De rol van moleculaire diagnostiek bij kinderen met een 'community-acquired pneumonia'.

Critically Appraised Topic

Wim Maurissen

15/05/2012

WHO Definitie pneumonie

Table 1 Clinical definition of pneumonia according to the WHO

	Fever[a]	Tachypnea[b]	Chest indrawing	Not able to drink and/or central cyanosis
Stage I (non-severe)	+	+		
Stage II (severe)	+	+	+	
Stage III (very severe)	+	+	+	+

[a] Fever≥38°C axillary

[b] Respiratory rate >50/min (2–11 months) and >40/min (1–5 years)

Manon CM, et al. Eur J Pediatr 2009, 168:1429-1436.

Incidentie

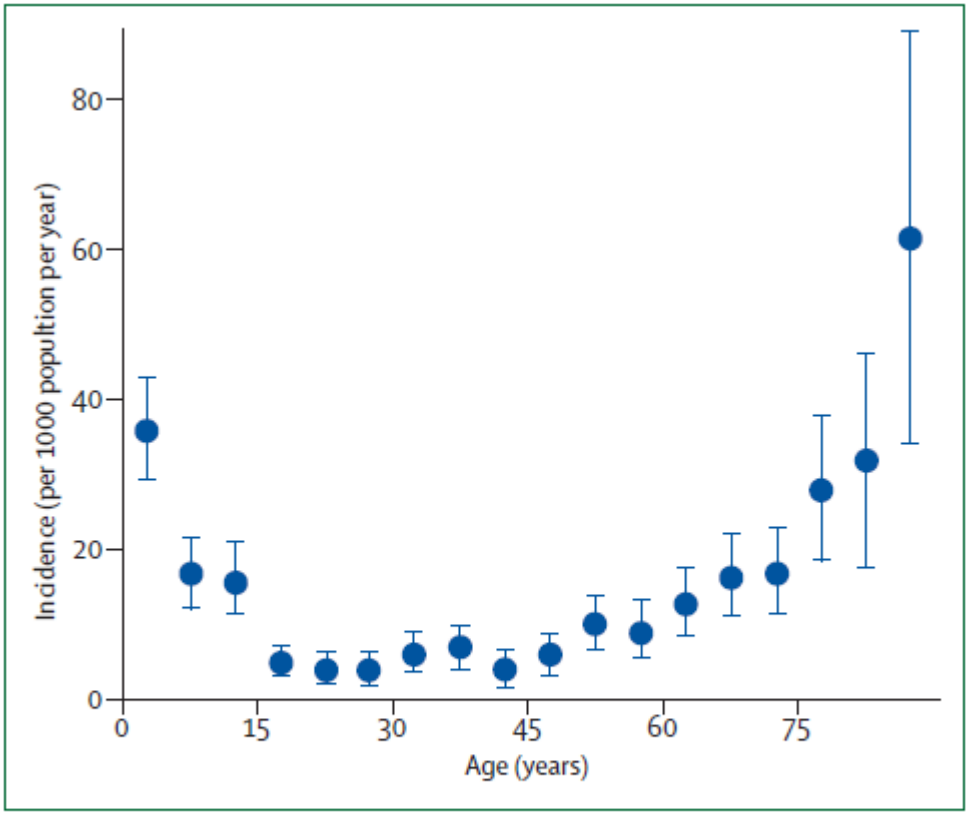


***Figure 1*: Age-specific incidence of community-acquired pneumonia**
Error bars=95% CIs. Modified from reference 8 with permission of Oxford University Press.

Ruuskanen O, et al. Lancet 2011, 377:1264-1275.

Table 2 Incidence per 10 000 population

Country	Disease	Definition of pneumonia	Age 0–1 year (95% CI)	Age 0–2 years (95% CI)	Age 0–3 years (95% CI)	Age 0–5 years (95% CI)	Age 0–16 years (95% CI)
Whole population data							
Norway	Pneumonia	Signs and CXR		42.1 (32 to 52.3)		32.8 (26.8 to 38.8)	14.7 (12.2 to 17.1)
UK	Pneumonia	Signs and CXR				33.8 (31.1 to 36.7)	14.4 (13.4 to 15.4)
Germany (PRI.DE)	Pneumonia	Clinical including comorbidity			137		
Germany (Schleswig-Holstein)	Pneumonia	Clinical by parental interview	181.1			150.1	
Admitted to hospital							
UK	Pneumonia	Signs and CXR				28.7 (26.2 to 31.4)	12.2 (11.3 to 13.2)
Germany (Kiel)	Pneumonia and bronchiolitis	Signs and CXR including comorbidity	111.3			65.8	30
Germany (PRI.DE)	Pneumonia	Clinical including comorbidity			107		
USA	All-cause pneumonia	Coding including comorbidity		129.6			

CXR, chest x-ray.

Harris M, et al. Thorax 2011, 66:1-23.

Etiologie

Bacteriën: *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes, Staphylococcus aureus.*
Atypische bacteriën: *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydia pneumoniae, Chlamydia trachomatis*.

Harris M, et al. Thorax 2011,66:1-23.

Virussen:

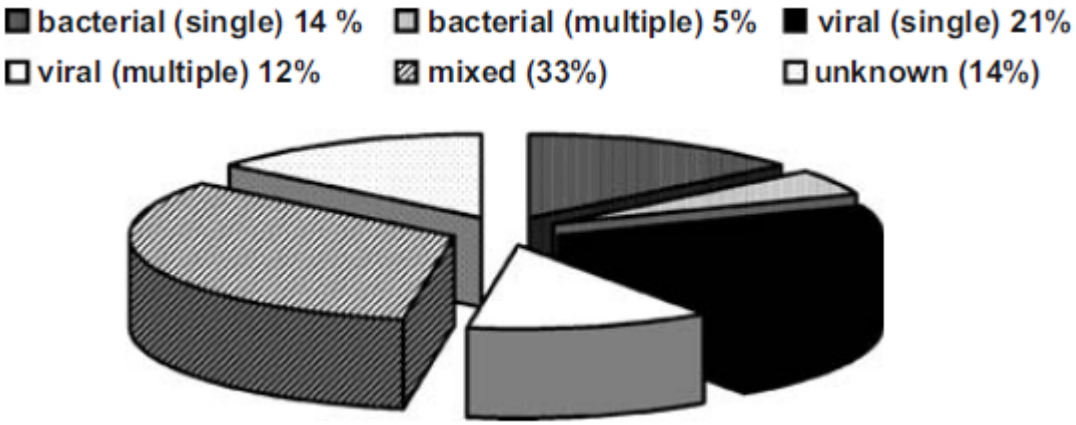
Panel: **Viruses linked to community-acquired pneumonia in children and adults**

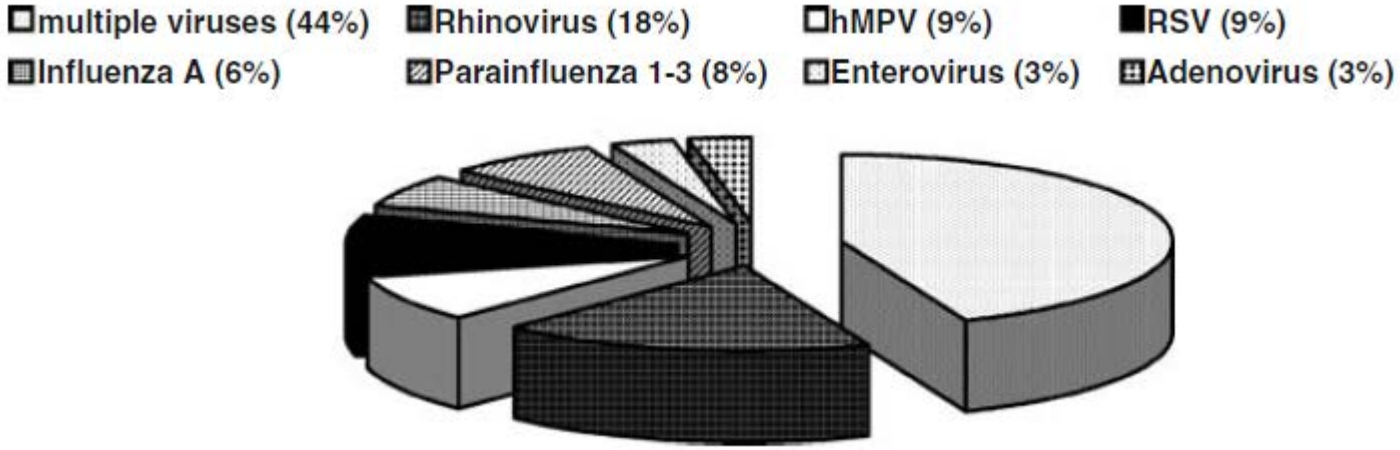
- Respiratory syncytial virus
- Rhinovirus
- Influenza A, B, and C viruses
- Human metapneumovirus
- Parainfluenza viruses types 1, 2, 3, and 4
- Human bocavirus*
- Coronavirus types 229E, OC43, NL63, HKU1, SARS
- Adenovirus
- Enteroviruses
- Varicella-zoster virus
- Hantavirus
- Parechoviruses
- Epstein-Barr virus
- Human herpesvirus 6 and 7
- Herpes simplex virus
- Mimivirus
- Cytomegalovirus†
- Measles†

*Mostly in children. †Mostly in developing countries.

Ruuskanen O, et al. Lancet 2011, 377:1264-1275.

Bij kinderen van 2 maanden tot 5 jaar met een CAP in Zwitserland





Manon CM, et al. Eur J Pediatr 2009, 168:1429-1436.

Bij kinderen van 2 maanden tot 35 maanden met een CAP in Spanje

TABLE I. Respiratory Viruses and Viral Coinfections Detected in 338 Episodes of Community-Acquired Pneumonia in Children Aged Less Than 3 Years Old in Gipuzkoa, Basque Country, Spain

	Age 1–11 months; no. of episodes = 78		12–23 months of age; no. of episodes = 116		Age >23 months; no. of episodes = 144		Total no. of episodes = 338	
Virus group	Total no. (%)[a]	Coinfections no. (%)[b]	Total no. (%)[a]	Coinfections no. (%)[b]	Total no. (%)[a]	Coinfections no. (%)[b]	Total no. (%)[a]	Coinfections no. (%)[b]
Respiratory syncytial virus	22 (28.2)	14 (63.6)	25 (21.6)	13 (52.0)	20 (13.9)	7 (35.0)	67 (19.8)	34 (50.7)
Human bocavirus	10 (12.8)	9 (90.0)	23 (19.8)	14 (60.9)	15 (10.4)	10 (66.7)	48 (14.2)	33 (68.8)
Rhinovirus	11 (14.1)	4 (36.4)	14 (12.1)	4 (28.6)	21 (14.6)	6 (28.6)	46 (13.6)	14 (30.4)
Human metapneumovirus	13 (16.7)	3 (23.1)	8 (6.9)	2 (25.0)	18 (12.5)	6 (33.3)	39 (11.5)	11 (28.2)
Parainfluenza viruses (1–4)	15 (19.2)	9 (60.0)	10 (8.6)	1 (10.0)	13 (9.0)	6 (46.2)	38 (11.2)	16 (42.1)
Influenza viruses (A, B, C)	8 (10.3)	4 (50.0)	12 (10.3)	6 (50.0)	5 (3.5)	3 (60.0)	25 (7.4)	13 (52.0)
Coronavirus[c]	4 (5.1)	2 (50.0)	7 (6.0)	3 (42.9)	11 (7.6)	4 (36.4)	22 (6.5)	9 (40.9)
Adenovirus	2 (2.6)	0 (—)	4 (3.5)	1 (25.0)	5 (3.5)	0 (0.0)	11 (3.3)	1 (9.1)
Episodes with viruses	60 (76.9)[d]	20 (33.3)[e]	81 (69.8)[d]	22 (27.2)[e]	85 (59.0)[d]	19 (22.4)[e]	226 (66.9)	61 (27.0)

[a]Percentage among the total number of pneumonia episodes investigated in the corresponding age group.
[b]Percentage of the total number of infections caused by this virus in the corresponding age group.
[c]Including coronavirus NL63, OC43, and 229E and those not typed.
[d]Chi-square for linear trend = 7.87, $P = 0.005$ on comparing the results obtained in children aged <12 months, 12–23 months, and >23 months.
[e]Chi-square for linear trend = 3.50, $P = 0.061$ on comparing the results obtained in children aged <12 months, 12–23 months, and >23 months.

Cilla G, et al. Journal of medical virology 2008, 80:1843-1849.

Bacterieel: 30-40% van de gevallen waarbij het etiologisch agens gekend is.
Pneumokok: 30% van alle gevallen (kinderen tot 5 jaar)

Table 1. Principal bacteria causing childhood pneumonia (community-acquired apart from the age group birth-1 month), by age.

Bacteria	Age group			
	Birth-1 month	1 month–3 months	3 months–5 years	5 years-18 years
Streptococcus pneumoniae	+	+++	++++	+++
*Haemophilus influenzae**	+	+	+	±
Streptococcus pyogenes	-	+	+	+
Staphylococcus aureus	++	++	+	+
Streptococcus agalactiae	+++	+	-	-
Escherichia coli	++	+	-	-
Mycoplasma pneumoniae	-	+	++	++++
Chlamydiophyla pneumoniae	-	+	+	++
Chlamydia trachomatis	+	++	-	-
Bordetella pertussis	±	++	+	+

++++ very common; +++ common; ++ relatively uncommon; + rare; ± very rare; - absent.
*Mainly untypeable because of wide use of Hib vaccine across Europe.
Adapted from Principi and Esposito [2,3].

Esposito S, et al. The Pediatric Infectious Disease Journal 2012, Publish Ahead of Print.

Seizoensgebonden distributie bij kinderen van 2 maanden tot 35 maanden met een CAP in Spanje

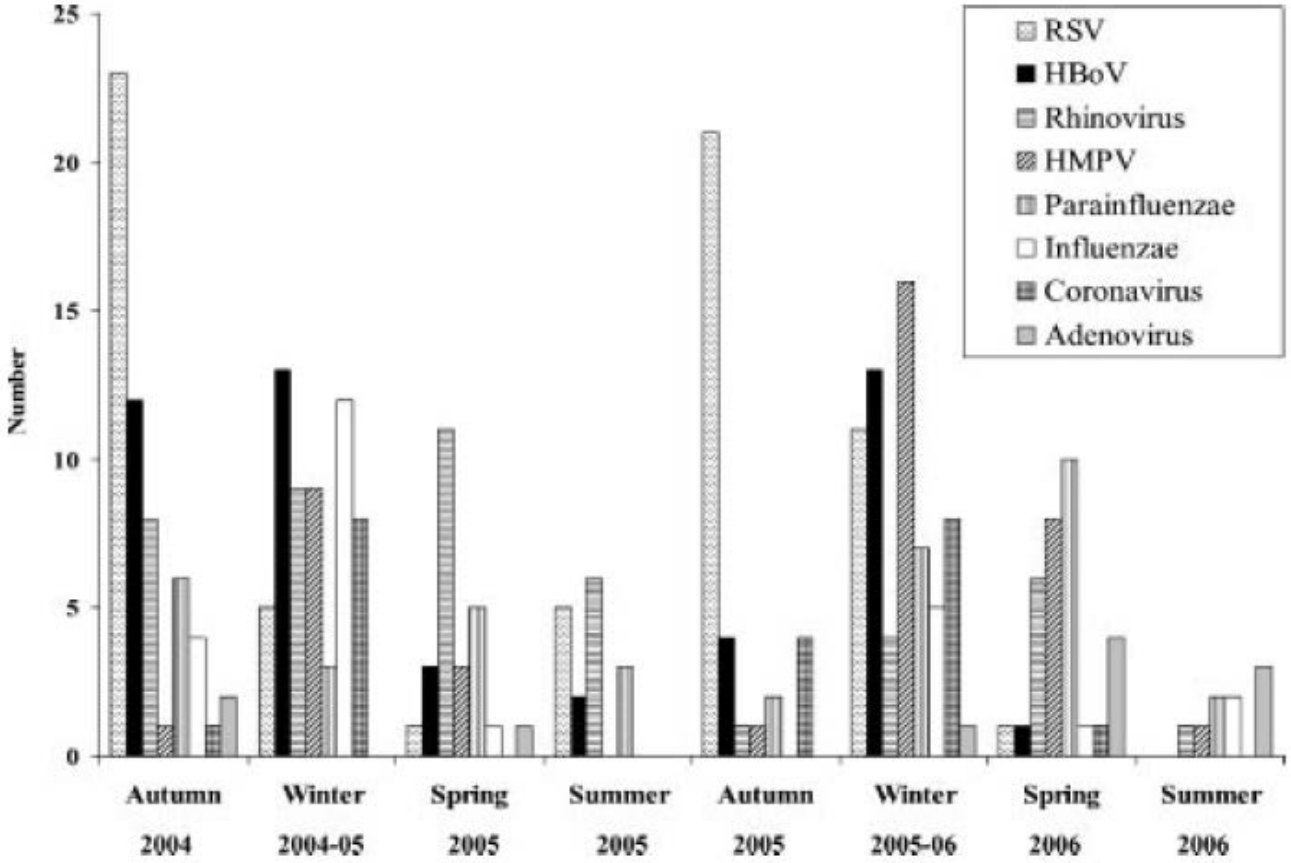


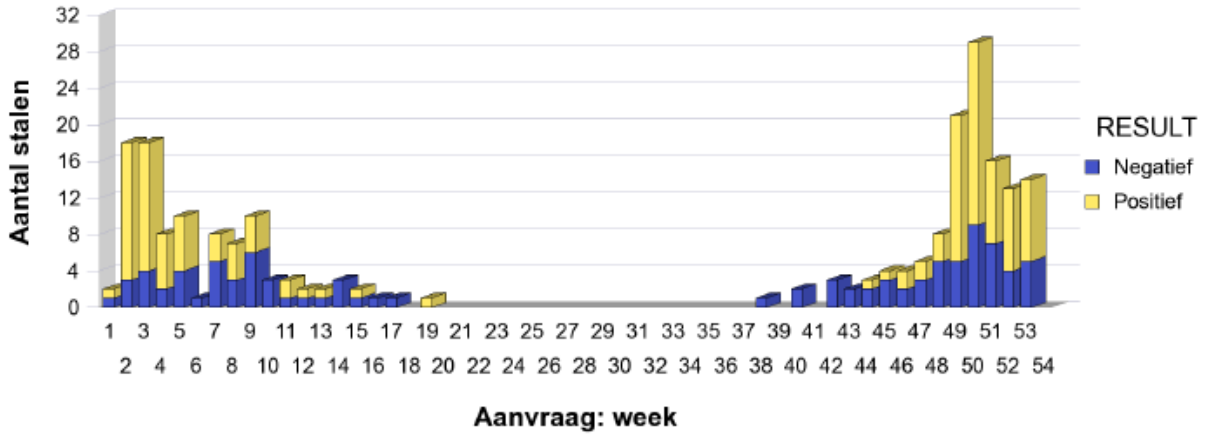
Fig. 2. Seasonal distribution of the most frequently detected viruses in 338 episodes of community-acquired pneumonia in Gipuzkoa (Basque Country, Spain) between October 2004 and September 2006.

Cilla G, et al. Journal of medical virology 2008, 80:1843-1849.

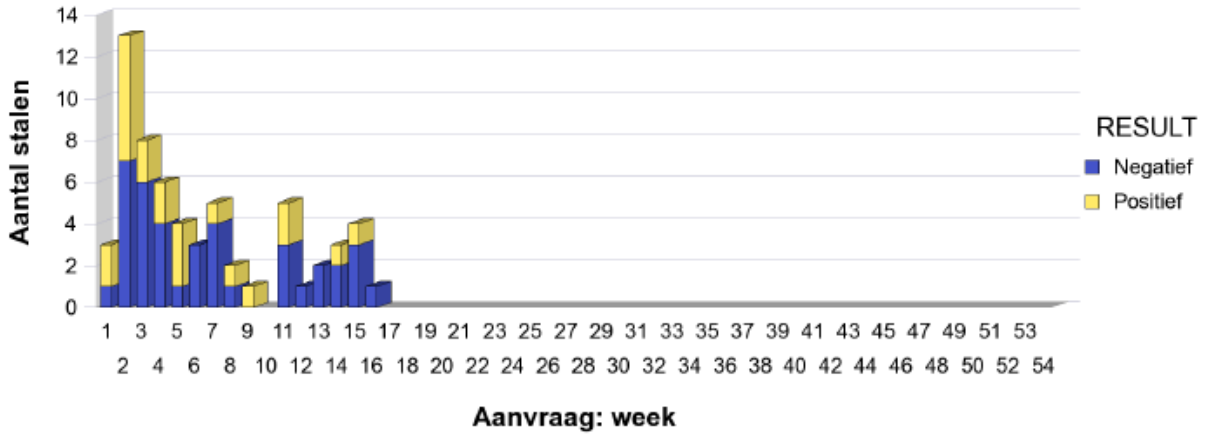
Ziekenhuis Oost-Limburg

RSV : antigeendetectie

2.011



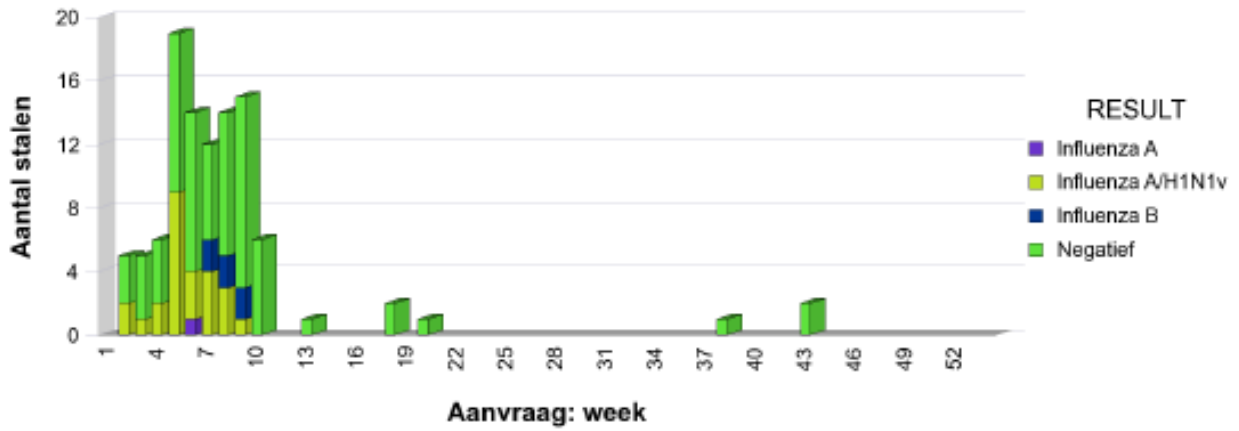
2.012



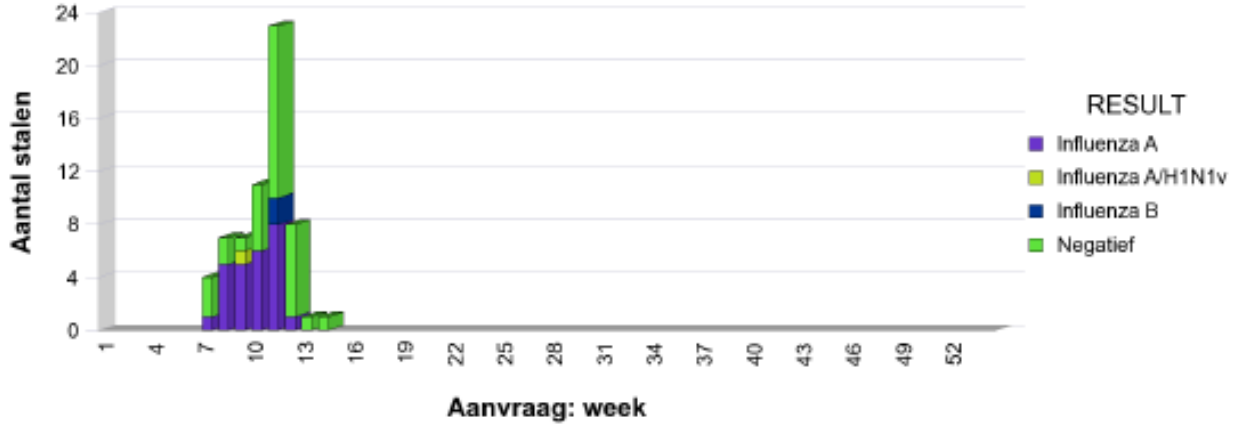
Ziekenhuis Oost-Limburg

Influenza

2.011



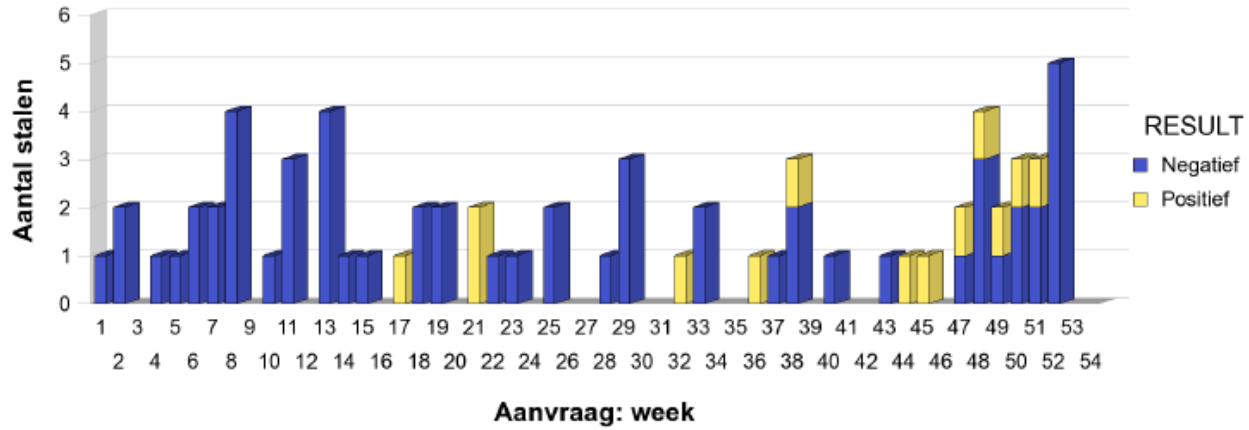
2.012



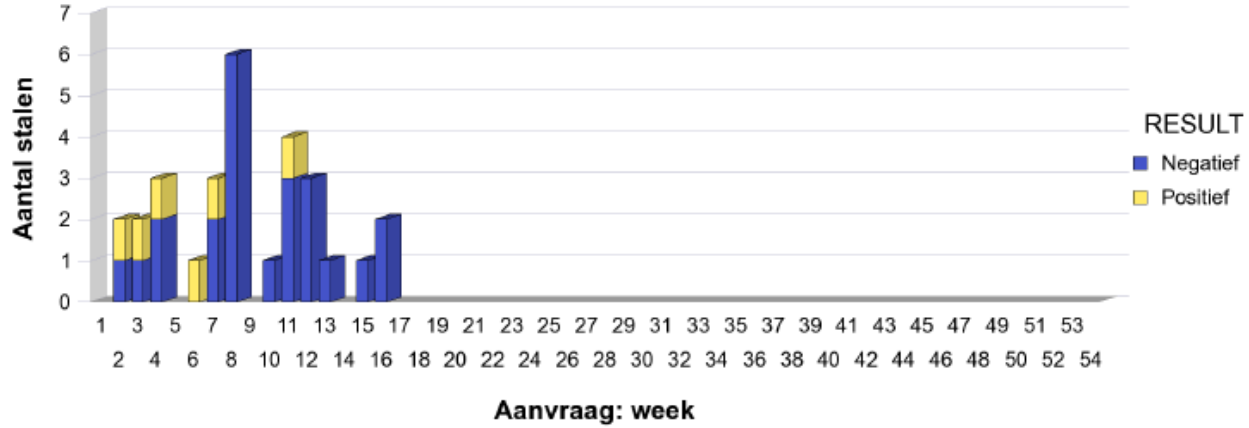
Ziekenhuis Oost-Limburg

PCR Mycoplasma pneumoniae

2.011



2.012



- Bepalen etiologisch agens bij kinderen met CAP?
- ZOL:

- Cultuur op respiratoire stalen
- Hemoculturen
- Real-time PCR *Mycoplasma pneumoniae* (kwalitatief) op een nasopharyngeaal aspiraat
- Serologie *Mycoplasma pneumoniae* (IgM, IgG)
- Influenza A, Influenza A subtype H1N1 en Influenza B op nasopharyngeale aspiraten of nasopharyngeale swabs (kwalitatief) via multiplex real-time PCR (Cepheid Xpert Flu)
- Respiratoir Syncytial Virus op nasopharyngeale aspiraten of nasopharyngeale swabs (kwalitatief) via snelle immuunchromatografische membraan assay (BinaxNOW RSV test)

Vragen CAT

Vraag 1: Wat zijn de performantie karakteristieken?

Vraag 2: Is moleculaire diagnostiek kost effectief?

Vraag 3: Voor welke indicaties?

Vraag 1: Wat zijn de performantie karakteristieken?

a. Viraal

   Influenza

   Respiratory syncytial virus

   Multiplex PCR

b. *Mycoplasma pneumoniae*

Influenza

- Snelle antigen testen
- Cultuur
- Nucleïnezuur testen

Snelle antigen testen

TABLE 1. Performance characteristics of QuickVue RAT compared to those of NAT

Parameter	H1N1 09 (n = 174)[a]	A/H3 (n = 88)[a]	Non-H1N1 09 (n = 97)[b]
No. of samples RAT positive	93[a]	68[a]	72
No. of samples RAT negative	81	20	25
Sensitivity (%)	53.4	77.2	74.2
Specificity (%)	100	100	100
PPV[c] (%)	100	100	100
NPV[d] (%)	76.2	92	90.2

[a] Includes two samples that were coinfected with H1N1 09 and influenza A/H3.
[b] Includes 88 influenza A/H3 and 9 others (3 seasonal influenza A/H1N1 and 6 untypeable).
[c] PPV, positive predictive value.
[d] NPV, negative predictive value.

Kok J, et al. *Journal of Clinical Microbiology* 2010, 48:290-291.

Cultuur en PCR

Table 5. Prospective Specimens: Xpert Flu Compared to Culture – Corrected After Discrepant Analysis by Sequencing

Virus	No. of Specimens[a]					Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
	N	Cs+X+	Cs-X+	Cs+X-	Cs-X-				
Nasal Aspirate/Wash									
Influenza A Virus	342	9	0	1	332	90.0 (55.5-99.8)	100 (99.1-100.0)	100 (71.7-100.0)	99.7 (98.3-100.0)
Influenza A Virus novel H1N1	342	7	1	0	334	100 (65.2-100.0)	99.7 (98.3-100.0)	87.5 (47.4-99.7)	100 (99.1-100.0)
Influenza B Virus	342	9	0	0	333	100 (71.7-100.0)	100 (99.1-100.0)	100 (71.7-100.0)	100 (99.1-100.0)
Nasopharyngeal Swab									
Influenza A Virus	297	12	0	0	285	100 (77.9-100.0)	100 (99.0-100.0)	100 (77.9-100.0)	100 (99.0-100.0)
Influenza A Virus novel H1N1	297	7	1	0	289	100 (65.2-100.0)	99.7 (98.1-100.0)	87.5 (47.4-99.7)	100 (99.0-100.0)
Influenza B Virus	297	8	0	0	289	100 (68.8-100.0)	100 (99.0-100)	100 (68.8-100.0)	100 (99.0-100.0)

[a]Cs+/- = culture positive/negative, sequencing of 2009 H1N1 target & sequencing of specimens that disagree with Xpert results; X+/-=Xpert Flu positive/negative.

Novak-Weekley SM, et al. *Journal of Clinical Microbiology* 2012, published online ahead of print.

Respiratory syncytial virus (RSV)

- Virale antigenen (vb. Binax Now)
- Cultuur
- Nucleïnezuur testen

Viraal antigen detectie

Table 1
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Binax NOW® RSV when reverse-transcriptase real-time polymerase chain reaction is used as the gold standard; Yukon–Kuskokwim Delta, Alaska; October, 2005–September, 2007.

	Sensitivity	Specificity	PPV	NPV
Overall (n = 311)	72% (57/79)	97% (226/232)	90% (57/63)	91% (226/248)
Age				
<1 year (n = 213)	79% (46/58)	97% (150/155)	90% (46/51)	93% (150/162)
1–<3 years (n = 98)	52% (11/21)	99% (76/77)	92% (11/12)	88% (76/86)
P value	0.025	0.67	1.0	0.35
Seasonality				
RSV season (n = 108)	78% (38/49)	97% (57/59)	95% (38/40)	84% (57/68)
Offseason (n = 203)	63% (19/30)	98% (169/173)	83% (19/23)	94% (169/180)
P value	0.17	0.65	0.18	0.02
Onset of illness before testing				
0–1 days (n = 38)	92% (12/13)	100% (25/25)	100% (12/12)	97% (35/36)
2–4 days (n = 81)	78% (14/18)	92% (58/63)	74% (14/19)	94% (58/62)
5+ days (n = 182)	65% (31/48)	99% (133/134)	97% (31/32)	89% (133/150)
P value for trend	0.04	0.24	0.62	0.07
Bronchiolitis				
Yes (n = 104)	89% (47/53)	92% (47/51)	92% (47/51)	89% (47/53)
No (n = 207)	38% (10/26)	99% (179/181)	83% (10/12)	92% (179/195)
P value	<0.001	0.02	0.32	0.58
Other pathogens present[a]				
Yes (n = 114)	56% (9/16)	100% (98/98)	100% (9/9)	93% (98/105)
No (n = 197)	76% (48/63)	96% (128/134)	89% (48/54)	90% (128/143)
P value	0.13	0.04	0.58	0.37

[a] Parainfluenza 1–3 (hPIV; n = 52), human metapneumovirus (hMPV; n = 39), influenza A/B (flu; n = 12), *Bordetella pertussis* (n = 5), hMPV and flu (n = 3), hPIV and hMPV (n = 2), hPIV and flu (n = 1)

Miernyk K, et al. *Journal of Clinical Virology* 2011,55:240-243.

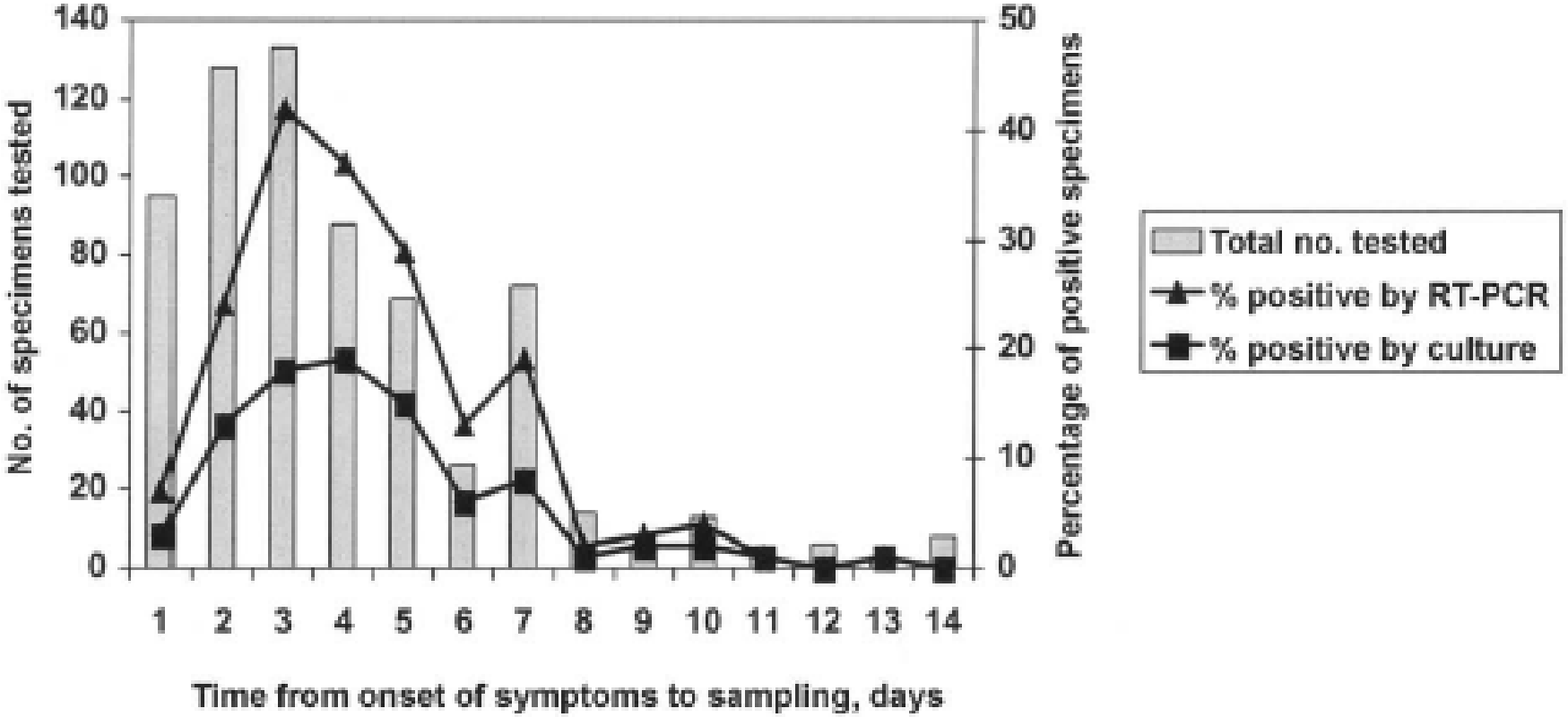
Table 1. Viral detection by reverse-transcription polymerase chain reaction (RT-PCR) and by conventional culture.

Results of detection by RT-PCR	Results of detection by conventional culture, no. of specimens							
	Respiratory syncytial virus		Influenza viruses A and B		Human parainfluenza viruses 1–3		Combined	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Positive	44	78	13	9	24	17	81	104
Negative	3	543	1	645	4	623	8	475

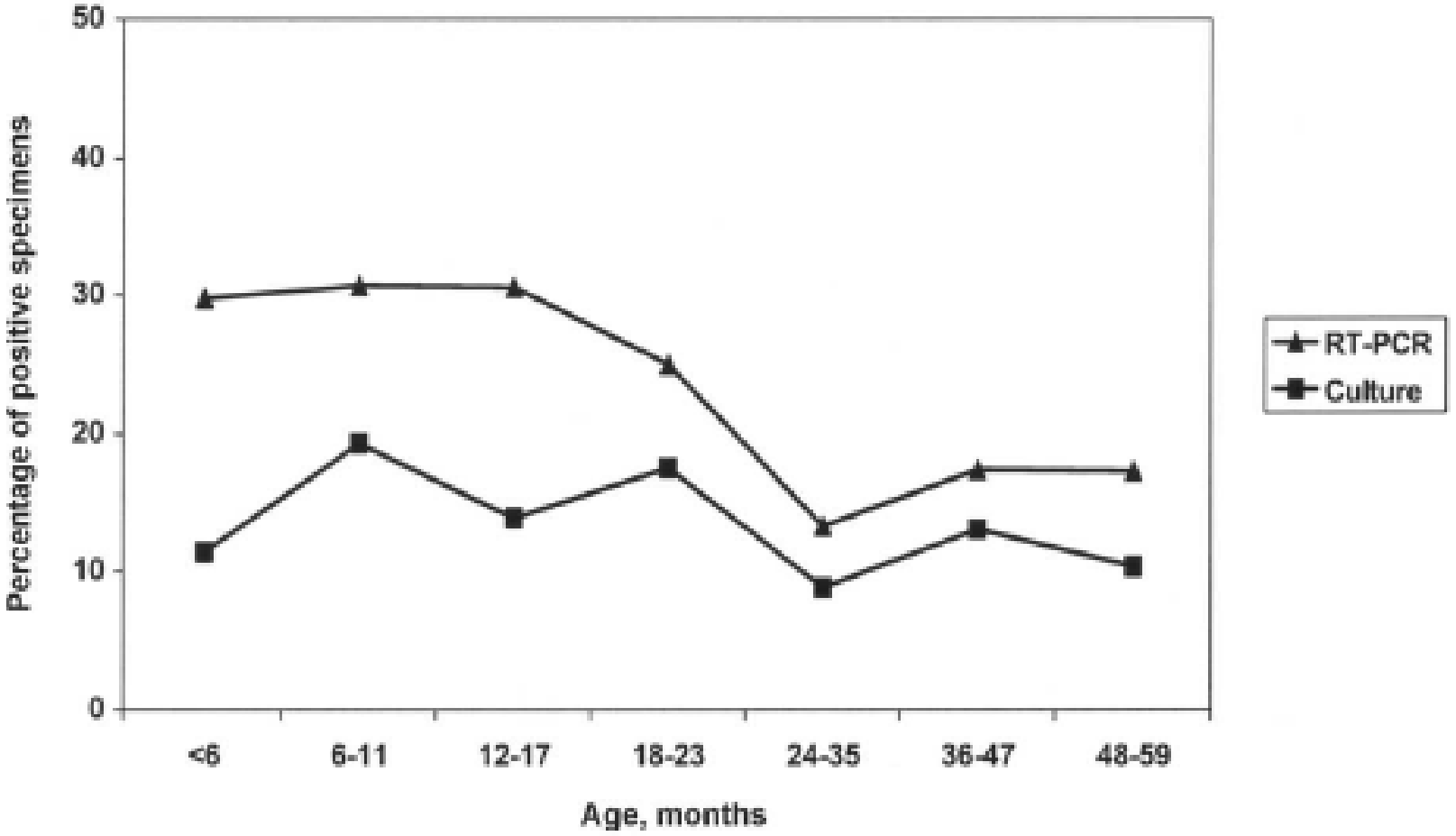
NOTE. The ratio of the no. of specimens positive by RT-PCR to the no. of specimens positive by culture was 2.6:1 for respiratory syncytial virus ($P < .0001$), 1.6:1 for influenza viruses A and B ($P \leq .01$), 1.5:1 for human parainfluenza viruses 1–3 ($P \leq .01$), and 2.1:1 for all combined ($P < .0001$). Statistical significance was calculated using the McNemar test for the comparison of the proportion of viruses detected by RT-PCR with the proportion of viruses detected by culture (paired χ^2 test).

Hogere opbrengst voor PCR vs cultuur

Weinberg GA, et al. *J Infect Dis* 2004,189:706-710.



Weinberg GA, et al. *J Infect Dis* 2004,189:706-710.



Weinberg GA, et al. *J Infect Dis* 2004,189:706-710.

Multiplex PCR

- Simultane detectie van een panel van virussen in één assay.
- 750 nasopharyngeale swabs werden genomen bij kinderen (<17j) met het vermoeden van een respiratoire infectie.
- De performantiekarakteristieken van 4 commerciële multiplex PCR assays, direct fluorescente antilichaam (DFA) kleuring en virale cultuur werd bepaald.

TABLE I. Targets and characteristics of multiplex assays for detection of respiratory viruses

Assay	Resplex II v2.0	Seeplex RV15	xTAG RVP	xTAG RVP Fast
Target	Influenza A, B	Influenza A, B	Influenza A (H1, H3, H5), B	Influenza A (H1, H3), B
	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B
	Parainfluenza 1–4	Parainfluenza 1–4	Parainfluenza 1–4	Parainfluenza 1–4
	Human metapneumovirus	Human metapneumovirus	Human metapneumovirus	Human metapneumovirus
	Adenovirus B,E	Adenovirus B,C,E, some A,D	Adenovirus A–F	Adenovirus A-F
	Bocavirus	Bocavirus	–	Bocavirus
	Coronavirus OC43	Coronavirus OC43/HKU1	Coronavirus OC43	Coronavirus OC43
	Coronavirus HKU1		Coronavirus HKU1	Coronavirus HKU1
	Coronavirus 229E	Coronavirus 229E/NL63	Coronavirus 229E	Coronavirus 229E
	Coronavirus NL63		Coronavirus NL63	Coronavirus NL63
	–	–	SARS coronavirus	–
	Enterovirus	Enterovirus	Enterovirus/Rhinovirus	Enterovirus/Rhinovirus
	Rhinovirus	Rhinovirus A/B/C		
Technology	End-point RT-PCR	End-point RT-PCR	End-point RT-PCR	End-point RT-PCR
	Microsphere-based detection	Dual priming oligo (DPO)	Microsphere-based detection	Microsphere-based detection
Equipment	LiquiChip (Luminex 200 system)	Lab901 ScreenTape system	Luminex 100 system	Luminex 100 system
Amplification/detection[a]	310 min	520 min	450 min	220 min

[a]For 24 specimens and excluding the nucleic acid extraction. Times are approximate.

Gharabaghi F, et al. *Clinical Microbiology and Infection* 2011,17:1900-1906.

- Echt positief: Virale cultuur positief of 2 positieven (DFA en de 4 moleculaire testen)
- Vals positief: Een positief resultaat in 1 enkele assay behalve virale cultuur.

TABLE 2. Distribution of respiratory viruses in paediatric nasopharyngeal samples

Virus	Virus subtype	Number	Single-infection number (%)	Dual-infection number (%)
Enterovirus/ rhinovirus	–	173	128 (21.7)	45 (7.6)
RSV	A	86	108 (18.3)	34 (5.8)
	B	56		
INFA	H1	40	58 (9.74)	6 (1.02)
	H3	22		
	Unidentified	2		
INFB	–	37	34 (5.8)	3 (0.51)
Parainfluenza	1	14	25 (4.23)	12 (2.04)
	2	9		
	3	6		
	4	8		
hMPV	–	39	27 (4.6)	12 (2.0)
Coronavirus	NL63	13	20 (3.28)	22 (3.69)
	OC43	15		
	HKU1	12		
	229E	2		
BoV	–	21	7 (1.2)	14 (2.4)
ADV	–	19	13 (2.2)	6 (1.0)
Total	–	574	420 (71.2)	154 (26.1)

Gharabaghi F, et al. *Clinical Microbiology and Infection* 2011,17:1900-1906.

TABLE 3. Sensitivity of direct fluorescent antibody (DFA), culture and four multiplex assays for detection and identification of respiratory viruses. Number of positives (within brackets)

Target	DFA	Culture	Resplex II Panel v2.0	Seeplex RV15	xTAG® RVP	xTAG® RVP Fast
INFA	76.7% (46)	60.3% (35)	96.9% (62)	96.9% (62)	98.4%[a] (63)	93.7%[b] (60)
INFB	78.4% (29)	75.0% (21)	100% (37)	100% (37)	100% (36)	64.9% (24)
PIV (1–4)	72.4% (21)	61.5% (16)	82.9% (34)	97.6% (40)	85.4% (35)	65.8% (26)
PIV1	76.9%	66.7%	86.7%	93.3%	71.4%	46.7%
PIV2	55.5%	44.4%	88.9%	100%	100%	77.8%
PIV3	100%	66.7%	100%	85.7%	71.4%	42.8%
PIV4	–	–	60.0%	100%	100%	100%
hMPV	68.6% (24)	43.3% (13)	82.0% (32)	97.4% (38)	97.4% (38)	92.3% (36)
RSV (A/B)	93.5% (130)	86.5% (96)	84.0% (121)	100% (144)	88.2% (127)	91.7% (132)
RSVA	–	–	90.4%	100%	85.5%	92.5%
RSVB	–	–	79.3%	100%	98.3%	94.8%
ADV	38.1% (8)	44.4% (8)	71.4% (15)	100% (21)	85.7% (18)	52.4% (11)
BoV	–	–	75.0% (18)	100% (24)	–	100% (24)
CoV OC43/HKU1	–	–	92.6% (25)	100% (27)	48.1% (13)	59.3% (16)
CoV 229E/NL63	–	–	100% (17)	100% (17)	88.2% (15)	88.2% (15)
Enterovirus/rhinovirus	–	–	96.7% (172)	71.7% (127)	93.8% (167)	97.7% (174)

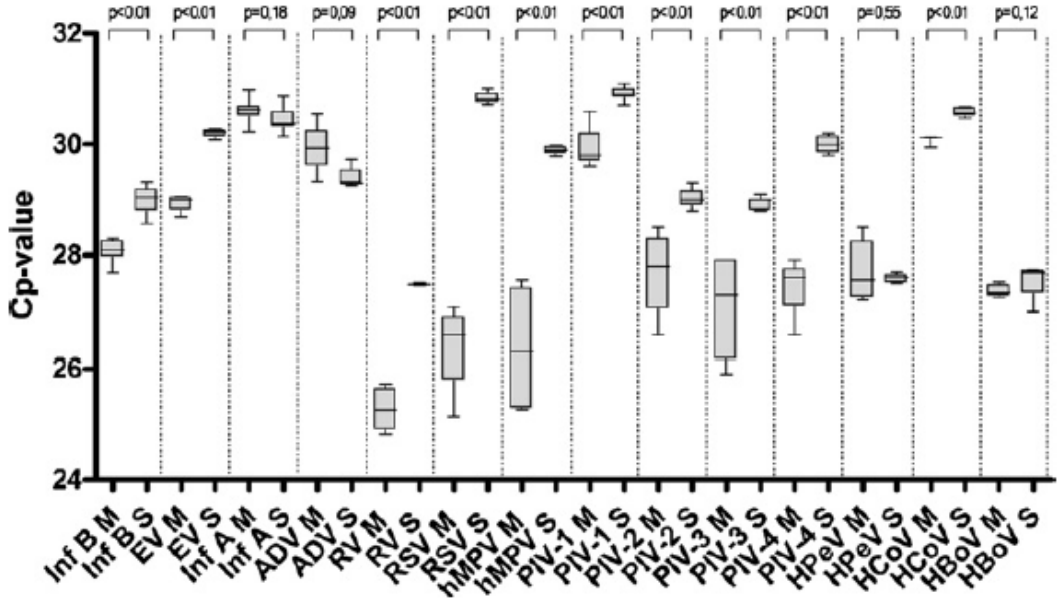
TABLE 4. Specificity of direct fluorescent antibody (DFA), culture and four multiplex assays for detection and identification of respiratory viruses

Target	DFA (%)	Culture (%)	Resplex II Panel v2.0 (%)	Seeplex RV15 (%)	xTAG® RVP (%)	xTAG® RVP Fast (%)
INFA	99.7	100	100	98.8	100	100
INFB	99.8	100	100	100	100	100
PIV (1–4)	99.8	100	100	99.0	99.6	97.6
hMPV	99.4	100	100	99.7	99.7	100
RSV (A/B)	99.6	100	100	97.7	100	100
ADV	100	100	99.9	98.1	99.9	100
BoV	–	–	100	100	–	99.6
CoV OC43/HKU1	–	–	100	99.3	99.9	100
CoV 229E/NL63	–	–	100	98.8	99.9	100
Entero/rhinovirus	–	–	99.3	99.1	96.0	99.8

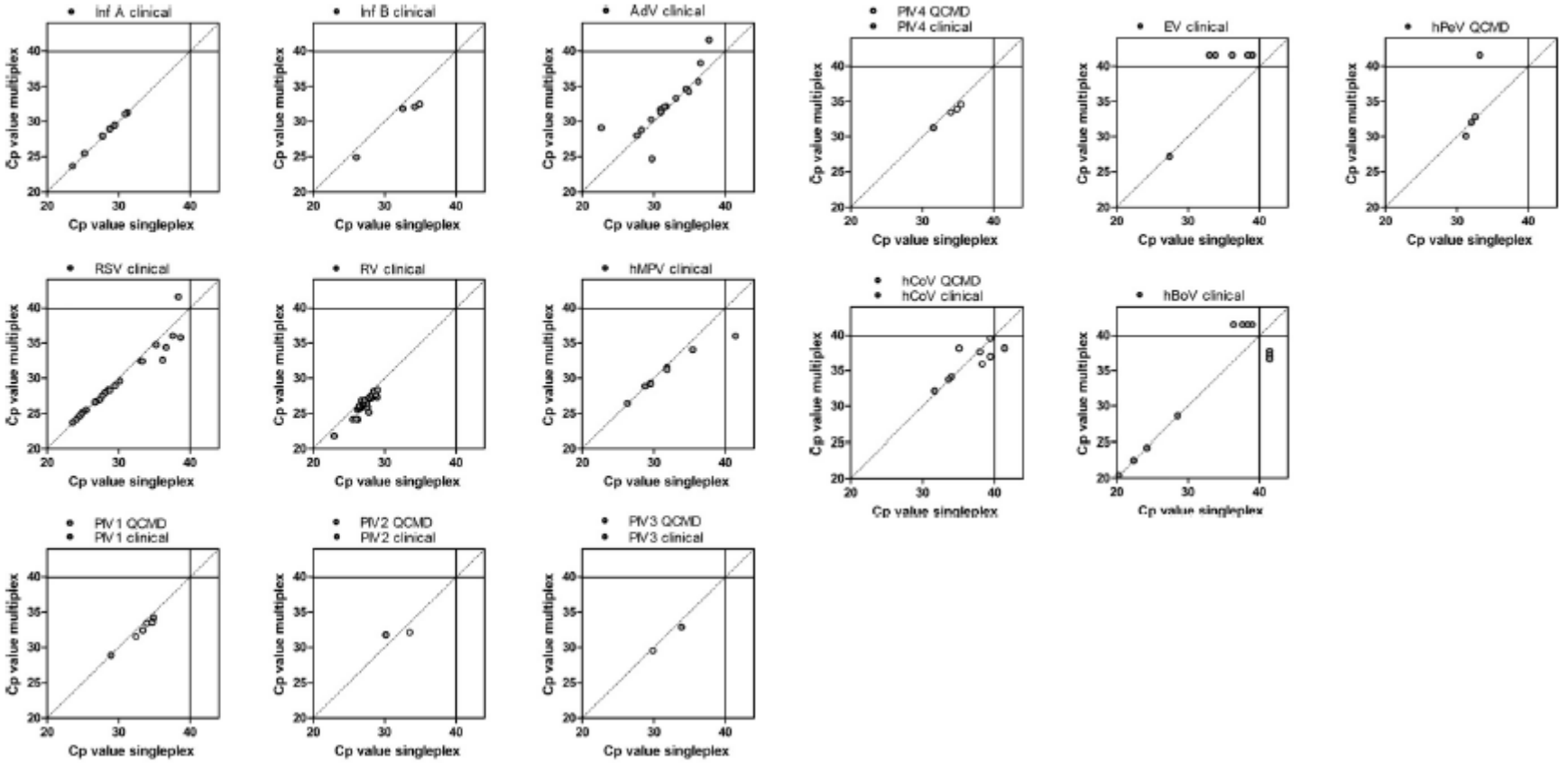
Gharabaghi F, et al. *Clinical Microbiology and Infection* 2011,17:1900-1906.

RT multiplex vs corresponderende single target PCR

- 133 kinderen met een acute respiratoire infectie.
- 70% (93/133) met een positief resultaat in minstens 1 single target assay.
- De variatie coëfficiënt (CV) range multiplex PCR (0.4% en 3.3%) was slechts matig hoger t.o.v. single target assays (0.3% en 1.0%).
- Mediane cycle threshold bij de multiplex PCR waren nooit hoger dan voor de single target assays.



Jansen RR, et al. *Journal of Clinical Virology* 2011,51:179-185.



Zeer hoge specificiteit en sensitiviteit.

Behalve een verminderde sensitiviteit voor Enterovirus detectie via multiplex PCR in vergelijking met de single target assay.

Jansen RR, et al. *Journal of Clinical Virology* 2011,51:179-185.

Mycoplasma pneumoniae

- Cultuur: duurt verschillende weken met een sensitiviteit van 60%.
- Serologie
- Nucleïnezuur testen

serologie

- 2 de serumstaal 3 tot 4 weken na start symptomen.
- Diagnose: seroconversie of viervoudige titer stijging van IgG antistoffen.
- IgM antistoffen: hogere sensitiviteit indien bepaling 7 dagen na start van de symptomen.

Blanco S, et al. *Diagnostic Microbiology and Infectious Disease* 2011,71:463-466.

- <u>Beperkingen:</u>

- Deel van de patiënten produceren geen IgM antistoffen.
- Patiënten met een reinfectie produceren niet altijd IgM antistoffen.
- IgM antistoffen kunnen later dan 7 dagen na start symptomen opkomen.
- IgM antistoffen kunnen maanden na een acute infectie nog persisteren.

Loens K, et al. *Eur J Clin Microbiol Infect Dis* 2010,29:1055-1069.

Nucleïnezuur testen

- Vergelijking 2 commerciële PCR assays (real-time en oligochromatografische)

Table 1
Sensitivity, specificity, PPV, and NPV of both molecular techniques compared with serology

PCR Assay	No. of positive specimens	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV	95% CI	NPV	95% CI
Cepheid kit	Group 1 30/32 Group 2 1/94 Group 3 0/19	93.7	77–98	98.9	93–99	96.7	81–99	97.8	91–99
Speed-oligo	Group 1 25/32 Group 2 0/94 Group 3 0/19	78.1	59–90	100	95–100	100	83–100	93	86–97

PPV = Positive predictive value; NPV = negative predictive value; CI = confidence interval.

Groep 1: 32 kinderen met pneumonie door *M. pneumoniae.*
Groep 2: 94 kinderen met respiratoire infectie van viraal of ongekend origine.
Groep 3: 19 gezonde kinderen.

Blanco S, et al. *Diagnostic Microbiology and Infectious Disease* 2011,71:463-466.

Nucleïnezuur testen

Meta-analyse:

Grote heterogeniteit:
- Vooral bij pediatrische patiënten
- Verschillende PCR methodes
- Verschillende serologie testen

Conclusies:
- Real-time PCR geeft betere performantie karakteristieken dan klassieke PCR.
- Ondanks de grote heterogeniteit concludeerden de auteurs dat er een belangrijke rol voor PCR weggelegd is, maar dat deze serologie (voorlopig) niet kan vervangen.

Zhang L, et al. *Indian J Med Res* 2011,134:270-280.

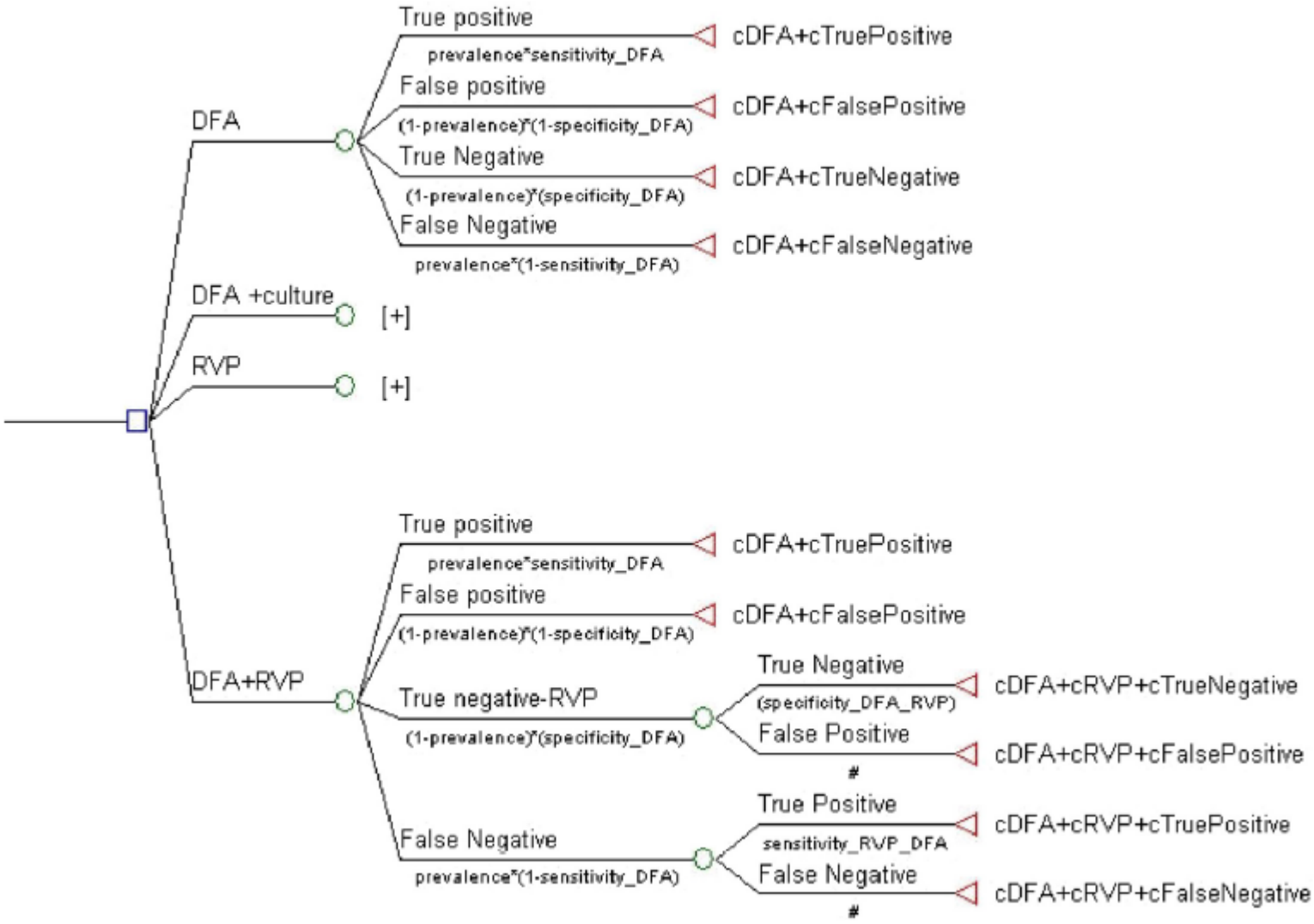
Vraag 2: Is moleculaire diagnostiek kost effectief?

Cost Analysis of Multiplex PCR Testing for Diagnosing Respiratory Virus Infections[▽]

James B. Mahony,[1,4]* Gord Blackhouse,[2,3] Jesse Babwah,[4] Marek Smieja,[2,4] Sonya Buracond,[4] Sylvia Chong,[4] William Ciccotelli,[1,4] Tim O'Shea,[1,4] Daifallah Alnakhli,[1,4] May Griffiths-Turner,[4] and Ron Goeree[2,3,4]

Mahony JB, et al. *Journal of Clinical Microbiology* 2009,47:2812-2817.

Vier diagnostische strategieën



Prevalentie van respiratoire virus infecties bij kinderen: 63.2%

Performantie karakteristieken:

TABLE 1. Sensitivity and specificity of the diagnostic tests used in the model[a]

Test	Sensitivity (%)	Specificity (%)
DFA	70	98
DFA + culture	72	98
xTAG RVP	94	98

Kost: Laboratorium test + Hospitalisatie

Laboratorium test: kosten materiaal + kosten arbeid

DFA: $13.80

Cultuur: $ 23.20

xTAG RVP: $ 80

(1 Canadese dollar = 0.76 Euro)

Hospitalisatie: kosten ziekenhuisopname + uitgevoerde onderzoeken + kosten antibiotica verbruik.

Kost ziekenhuisopname per dag:
Afdeling pediatrie: $ 690.72
Pediatrische intensieve zorgen: $ 1548
Droplet isolatie: $ 1662.12

De gemiddelde hospitaalkost per diagnostische status:

TABLE 2. Breakdown of cost per case by diagnostic status

Diagnostic status	Cost ($)			
	Hospitalization	Tests and investigations	Antibiotics	Total
True positive	2,347	60	6	2,413
False negative	4,697	53	6	4,756
True negative	5,166	55	6	5,228
False positive	5,186	55	6	5,248

Er is een duidelijke meerkost indien de diagnose gemist wordt.

Gemiddelde gewogen kosten per case voor elk van de vier diagnostische strategieën

TABLE 3. Calculation of weighted costs for each diagnostic strategy by using proportions and actual costs

Test	Viral test result	Diagnostic status	Proportion	Cost ($)	Weighted cost ($)[a]
RVP[b]	Positive	True positive	0.592	2,493.00	1,476
	Positive	False positive	0.007	5,327.00	39
	Negative	True negative	0.363	5,307.00	1,926
	Negative	False negative	0.038	4,836.00	61
	Total		1.000		3,623
DFA	Positive	True positive	0.441	2,426.80	1,070
	Positive	False positive	0.007	5,260.80	39
	Negative	True negative	0.363	5,240.80	1,900
	Negative	False negative	0.189	4,769.80	901
	Total		1.000		3,911
DFA + culture	DFA positive	True positive	0.441	2,426.80	1,070
	DFA positive	False positive	0.007	5,260.80	37
	DFA negative, culture positive	True positive	0.004	2,447.45	9
	DFA negative, culture positive	False positive	0.007	5,281.45	38
	DFA negative, culture negative	True negative	0.356	5,261.45	1,872
	DFA negative, culture negative	False negative	0.185	4,790.45	887
	Total		1.000		3,914
DFA + RVP	DFA positive	True positive	0.441	2,426.80	1,070
	DFA positive	False positive	0.007	5,260.80	37
	DFA negative, RVP positive	True positive	0.045	2,506.80	114
	DFA negative, RVP positive	False positive	0.007	5,340.80	39
	DFA negative, RVP negative	True negative	0.356	5,320.80	1,893
	DFA negative, RVP negative	False negative	0.144	4,849.80	697
	Total		1.000		3,849

[a] The weighted cost is proportion × cost.
[b] RVP, xTAG RVP test.

Hospitaalkost + laboratoriumkost

Conclusie:

- xTAG RVP test alleen is de goedkoopste strategie indien de prevalentie van respiratoire virus infecties hoger is dan 11%.
- Prevalentie respiratoire virus infecties bij kinderen:
  - overall positivity rate: 63%
  - monthly low: 36%
  - monthly high: 87%
- OORZAAK: VERMINDERING HOSPITAALDUUR

Mahony J, et al. *107th Gen Meet Am Soc Microbiol* 2007, Abstr. C-070

DUS: xTAG RVP test alleen is heel het jaar door de goedkoopste strategie.

- Geen verminderd antibiotica verbruik bij de xTAG RVP test strategie.

Mahony JB, et al. *Journal of Clinical Microbiology* 2009,47:2812-2817.

- 53% reductie in antibiotica gebruik indien een snelle diagnose van respiratoire virale infectie bij kinderen.

Woo PCY, et al. *Journal of Clinical Microbiology 1997*,35:1579-1581.

Vraag 3: Voor welke indicaties?

IDSA guidelines:

Bradley JS, *et al.* The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the pediatric infectious diseases society and the infectious diseases society of America. *Clinical Infectious Diseases* 2011;53:25-76.

BTS guidelines:

Harris M, *et al*. British thoracic society guidelines for the management of community acquired pneumonia in children: update 2011. *Thorax* 2011;66:1-23.

Onderscheid viraal vs bacterieel vs mixed

Table 2 Demographic and clinical results of 99 patients hospitalized for community-acquired pneumonia and correlation with etiology

Characteristics	Total	Bacterial	Viral	Mixed	Unknown pathogen	*P* Value
No. of patient (%)	99	19 (19)	33 (33)	33 (33)	14 (14)	
Age, months (0.25; 0.75)[a]	29.4 (17; 48)	23.4 (19; 48)	25.1 (15; 38)	42.9 (24; 52)	22.4 (11; 34)	0.033[c,e]
Gender (m/f)	48/51	11/8	17/16	14/19	6/8	0.692[d]
Antibiotics before hospitalization	27	8	8	9	2	0.327[d]
Pneumonia stage I[b] (non-severe)	9	2	5	2	0	0.650[d]
Pneumonia stage II[b] (severe)	67	13	19	24	11	
Pneumonia stage III[b] (very severe)	23	4	9	7	3	
Temperature at admission (0.25; 0.75)[a]	39.1 (38.5; 39.8)	39.0 (38.5; 39.8)	39.2 (38.7; 39.8)	39.1 (38.3; 39.6)	38.8 (38.5; 39.5)	0.308
Symptoms of pain[b,f]	22	2	6	13	1	0.026[d,g]
Vomiting[b]	45	8	13	18	6	0.634[d]
URS[b]	11	1	4	3	3	0.505[d]
Dehydration[b]	25	9	2	8	6	0.003[d,h]
Oxygen requirement[b]	26	5	11	7	3	0.693[d]
Radiological consolidation[b]	77	16	25	26	10	0.911[d]
CRP mg/l[a]	167 (60; 200)	200 (100; 204)	88 (21; 194)	200 (117; 250)	142 (23; 192)	0.009[c,i]
PCT ng/ml[a]	6.0 (1; 14)	11.5 (5; 18)	2.0 (0.5; 7.5)	11.0 (3; 18)	3.0 (0.5; 4)	0.018[c,j]
WBC count (G/L)[a]	15.0 (9; 21)	15.0 (11; 19)	12.0 (7; 21)	16.0 (10; 21)	15.5 (11; 18)	0.591[c]
Band forms (G/L)[a]	1.85 (0.6–3.8)	2.65 (1.3; 3.8)	1.4 (0.5; 2.6)	1.82 (1.0; 4.0)	1.31 (0.6; 3.1)	0.417[c]
Duration of hospitalization, days[a] (0.25; 0.75)	2 (2; 4)	3 (2; 9)	2 (1; 4)	2 (2; 4)	2 (1; 3)	0.255[d]

Manon CM, et al. Eur J Pediatr 2009, 168:1429-1436.

Table 3 Clinical signs in relation to the aetiology of pneumonia, classified as pneumococcal, atypical bacterial and viral aetiology

Finding	Pneumococcal (N = 18)	Atypical bacterial (N = 28)	Viral (N = 22)	p-value[a]	Unknown (N = 33)
Fever, mean (SD)	39.1 (0.9)	38.5 (1.1)	38.4(1.0)	0.0880[b]	38.7(1.1)
Fever >39,5°C	4(22.5%)	6/2(1.4%)	3(13.6%)	0.7907[c]	8(18.2%)
Respiratory rate mean[#] (SD)	39.4 (13.3)	37.8 (15.8)	39.3 (12.8)	0.9320[b]	35.3(14.5%)
Tachypnoea (N = 25)	7 (46.7%)	9 (47.4%)	9 (47.4%)	1.000[c]	7 (25.0%)
Crackles (N = 33)	9 (50.0%)	10 (37.0%)	14 (66.7%)[g]	0.1316[c]	16(48.5%)
Dullness (N = 7)	3 (23.1%)	4 (20.0%)	0	0.1552[c]	6(23.1%)
Decreased breath sounds (N = 40)	8 (44.5%)	19 (67.9%)	13 (59.1%)	0.1764[c]	20(60.6%)
Alveolar infiltration[d] (N = 44)	12(66.7%)	22 (78.6%)	10 (45.5%)[h]	0.0547[c]	26(78.8%)
Atelectatic areas (N = 6)	1 (5.6%)	4 (14.3%)	1 (4.5%)	0.5570[c]	3(9.1%)
Pleural fluid (N = 7)	3 (16.7%)	3 (10.7)	1 (4.5%)	0.4626[c]	7(21.2%)
Procalcitonin >1.0 ng/mL[e] (N = 54)	10 (55.6%)	17 (60.7%)	8 (36.4%)[i]	0.2422[c]	19(59.4%)
Hospital treatment[f] (N = 27)	3 (16.7%)	6 (21.4%)	4 (18.2%)	1.000[c]	14(42.4%)

Korppi M, et al. *Eur J Clin Microbiol* 2008, 27:167-175.

- Geen microbiologische onderzoeken bij kinderen die niet in het ziekenhuis dienen te worden opgenomen.

Harris M, et al. *Thorax* 2011,66:1-23.

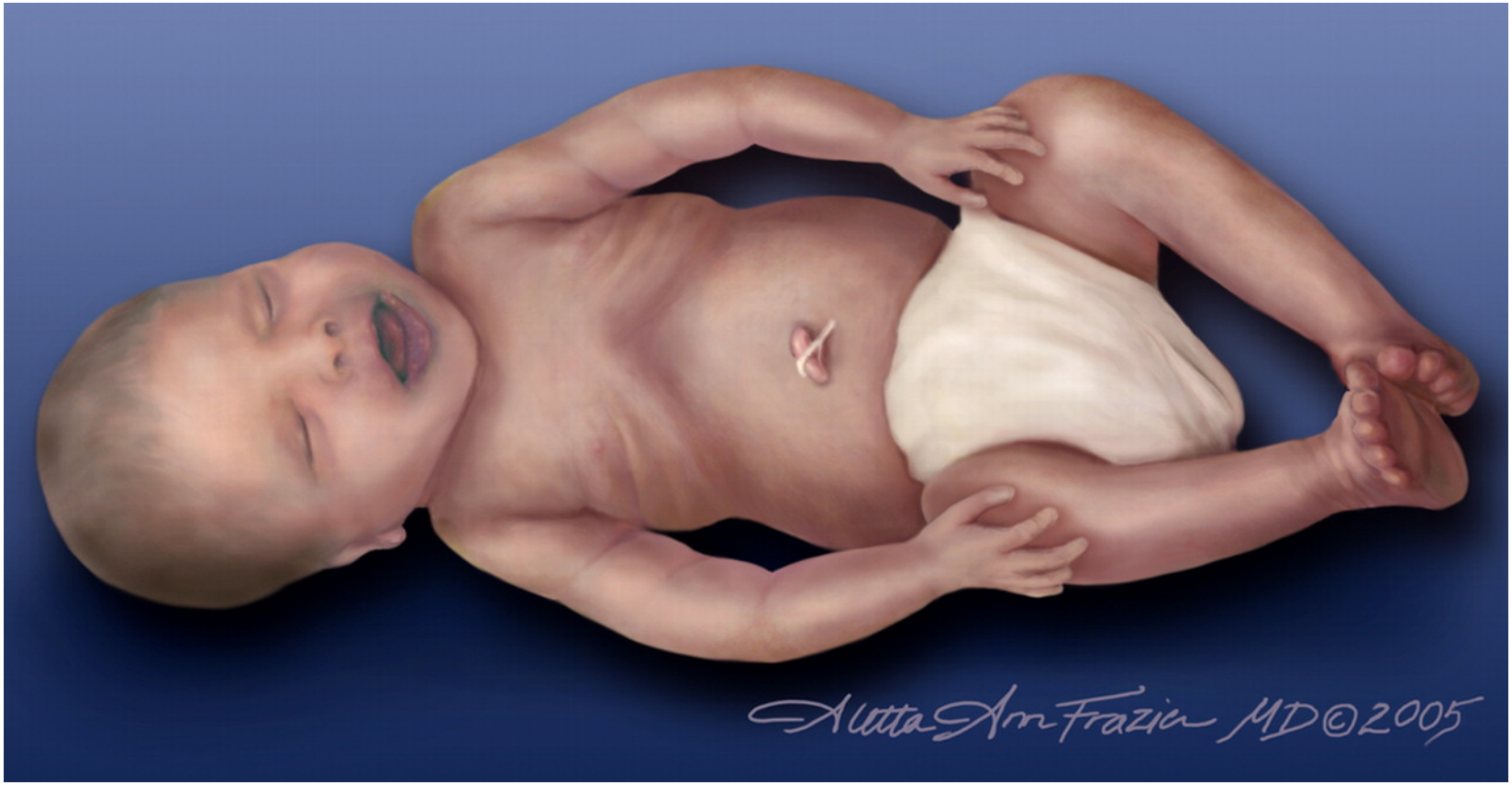
Opname bij ernstige pneumonie

Table 3. Criteria for hospitalisation of children with CAP.

Respiratory distress	Age-adjusted tachypnea
	SpO2 <90-93% in room air (if FiO_2>0.50, ICU or continuous cardiorespiratory monitoring are required to maintain saturation >92%)
	Cyanosis
	Retractions
	Grunting
	Nasal flaring
Capillary refill time >2 min	
Dehydration	
Vomiting/not feeding	
Comorbidities (e.g., congenital heart disease, chronic lung disease of prematurity, chronic respiratory conditions leading to infection such as cystic fibrosis, bronchiectasis, immunodeficiency)	
Etiological agent (e.g. MRSA, bacterial/viral co-infections)	
Unreliable family environment	

Esposito S, et al. The Pediatric Infectious Disease Journal 2012, Publish Ahead of Print.

Clinical features of respiratory distress.



Drawing depicts a representative preterm newborn with RDS exhibiting substernal and intercostal retractions, nasal flaring, and circumoral cyanosis.

Agrons GA, et al. Radiographics 2005;25:1047-1073

Gehospitaliseerde kinderen:

- Hemoculturen: Prevalentie bacteriëmie bij kinderen met CAP = <10%

  Shah SS, et al. *Pediatr Infect Dis* J 2011, 30:475-479.

- Sputum: Enkel bij oudere kinderen en adolescenten aanbevolen.
- Nasopharyngeale bacteriële cultuur: Niet nuttig bij kinderen.
- Cultuur pleuravocht: 9% positief (PCR Pneumokokken: 68% positief, Pneumokokken Ag test: 25% positief) van bewezen pneumokokken pneumonie.

  Eastham KM, et al. *Thorax* 2004, 59:522-525.

- Urinaire antigentest: Niet aanbevolen bij kinderen. Geen onderscheid tussen pneumokokken pneumonie en nasopharyngeale kolonisatie.
- Serologie op serum en PCR *Mycoplasma pneumoniae* op nasopharyngeaal aspiraat: De IDSA guidelines raden deze testen aan indien de pretest probabiliteit voor een *M. pneumoniae* infectie intermediair of hoog is.

- Antigentest Influenza op nasopgaryngeaal aspiraat of wisser: Lage NPW en sensitiviteit. Opgepast met een negatief resultaat.

Kok J, et al. *Journal of Clinical Microbiology* 2010, 48:290-291.

- Antigentest RSV op nasopgaryngeaal aspiraat of wisser: Hoge sensitiviteit indien bronchiolitis klachten (89%). Lage sensitiviteit indien andere klachten (38%). Hoge specificiteit. Een positief resultaat hoeft niet geconfirmeerd te worden.

Miernyk K, et al. *Journal of Clinical Virology* 2011,55:240-243.

- PCR virussen op nasopharyngeaal aspiraat of nasopharyngeale wisser. Klinische relevantie van een positief resultaat. Asymptomatisch kolonisatie van een virus is mogelijk.

Jansen R, et al. *Journal of Clinical Microbiology* 2011,49:2631-2636.

Case-control studie

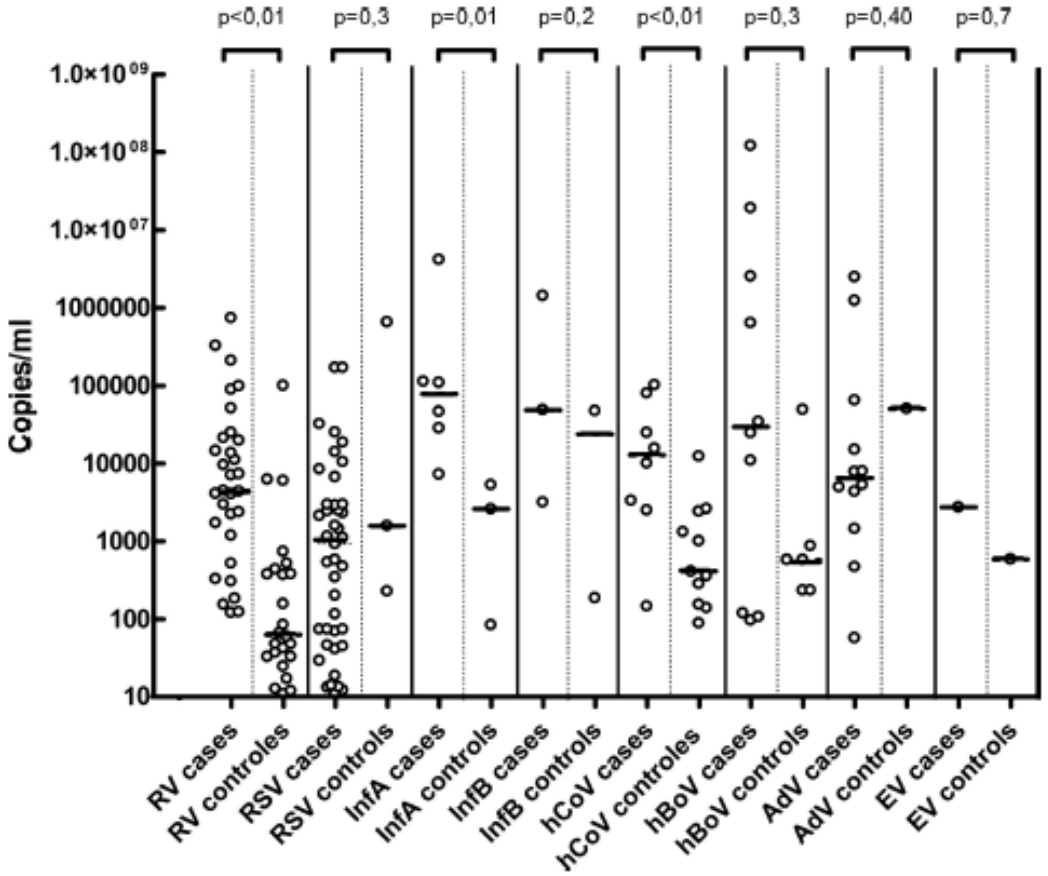


FIG. 2. Crossing point values for each positive sample. White diamonds denote single infections, and black diamonds denote positive samples as part of a double or triple infection. Horizontal lines represent the median. PIV 1, PIV2, PIV3, PIV4, and hMPV are not shown in this figure because no positive samples were found among controls for these species.

Jansen R, et al. *Journal of Clinical Microbiology* 2011,49:2631-2636.

Antibiotica gebruik:

IDSA: Kinderen die niet respiratoir falen met een positieve snelle test voor een virus hebben geen antibiotica nodig.

Bradley JS, et al. *Clinical Infectious Diseases* 2011,53:25-76.

Geen consensus hierover: Alle kinderen met een CAP behandelen met antibiotica wegens bacteriële (sur)infectie onmogelijk uit te sluiten.

Ruuskanen O, et al. Lancet 2011, 377:1264-1275.

Praktijk:

Significante reductie indien influenza positief in influenza seizoen.

Bonner AB, et al. Pediatrics 2003, 112:363-367.

Significante reductie indien positief voor RSV, parainfluenza 1,2,3 of adenovirus.

Byington CL, et al. Arch Pediatr Adolesc Med 2002, 156:1230-1234.

Geen significante reductie indien positief (multivirus testing) vs negatief of niet getest.

Doan QH, et al. J Pediatr 2009, 154:91-95.

Conclusies detectie respiratoire virussen

IDSA: Sensitieve en specifieke testen voor de snelle etiologisch diagnose van een ernstige CAP bij kinderen.

Multiplex PCRs voor respiratoire virussen hebben goede performantiekarakteristieken.

Nut:
- Opstarten van antivirale therapie. (Neuraminidase inhibitoren tegen influenza)
- Isolatiebeleid in het ziekenhuis.
- Beter in kaart brengen van de epidemiologie.

Bedenkingen:
- Asymptomatische kolonisatie (bepalen van Cutoff waardes)
- Snelle diagnose is nodig (enkel mogelijk via automatisch systeem (recent op de markt maar kostelijk))
- Mogelijks geen verminderd antibiotica verbruik.
- Volgens studie (vraag 2) goedkoper wegens kortere hospitalisatie. Output in de studie is niet te veralgemenen wegens andere input parameters.
- Geen terugbetaling voorzien in België.