORF2 en ORF3
	HEV IgM/IgG	Dia.Pro	Italy	gt 1 en 2	synthetic, ORF2 en ORF3
	HEV IgM/IgG	RPC Diagnostic systems **/ DSI**	Italy	gt 1 en 2	artificial ag. Composed of 12 antigenic regions derived from ORF2 and ORF 3
	HEV IgM/IgG	DiaCheck			
	HEV IgM/IgG	Wantai HEV IgG PE2	Singapore		PE2 peptide from structural region of ORF2

Pas et al, in preparation

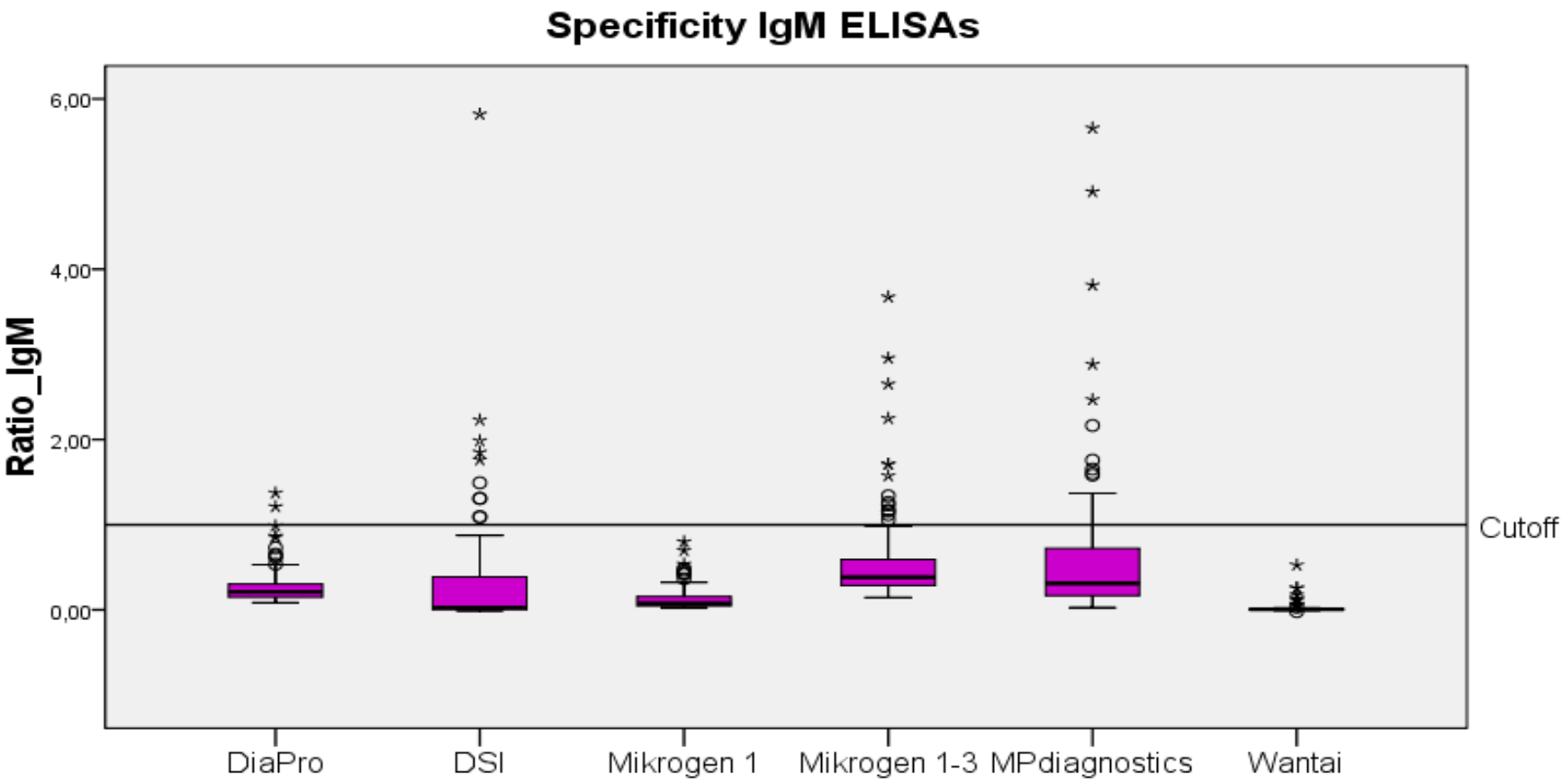
Erasmus MC

Sensitivity panel : HEV PCR confirmed patients

Time of Drawal	# samples	# patients	# samples immune status			genotypes included		
			ID	non-ID	unknown	gt 1	gt 3	unknown
Prior to infection	12	12	12	0	0	0	9	3
< 6 wks	34	31	16	14	4	7	18	9
6 wks <t< 6 months	22	19	15	4	3	0	16	6
>6 months	20	16	16	3	1	1	17	2
total	88							

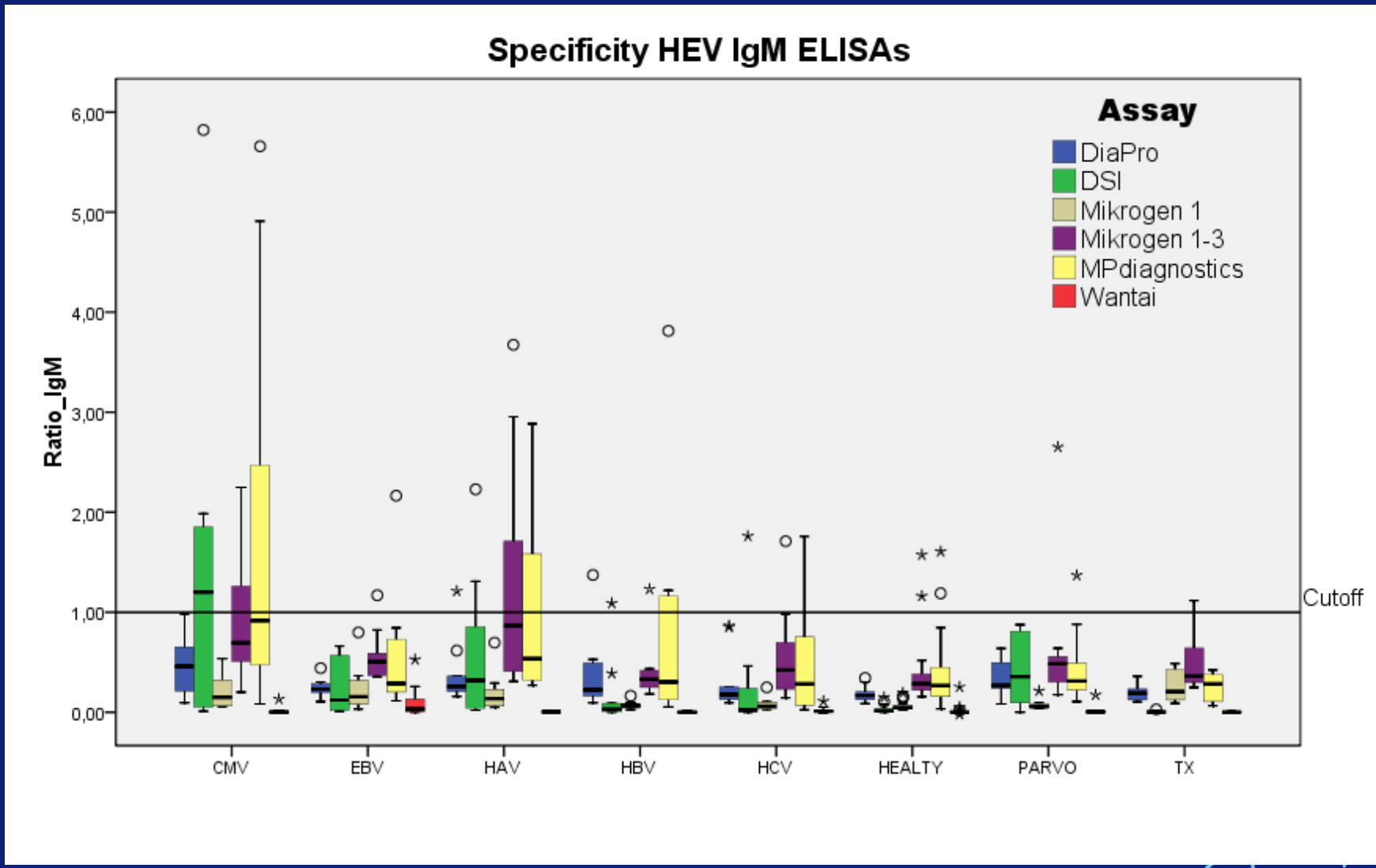
Division was made on basis of clinical symptoms in combination with retrospective HEV RT-PCR analysis

Specificity panel HEV- IgM



N= 89 samples

Specificity panel HEV- IgM



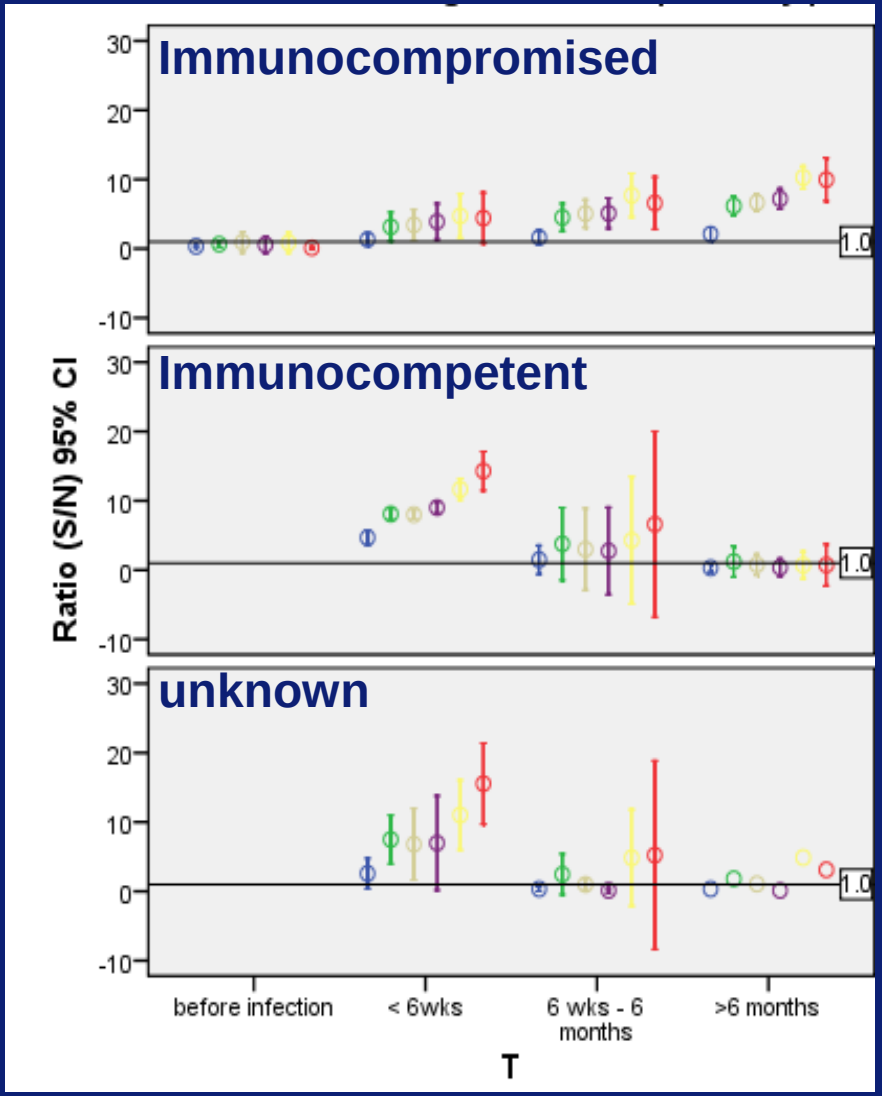
HEV serology – validation of commercial assays

	Analytical sensitivity (titers at cutoff)					Clinical performance	
	IgM		IgG				
	geno 1	geno 3	WHO	geno 1	geno 3	Sens. IgM	Spec. IgM
Mikrogen old	4000	250	1600	6400	800	52%	**>99%**
Mikrogen new	32000	16000	**3200**	**>12800**	**3200**	79%	90%
MP diagnostics	**>64000**	4000	800	3200	100	74%	84%
DSI	8000	4000	800	3200	800	71%	90%
DiaPro	32000	32000	800	6400	100	**81%**	98%
Wantai	**>64000**	**>64000**	1600	**>12800**	800	75%	**>99%**
DRG	32000	32000	800	6400	100	excluded	
Diacheck	4000	250	400	3200	100		

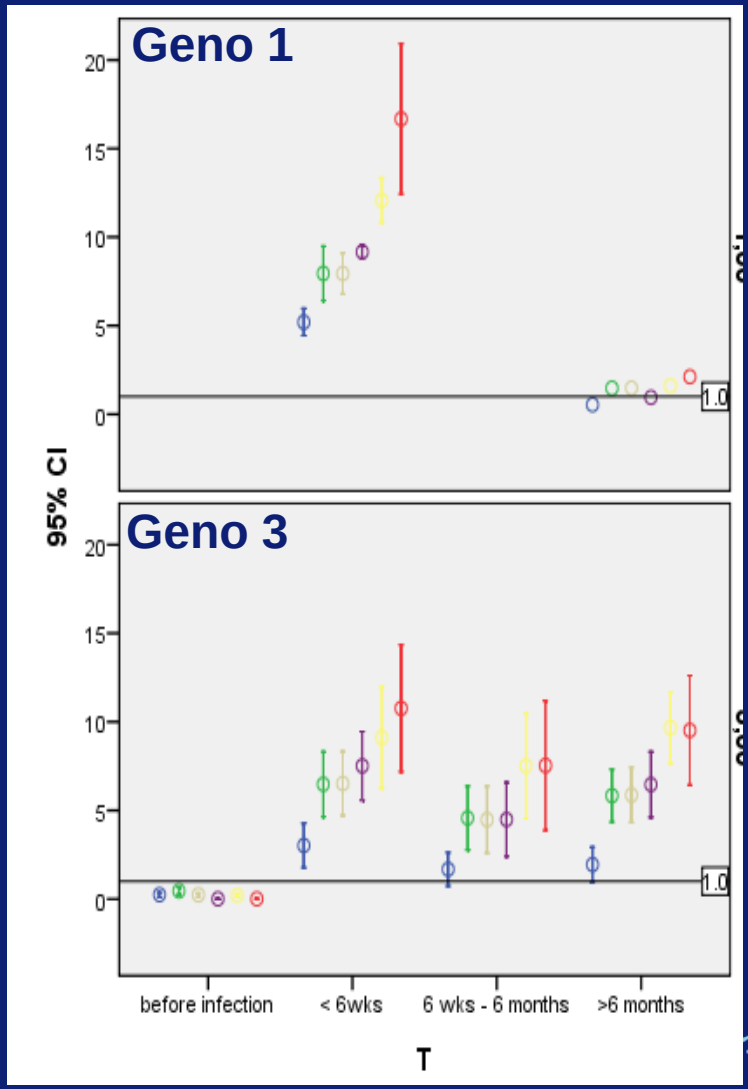
IgM ratio's sensitivity panel

Ratio_Mikrogen_1_IgM
Ratio_Mikrogen_13_IgM
Ratio MP IgM
Ratio_DSI_IgM
Ratio_DiaPro_IgM
ratio_wantai_IgM

per immune status group



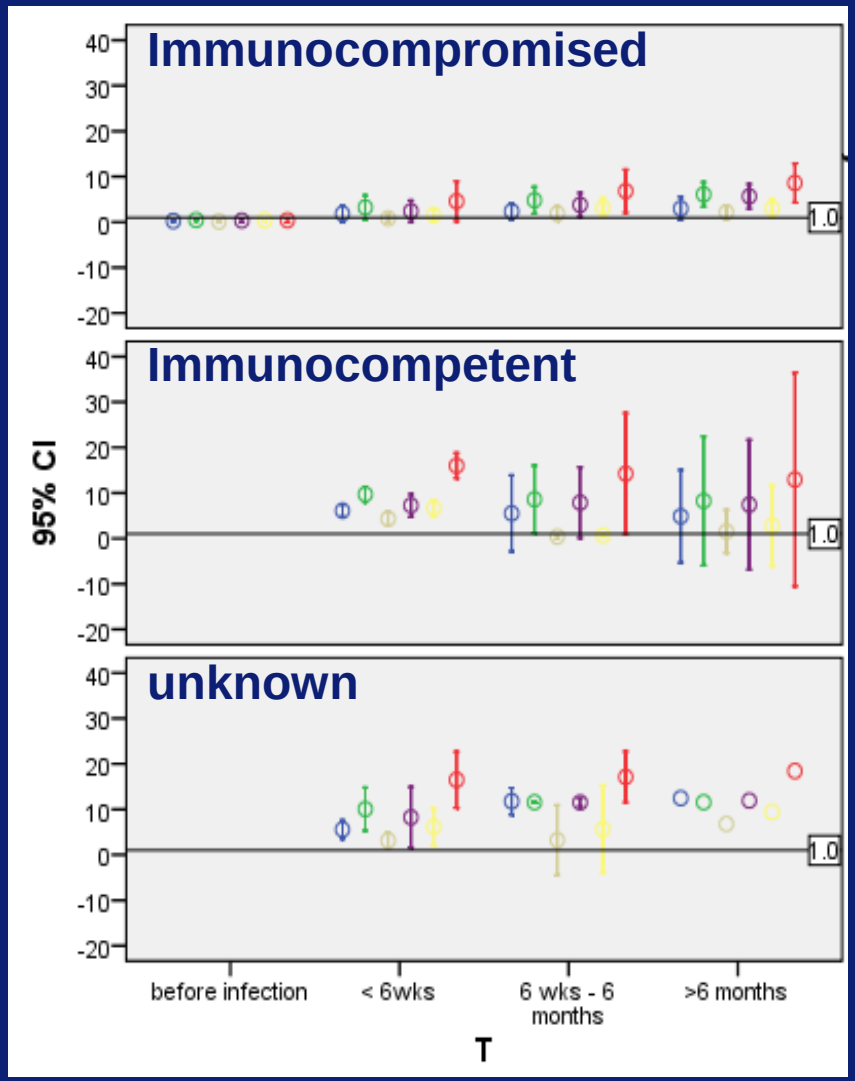
per genotype



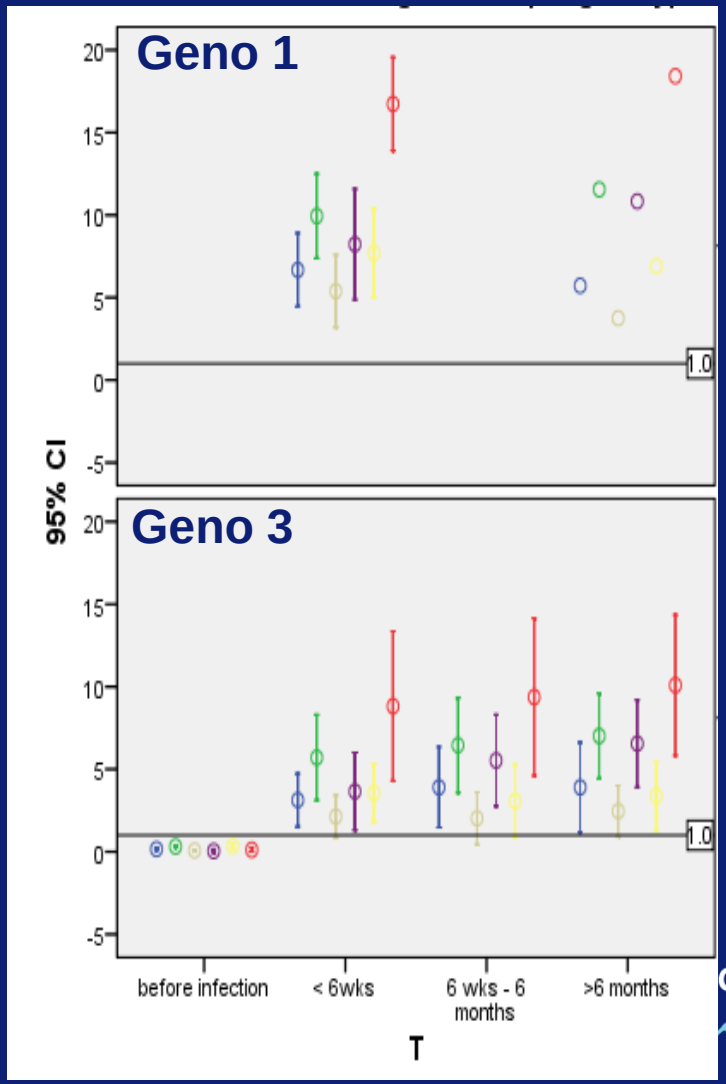
IgG ratio's sensitivity panel

Ratio_Mikrogen_1_IgG
Ratio_Mikrogen_13_IgG
Ratio_MP_IgG
Ratio_DSI_IgG
Ratio_DiaPro_IgG
ratio_wantai_IgG

per immune status group

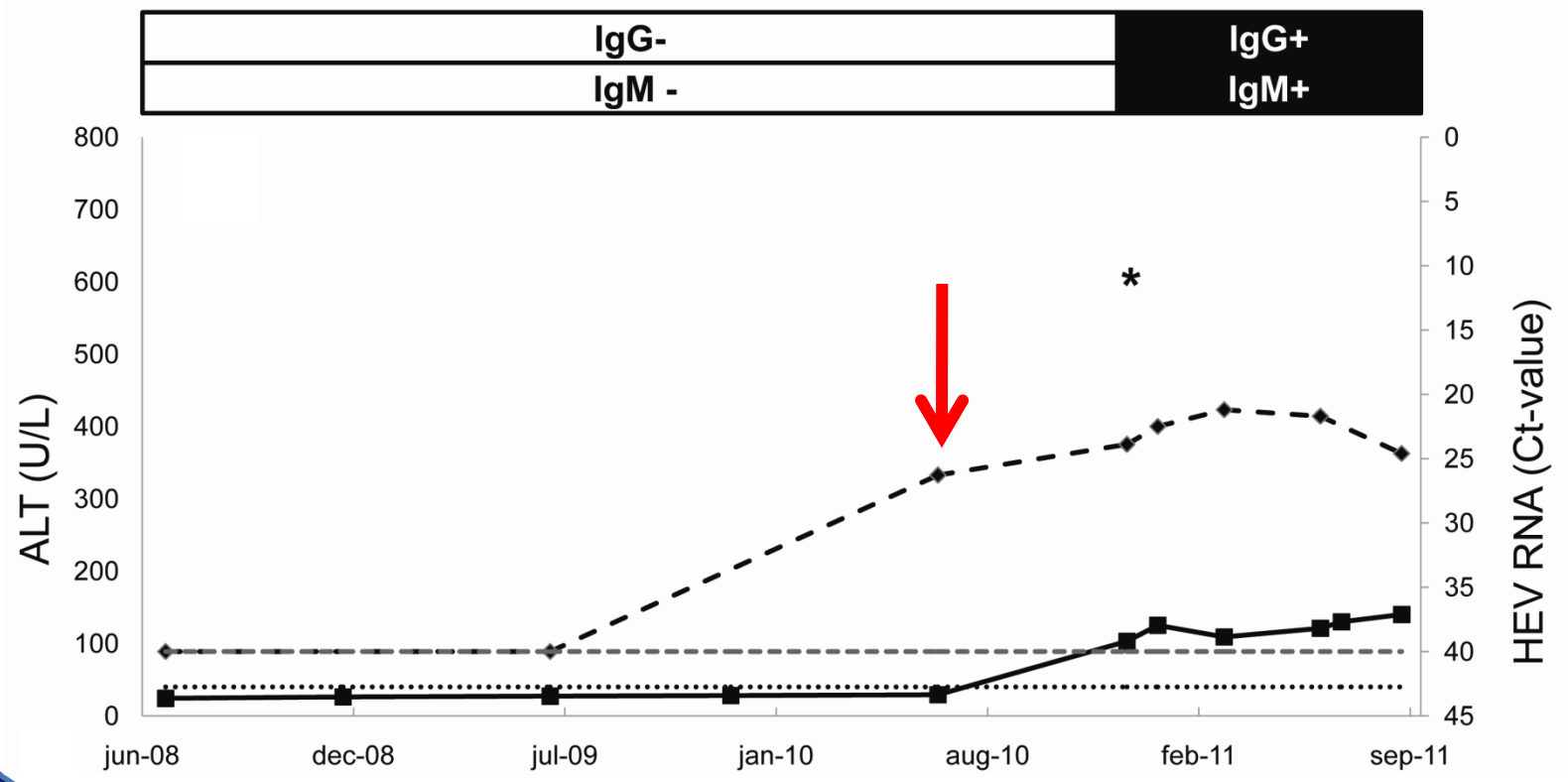


per genotype



Pas et al, in preparation

Course of chronic HEV infection



ALT
HEV PCR
ULN ALT
LOD HEV PCR

Erasmus MC

Parameters of HEV cases of SOT group

	median	range	ULN (F/M)
Peak ALT (U/L)	301	81 - 909	30/40
Peak AST (U/L)	172	66 - 1016	30/36
Peak γ-GT (U/L)	299	72 - 1740	34/49
Peak Billirubine (µmol/l)	16	5 - 100	16/16
Peak HEV-RNA (Ct values)	20.0	16.7 - 26.6	NA
Period of HEV-RNA positivity (months)	16	6 - 55	NA
Time between the SOT and first HEV-RNA positive (months)	2.0	-0.3 - 20.1	NA
Time of HEV-RNA positivity prior to HEV IgM (days)	32	0 - 826	NA
Time of HEV-RNA positivity prior to HEV IgG (days)	124	0 - 826	NA

** HEV IgM and IgG (Wantai Biochemicals)*

Erasmus MC

Pas et al, EID 2012

Current status of HEV diagnostics

* Pathology not specific

  Invasive

* Virus culture inefficient

* HEV serology

  - validation of commercial assay

  - conformational testing using blot

*** Molecular diagnostics**

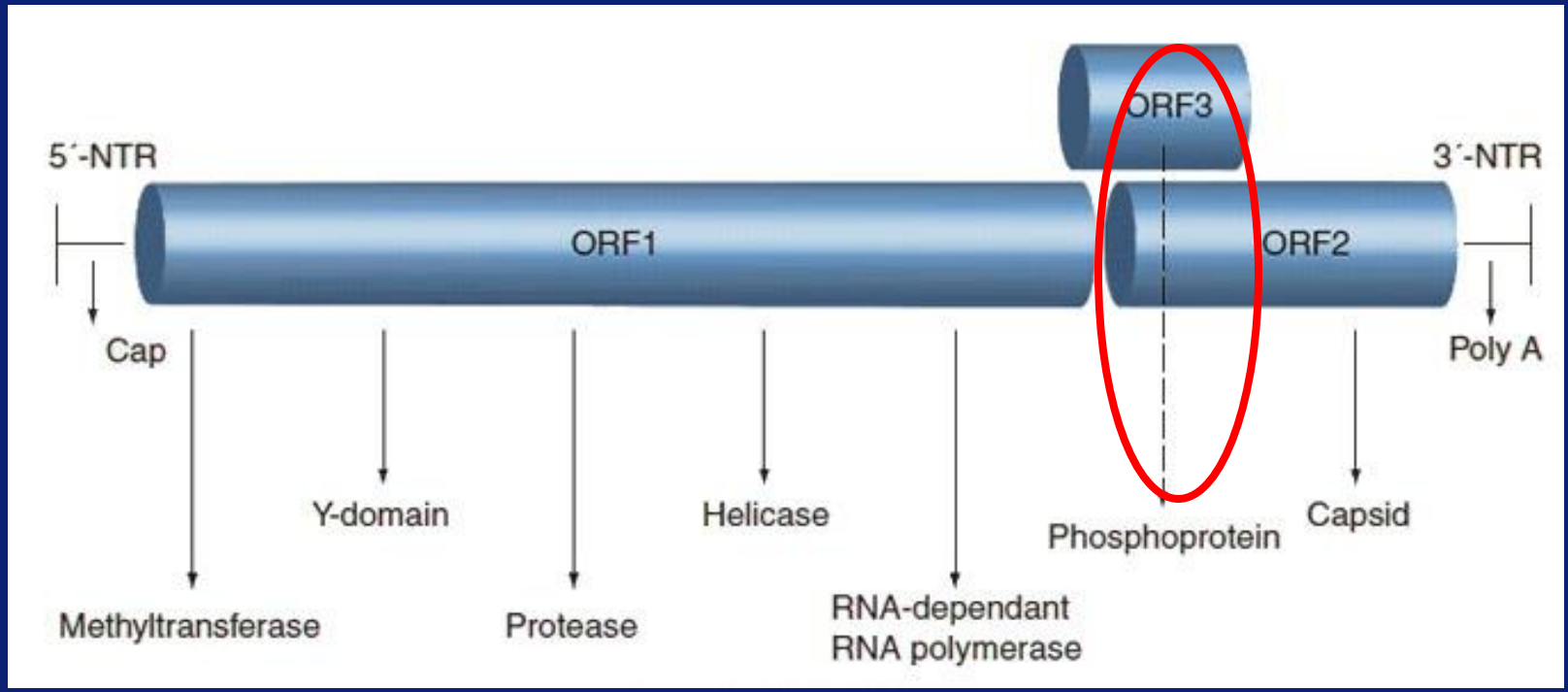
- real time vs conventional RT-PCR

-standardisation

- genotyping

Erasmus MC

HEV real time RT-PCR



Erasmus MC

HEV real time RT-PCR

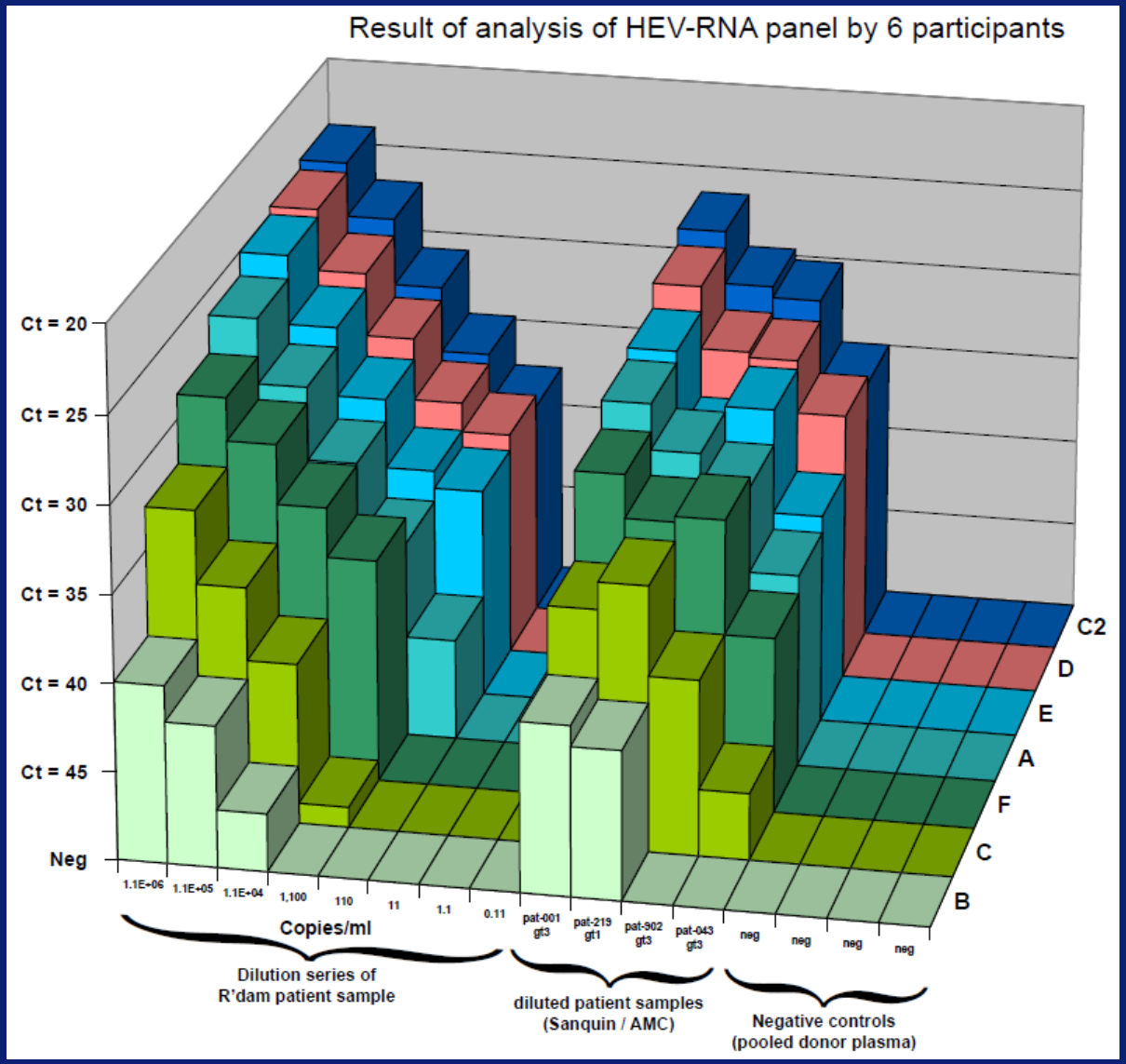
1st Author	YoP	Validated	Target	Principle	Length	Limit of Detection
Mansuy et al	2004	Gt1 and 3		Two-step Taqman on Light cycler		1E3copies/ml
Orru et al	2004			SyBRgreen		10GEC
Jothukumar et al	2004	Gt1-4	ORF2/ORF3	taqman	70 bp	4GEC, others claim no quant.
Ahn et al	2006	gt3	ORF2/ORF3	taqman	103bp	16 copies/ml
Enouf et al	2006	Gt1-4	ORF2/ORF3	Taqman on Light cycler	86bp	10 copies/rx
Li et al	2006	GT 1-2,macaques	ORF2	SyBRgreen	207bp	4.5x103 copies/rx
Gyarmati et al	2007	Gt1-4 2 human samples	ORF2	Taqman on Light cycler	113 bp	1-20 geq/rx
Zhao et al	2007	Gt1-4, theoretical	ORF2/ORF3	taqman	103bp	5,6E3 copies/ml pseudovirus
Ward et al	2009	swine isolates only	comparison of four of the above ; Jothukumar et al most sensitive			
Vasichova et al	2012	swine isolates only	**dual target** Jothukuman2006+ Gyarmati2007	taqman in LC480	70/113bp	50 copies/ml

HEV NAT quality assessment 2011 – Sanguin/ R'dam

- *6 participating labs of Dutch HEV workgroup*
- *Samples were randomized and included:*
  - *10 log dilution series of genotype 3 sample*
  - *calibrated against candidate WHO HEV standard**
  - *four (diluted) patient samples, 3 x gt3 and 1x gt1*
  - *four negative controls (EDTA-(mini) pools)*

** Baylis SA, et al. J Clin Microbiol. 2011 Apr;49(4):1234-9.*

HEV NAT quality assessment – Results



Sanquin

Erasmus MC

Quality of HEV nucleic acid amplification assays

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1234–1239 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.02578-10

Standardization of Hepatitis E Virus (HEV) Nucleic Acid Amplification Technique-Based Assays: an Initial Study To Evaluate a Panel of HEV Strains and Investigate Laboratory Performance▽†

Sally A. Baylis,* Kay-Martin Hanschmann, Johannes Blümel, and C. Micha Nübling
on behalf of the HEV Collaborative Study Group‡

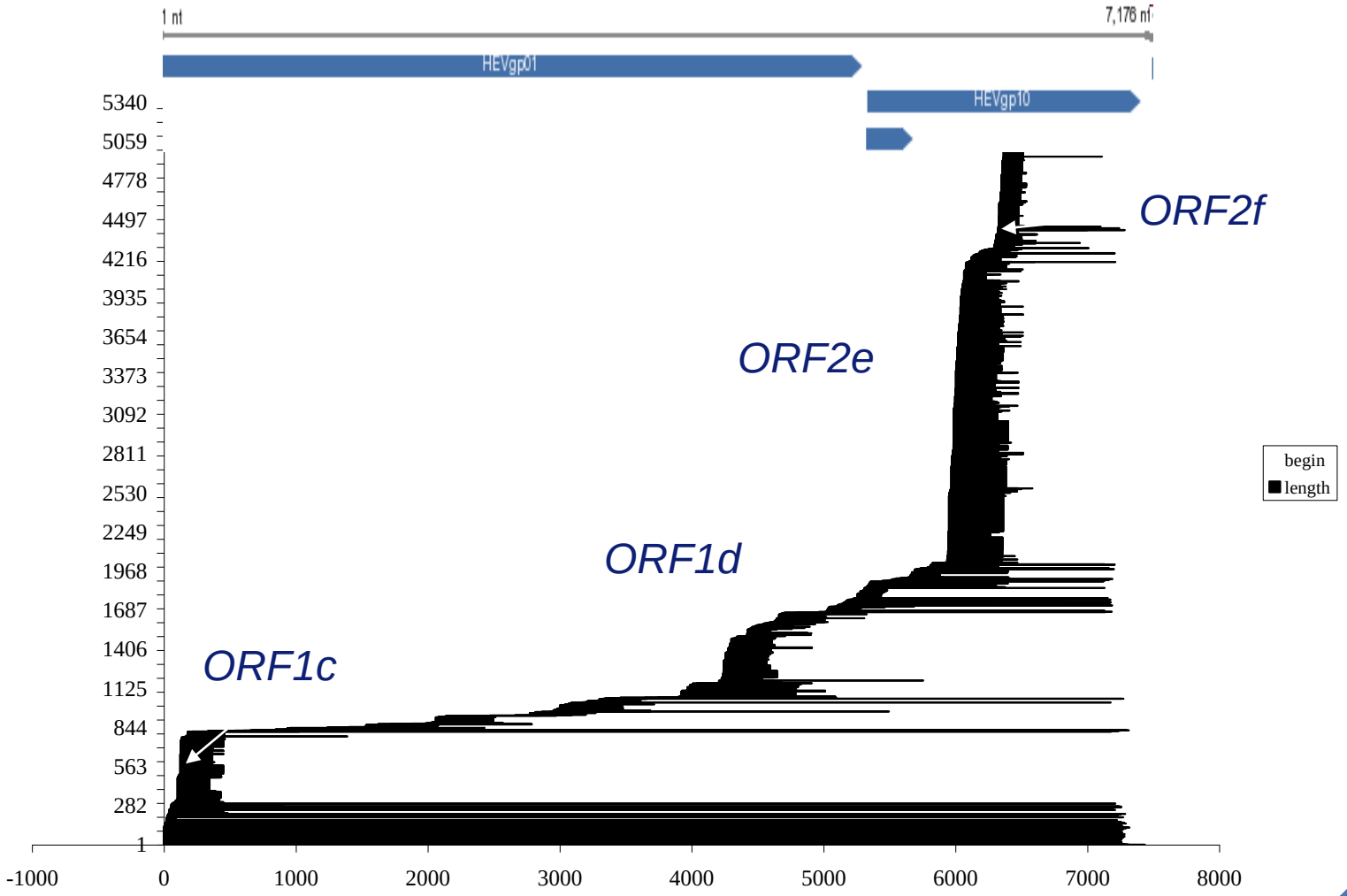
24 laboratories, 22 HEV-positive plasma, 10-fold serial dilutions of HEV genotypes 3a, 3b, 3f, and 4c.

International standardisation needed

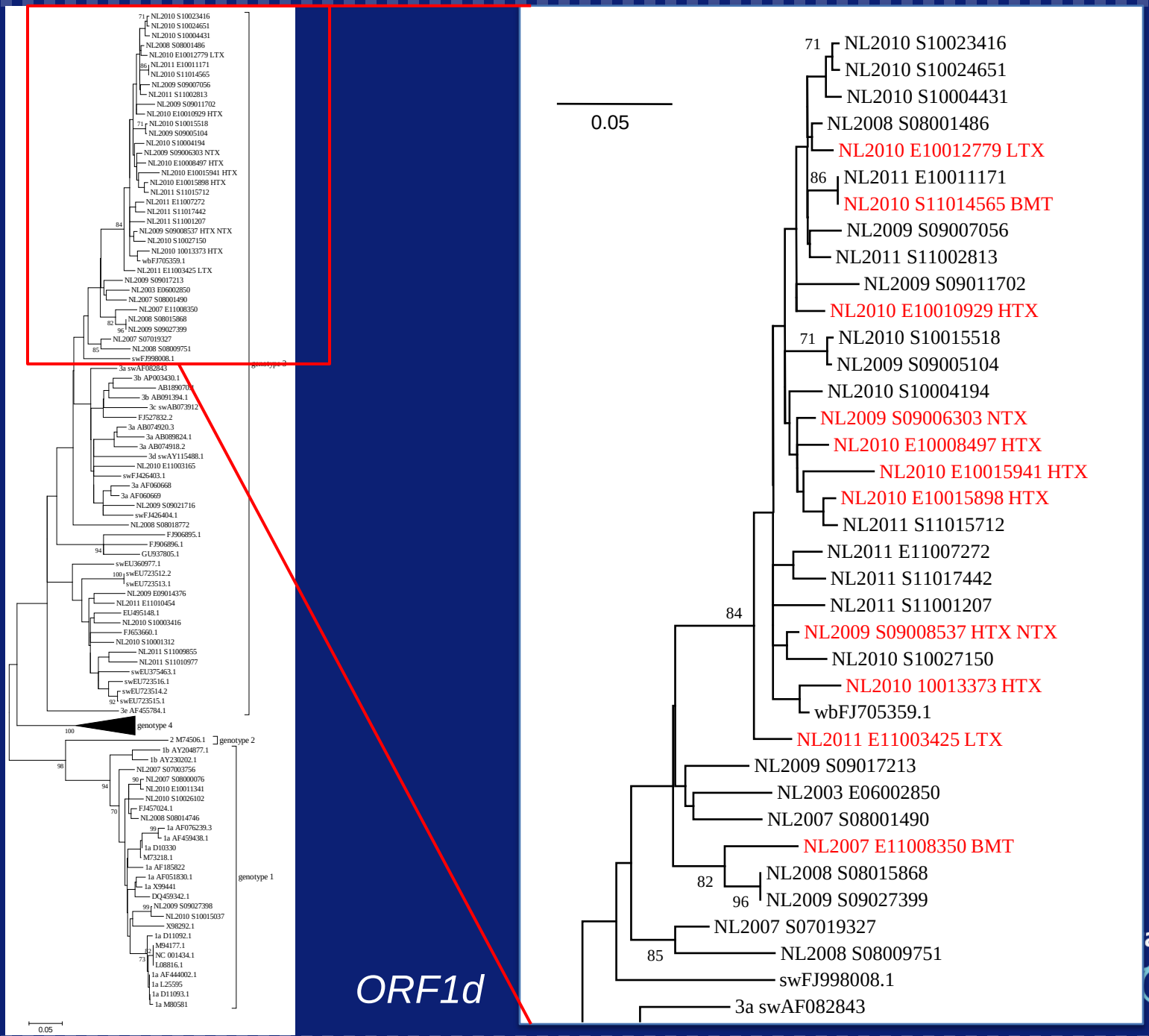
→ 1st HEV RNA WHO standard available (Gt3, 250,000 IU/ml)!

Erasmus MC

Distribution of available sequences along the genome



Courtesy: Harry Vennema, RIVM, The Netherlands


0.05
71 NL2010 S10023416
NL2010 S10024651
NL2010 S10004431
NL2008 S08001486
NL2010 E10012779 LTX
86 NL2011 E10011171
NL2010 S11014565 BMT
NL2009 S09007056
NL2011 S11002813
NL2009 S09011702
NL2010 E10010929 HTX
71 NL2010 S10015518
NL2009 S09005104
NL2010 S10004194
NL2009 S09006303 NTX
NL2010 E10008497 HTX
NL2010 E10015941 HTX
NL2010 E10015898 HTX
NL2011 S11015712
NL2011 E11007272
NL2011 S11017442
84 NL2011 S11001207
NL2009 S09008537 HTX NTX
NL2010 S10027150
NL2010 10013373 HTX
wbFJ705359.1
NL2011 E11003425 LTX
NL2009 S09017213
NL2003 E06002850
NL2007 S08001490
NL2007 E11008350 BMT
82 NL2008 S08015868
96 NL2009 S09027399
NL2007 S07019327
85 NL2008 S08009751
swFJ998008.1
3a swAF082843
ORF1d
genotype 4
genotype 2
genotype 1
0.05

Conclusies

- Bewustzijn van HEV infecties in transplantatie settings is stijgende, daarom is accurate en snelle diagnostiek nodig voor de juiste interventie strategie

- Om een HEV infectie te diagnosticeren is zowel serologie als moleculaire diagnostiek (real time RT-PCR) van belang

- De nauwkeurigheid van de huidige HEV serologsiche assays varieert enorm. In onze validatie waren voor IgM DiaPro en Wantai de beste testen, en voor IgG de nieuwe Mikrogen en Wantai assay de beste.

- Kwaliteit van moleculaire assays varieert tussen de verschillende labs, daarom zijn kwaliteitsrondzendingen en standardisatie van belang

Erasmus MC

Acknowledgements

ErasmusMC

Claudia Mulders

Sevgi Deniz

Manon Briede

Mark Pronk

Sandra Scherbeijn

Drs. Roel Streefkerk

Dr. M. Beersma

Prof. Dr. Marion Koopmans

Prof Dr. Ab Osterhaus

Dr. A. A. van der Eijk
Dr. R.A. de Man
Dr. P. Th.W van Hal
Prof. Dr. W. Weimar
Dr. A.H.M.M. Balk

RIVM
Dr. H. Vennema
Dr. J. Reimerink

Sanquin
Dr. B. Hogema