

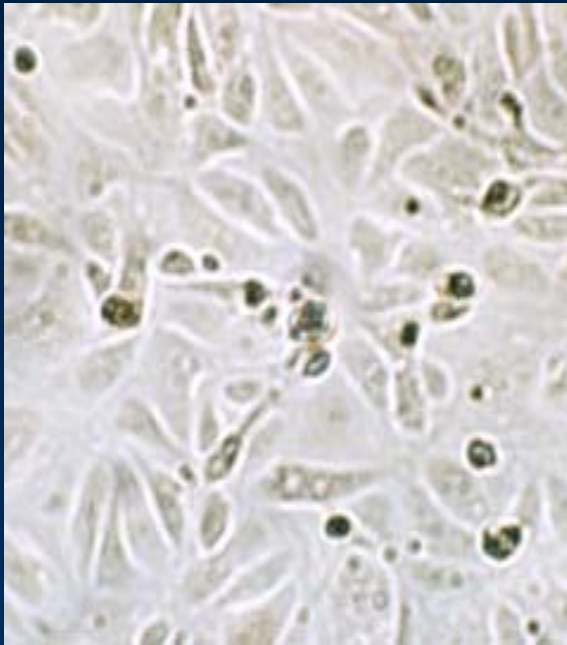
# A newly discovered human pneumovirus isolated from young children with respiratory tract disease

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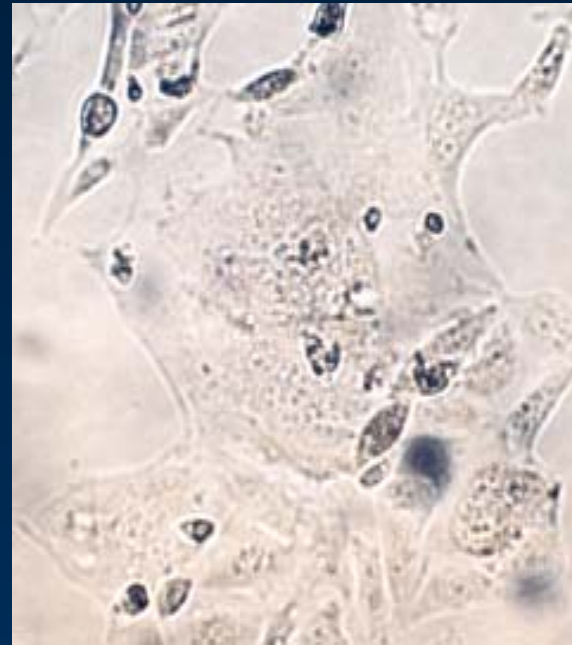
Bernadette G. van den Hoogen, Jan C. de Jong, Jan Groen, Thijs Kuiken, Ronald de Groot,  
Ron A.M. Fouchier and Albert D.M.E. Osterhaus

# Cytopathic effects in tMK cells

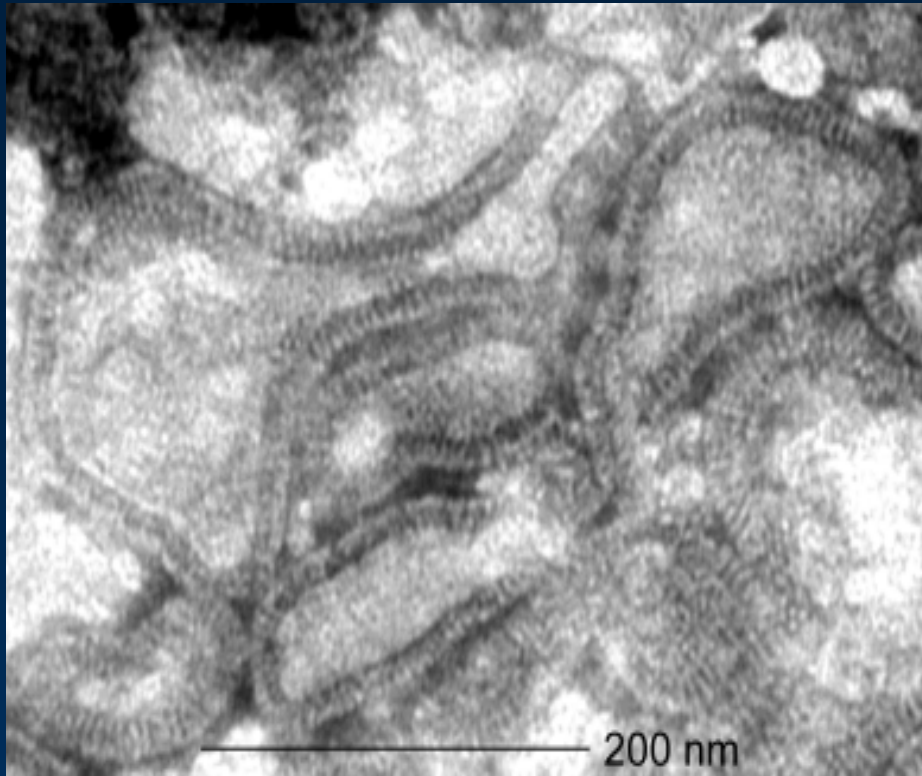
Control



hMPV-infected



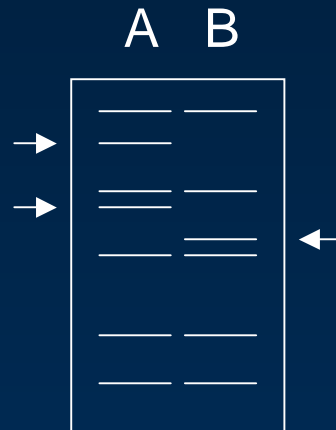
# Electron microscopy



- Pleiomorph
- Average size 100 - 600 nm
- Nucleocapsids rarely observed
- Envelope projections of 13 -17 nm
- Paramyxovirus

# RAP-PCR procedure

1. RNA isolation
2. RAP-PCR
3. Gel electrophoresis



4. Cloning (pCR2.1)
5. Sequencing
6. BLAST

# PCR and sequence analysis of vRNA



# Genomic organisation of pneumoviruses



APV, 13.373 bp

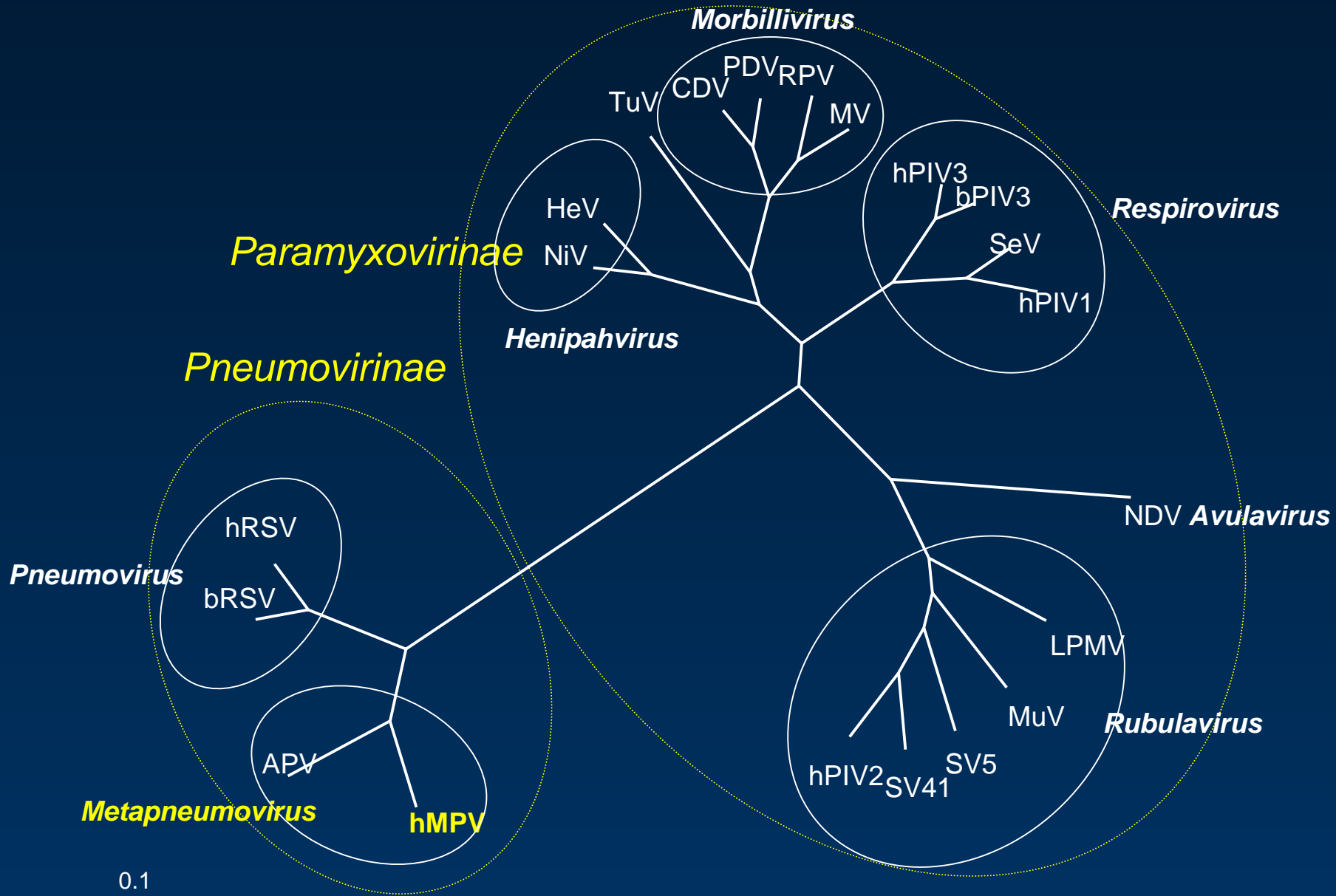


hMPV, 13.378 bp



hRSV, 15.225 bp

# Order Mononegavirales, family Paramyxoviridae

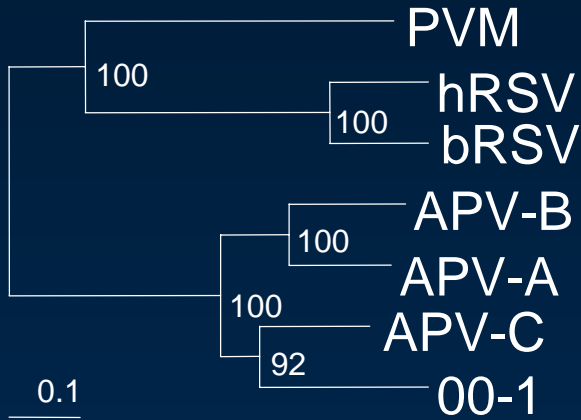


0.1

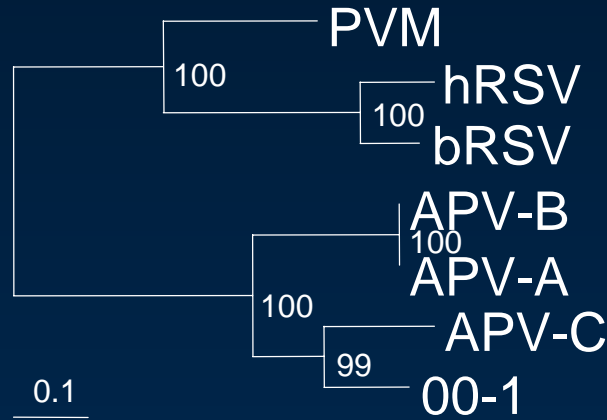
DNA Maximum likelihood, Polymerase ORF

# Phylogenetic analysis

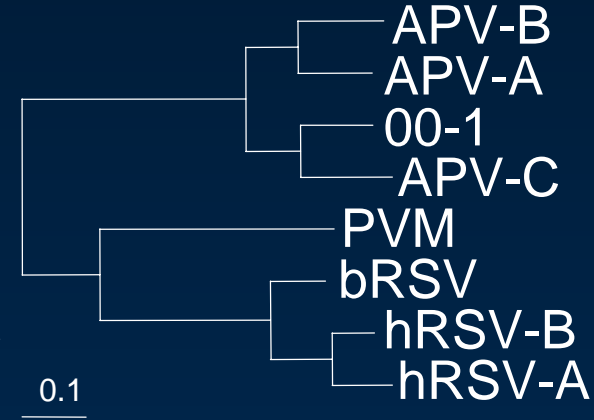
**F**



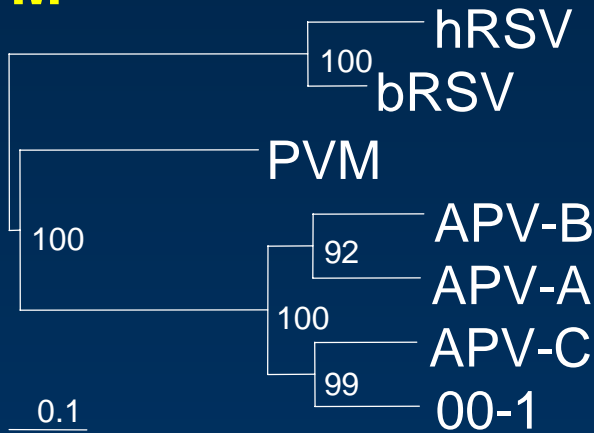
**N**



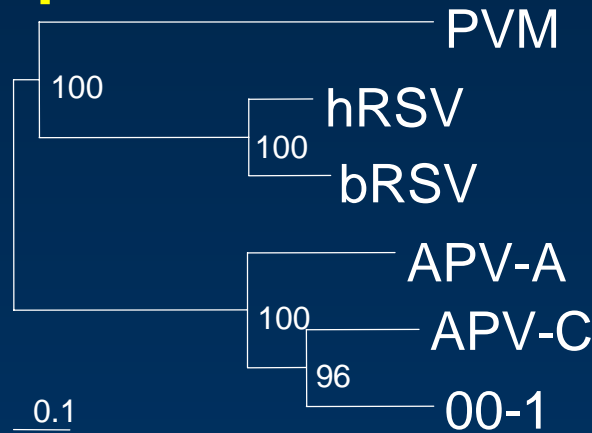
**M2-1**



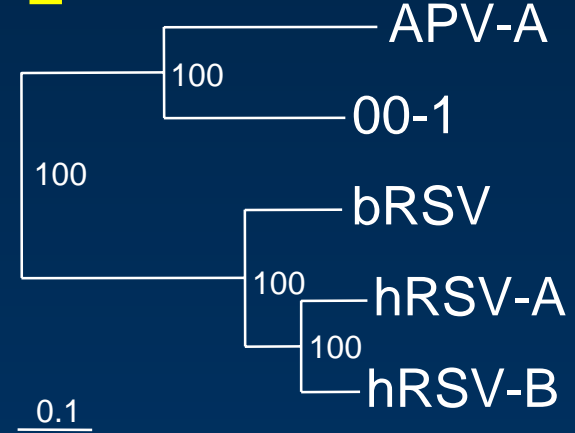
**M**



**P**



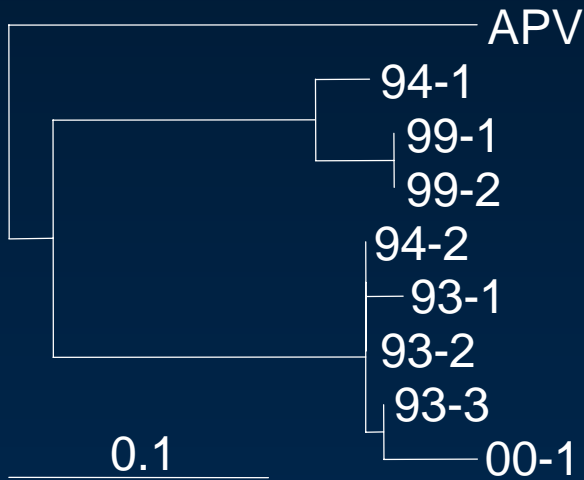
**L**



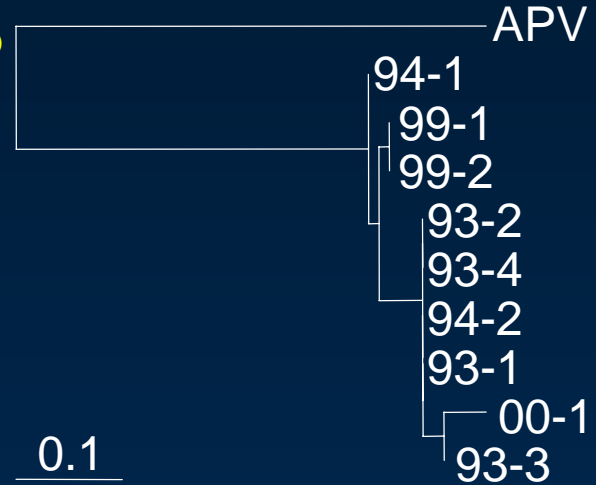


# Genetic diversity of hMPV isolates

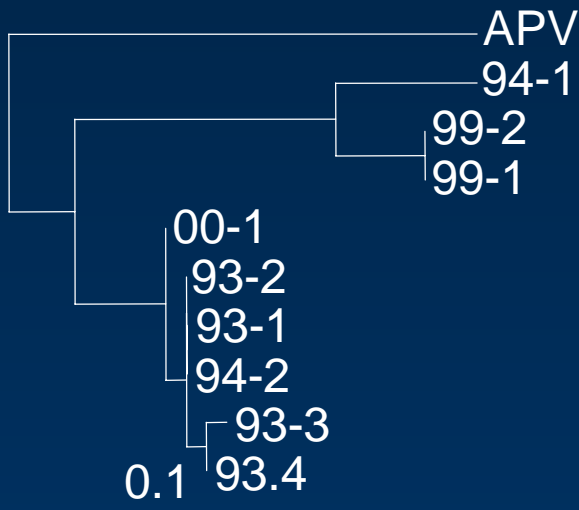
F, 142 bp



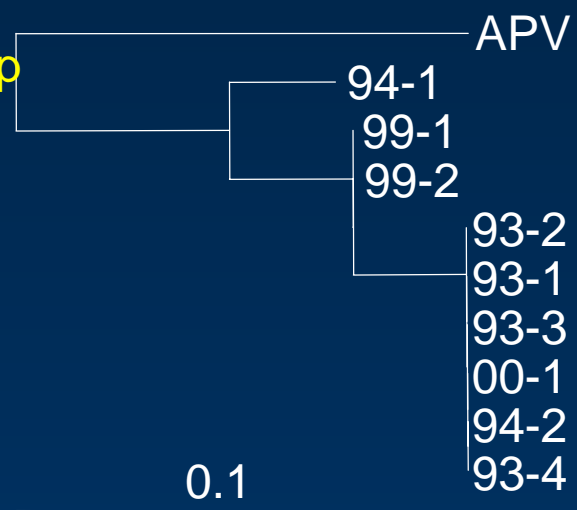
N, 71 bp



M, 143 bp



L, 102 bp



## Amino acid sequence homology of metapneumovirus ORFs

Sequence identity with ORFs of hMPV isolate 00-1

	N	P	M	F	M2.1	M2.2	SH	G
hMPV 99-1	95	86	98	94	95	90	57	33
APV-C	88	68	87	81	84	56	N.A.	N.A.
APV-A	69	55	78	68	72	25	18	9

## Seroprevalence of hMPV in The Netherlands

Age (Years)	Immunofluorescence assays		Virus neutralization assays		
	<i>n</i> tested	<i>n</i> positive (%)	<i>n</i> tested	<i>n</i> positive (%)	Titre range
0.5 - 1	20	5 (25)	12	3 (25)	16-32
1 - 2	20	11 (55)	13	4 (31)	16-32
2 - 5	20	14 (70)	8	3 (38)	16-512
5 - 10	20	20 (100)	4	4 (100)	32-256
10 - 20	20	20 (100)	4	3 (75)	32-128
> 20	20	20 (100)	4	3 (75)	32-128
8 - 99 <sup>1</sup>	72	72 (100)	11	11 (100)	16-128

## Conclusions-1

### a newly discovered human metapneumovirus (hMPV)

- hMPV is associated with respiratory tract illnesses:
  - In young children (& elderly, immunocompromised, others)
  - Primarily in the winter season
  - Symptoms similar to those caused by RSV (ranging from mild respiratory symptoms to severe bronchiolitis and pneumonia)
  - From children infected with hMPV, no other human pathogens were isolated
  - hMPV vRNA is not detected in throat swabs from healthy children
  
- Experimental infections of animals with hMPV:
  - hMPV does not replicate in chickens and turkeys
  - hMPV replicates in macaques, and causes mild respiratory symptoms
  - hMPV replicates in guinea pigs and ferrets

## Conclusions-2

### a newly discovered human metapneumovirus (hMPV)

- hMPV is a putative new member of the *pneumovirinae*, genus *metapneumovirus*
  - Virus characteristics (EM, CPE, HA<sup>-</sup>, CHCl<sub>3</sub>-sensitive, trypsin-dependence)
  - Sequence homology
  - Genomic organisation
- hMPV is a virus that commonly infects humans
  - Sero-prevalence reaches 100 % of humans by the age of five years
  - In the 2000 winter season, hMPV was isolated from 10 % of children with respiratory tract illnesses (negative for other pathogens)
  - Human sera collected in 1958 were all positive for antibodies to hMPV
  - Reinfection with homologous/heterologous virus strains?
- hMPV diversity
  - Two genetic clusters of hMPV isolates were found in the Netherlands
  - The genetic clusters may represent different serotypes of hMPV

# Acknowledgements

## Studies:

### Molecular

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C. Verheyen  
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R. Dias-d'Ullois

### Diagnostic

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J. de Jong

### Paediatric

R. de Groot

### Pathology

T. Kuiken

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