Role of multiplex PCR in the diagnostics of respiratory viruses

PIGS course November 25th-26th 2016

Julia Bielicki AND Sandra Asner

Overview

- Introduction
- Molecular assays available (including POC)
- PROs and CONs
- Summary and suggested use of molecular assays (target patient-populations)

Introduction

- Acute respiratory tract infections (ARTI)

- 4.25 million deaths/ yr

- Third most common cause of death worldwide

- Leading cause of hospitalization for infants and young children in DV countries

- 12-32 million episodes of URTI/yr in children 1-2 yrs of age

- NAT recognized as important tools since mid-90s

Mahony J.B, Critical Reviews in Clinical Laboratory Sciences, 2011

Overview of diagnostic procedures

- Conventional diagnostic methods:

1. viral culture, acid lability testing

- Limitations: decreased sensitivity, labor intensive, prolonge

2. Serologic testing

- Limitations: results not in real-time, numerous serotypes

3. Direct fluorescent antibody (DFA) testing for multiple serotypes

Santti et al, 1999; Lu et al, 2008

Molecular assays

- Reverse-transcription-PCR (RT-PCR)

- Target 5’untranslated region (5’-UTR)

- Commercial assays

- Resplex IIv2.0 (Qiagen, Mississauga, ON, Canada)
- Seeplex RV15 kit (Seegene Inc.,Seoul, Korea)
- xTAG-RVP/xTAG-RVP Fast (Luminex , Austin Texas)
- MassTag PCR-> (discovery of HRV-C)
- Respiratory MultiCode-Plx Asasy

Lu et al, 2008; Savolainen et al, 2003, Schibler et al, 2012; Utokaparch et al, 2011; Franz et al, 2010 Gambarino et al, 2009

Molecular platforms with point-of-care testing potential

Table 1 Comparison of molecular platforms with point-of-care potential for detecting respiratory viruses.

System and panel	Benefits	Limitations	References
Alere i Influenza A&B	15 min run-time 2 min "hands on" time Simplicity	Moderate sensitivity for Influenza A Only influenza viruses detected	62–64
Biofire FilmArray Respiratory Panel	60 min run-time 2 min "hands on" time Wide range of viruses detected	Unable to process multiple samples simultaneously	65–71
Cepheid GeneXpert (Xpert Flu and Flu/RSV)	75 min run-time 2 min "hands on" time Modular system allows multiple simultaneous tests	Limited range of viruses detected	72–75

Alere i influenza AEB
+ Highly specific & simple use, fast turn-around

Biofire
+ Highly sensitive>90%
- no RCTs, not tested among adults

Cepheid GeneXpert
Evaluated retrospectively among adult & ped patients
-Sensitivity 97% FLU-A; 100% FLU-B; 98%, RSV
-Specificity 100% for all three viruses

Journal of Infection (2015) 71, 501–510

What is the situation in your centre?

In patients with ARI

- We use multiplex PCR being admitted to hospital
- We use rapid diagnostics for single pathogens
- We use a stepwise approach to multiplex PCR
- We use multiplex PCR only in selected patient groups

How do you think multiplex PCR should be used?

- Systematically in all patients admitted to hospital
- Systematically in all patients – we deserve to know what is causing it!
- A stepwise or selective approach is best
- Should not be used – knowing what pathogens can be detected e.g. from URT does not impact management

PRO

THIS HOUSE BELIEVES THAT ALL CHILDREN WITH AN ACUTE RESPIRATORY INFECTION SHOULD ROUTINELY UNDERGO MULTIPLEX PCR TESTING

WHY?

- It will help you to identify causative organisms of LRTI
- It will help you to differentiate between viral and bacterial aetiology of RTI
- It will improve anti-infective use for RTI in children
- It will improve cohorting and prevent nosocomial infeciton

PRO

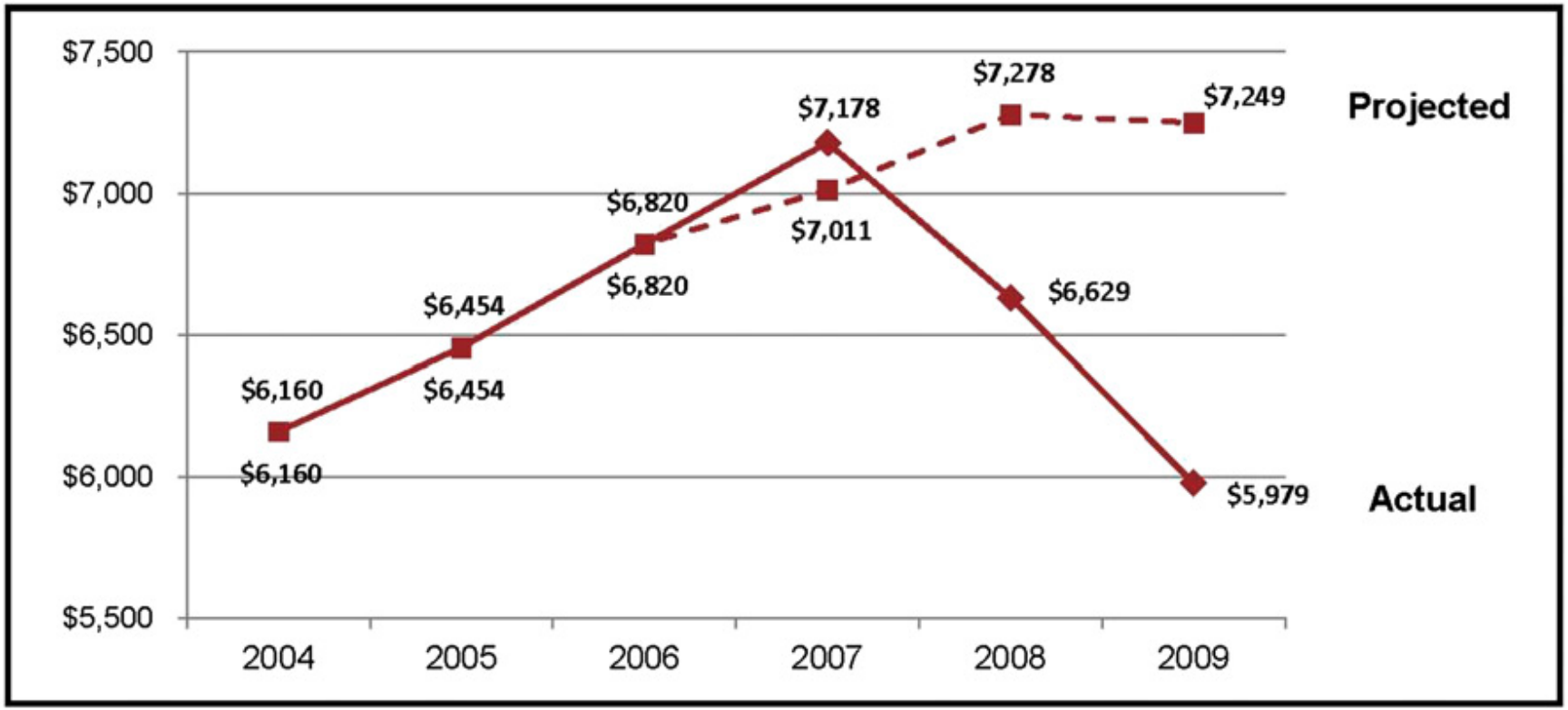
- Increased sensitivity of molecular tests *

-> More diagnosed resp. viral infections

-> Reduction in unnecessary antibiotic use, number of investigations performed and LOS

OVERALL health economic benefit

Routine implementation of rapid diagnostics reduces costs



Byington CL et al. Pediatrics. 2012; 130:e16-24

Routine implementation of rapid diagnostics reduces antibiotic use

Phase 1 (first season) → Phase 2 (second season)

	Pos pts	Neg pts
AB use	44%	45%
IV AB duration	2.5 d	4.0 d
AB on discharge	37%	52%

	Pos pts	Neg pts
IV AB initiation	26%	44%

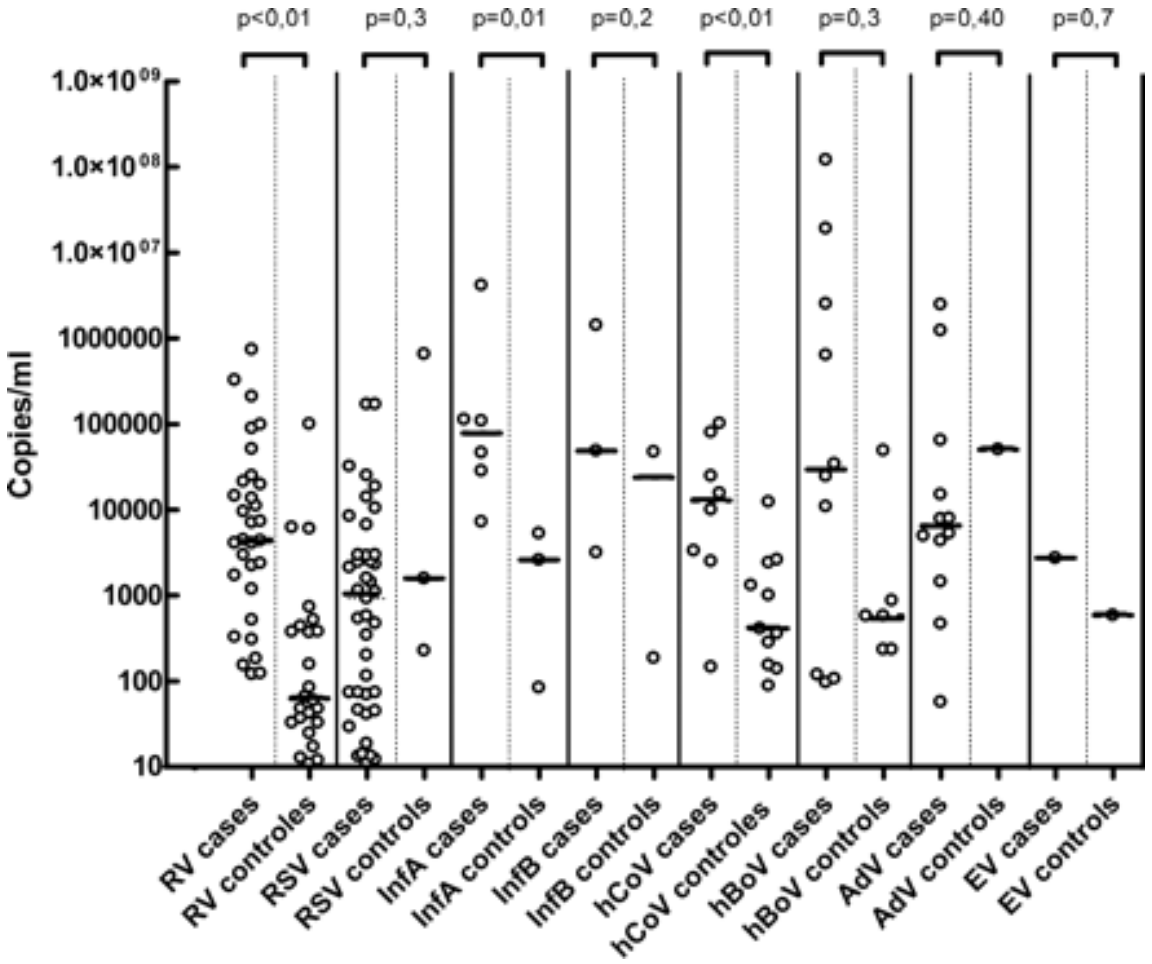
Byington CL et al. Ped Arch Pediatr Adolesc Med. 2002; 156(12):1230-1234

PRO

- Quantitative assays-> correlation viral load and clinical severity

- Genotyping assays-> antiviral resistance detection

Viral load may help clinical interpretation



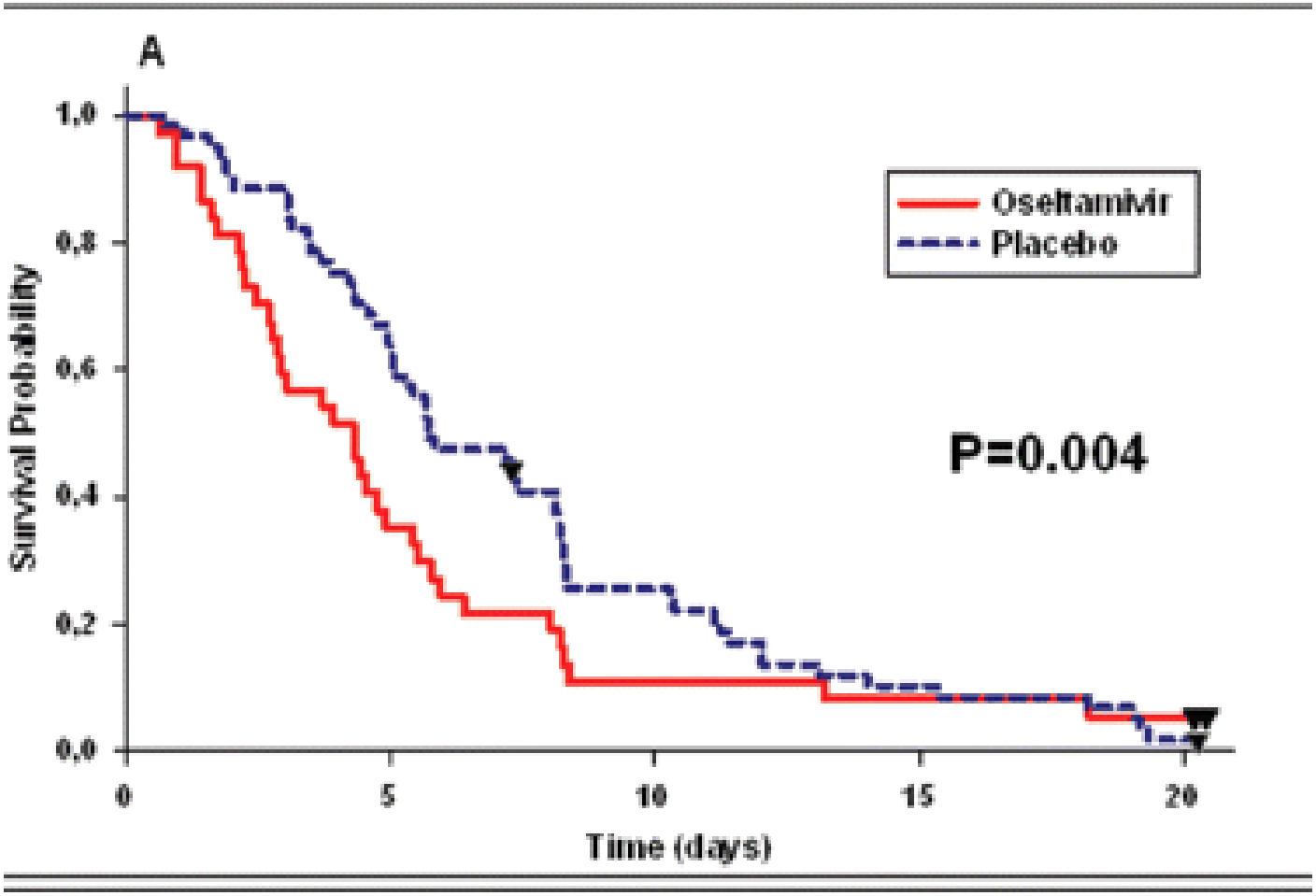
Jansen RR et al. J Clin Microbiol. 2011; 49(7):2631-36

PRO

- Improved use of directed antiviral therapy for influenza:
- Oseltamivir
- Ribavarin: Selective cases of adult HSCT with RSV UTI
- Other molecules targetting RSV

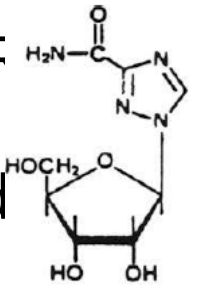
James B. Mahony Critical reviews in Coinical Laboratory Sciences,2011

Provides the opportunity to institute antiviral therapy for influenza



Heinonen S et al. Clin Infect Dis. 2010; 51(8):887-94

Ribavarin aerosolized or oral



- FDA-approved drug for treatment of Pneumonia in HF children
- Licensed for aerosol treatment of RSV in children and
- $50 000 (10-day course of A-R)

- RIB +/- immunomodulators vs no treatment

Shah et al, Blood 2011 (SR), Shah DP et al, J Antimicrob Chemother 2013

- ❑ prevention of progression URTI->LRTI (45%-16%)
- ❑ mortality in adult HSCT vs no therapy (70%-35%)

- RIB iv =po efficacity in preventing RSV LRTI in 10 adults HSCT recipients with severe lymphopenia

Gueller S et al, Transpl Infect Dis, 2013

Other molecules for RSV

- **Small-interfering RNA's (si-RNA)**

  Short lengths of double-stranded RNA->directed against mRNA RSV nucleocapsid protein that regulates gene expression

- **RSV-specific siRNAs [ALN-RSV01 (siRNA), Alnylam Pharmaceuticals, Cambridge, MA] - 2 RCT**
- Targets two nucleocapsid protein genes, the P protein and N protein genes
- Two phase II trials completed

1) Prophylaxis prior RSV inoculation in healthy volunteers -> 38% decrease in N of infections

   Zamora et al, Am J Respir Crit Care Med, 2011:

2) Lung transplant with confirmed RSV infection-> reduction of daily scores and bronchiolitis obliterans

   DeVincenzo J et al, Proc Natl Acad Sci USA, 2010

PRO

- Appropriate infection-control measures-> lower the rate of nosocomial spread

- Key roles in future pandemics->More accurate infromation to PHA regarding viruses in circulation;

  SARS CoV, 2009A/H1N1influenza

James B. Mahony Critical reviews in Clinical Laboratory Sciences 2011

CON

THIS HOUSE DOES NOT BELIEVE THAT CHILDREN WITH AN ACUTE RESPIRATORY INFECTION SHOULD NOT ROUTINELY UNDERGO MULTIPLEX PCR TESTING

How do you think multiplex PCR should be used?

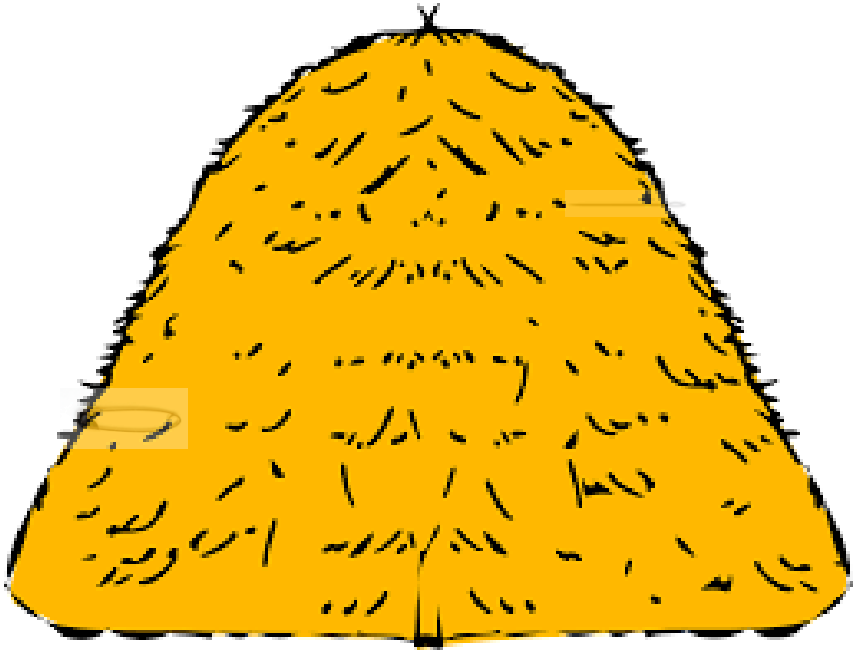
- Systematically in all patients admitted to hospital
- Systematically in all patients – we deserve to know what is causing it!
- A stepwise or selective approach is best
- Should not be used – knowing what pathogens can be detected e.g. from URT does not impact management

WHY?

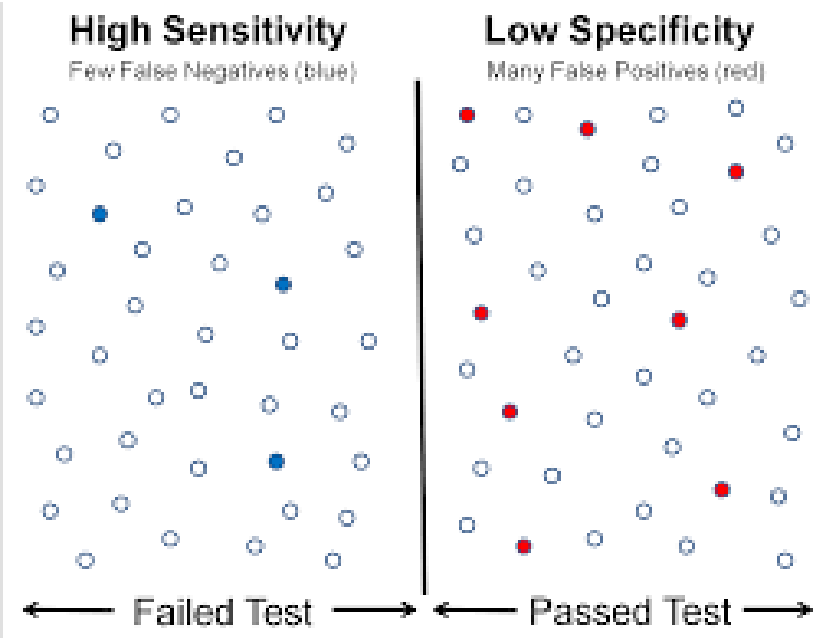
- It will not help you to identify causative organisms of LRTI
- It will not help you to differentiate between viral and bacterial aetiology of LRTI
- It will not improve anti-infective use for LRTI in children
- It will not improve cohorting and prevention of nosocomial infection

Analytical and diagnostic sensitivity and specificity

Analytical

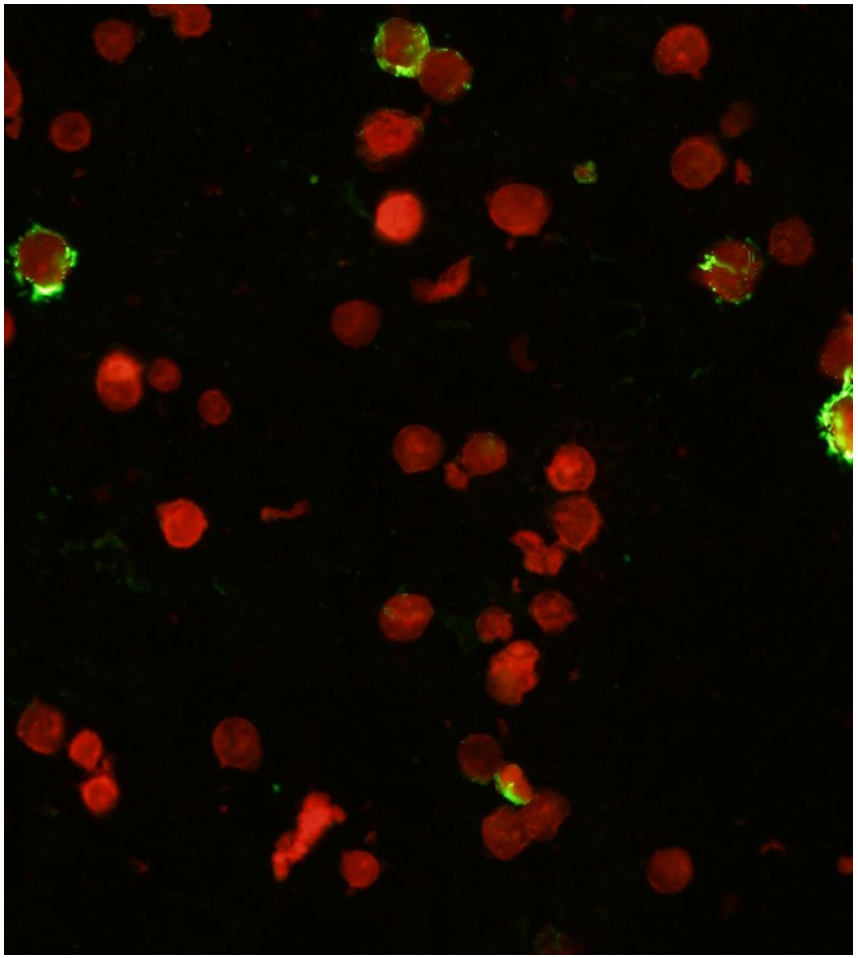


Diagnostic



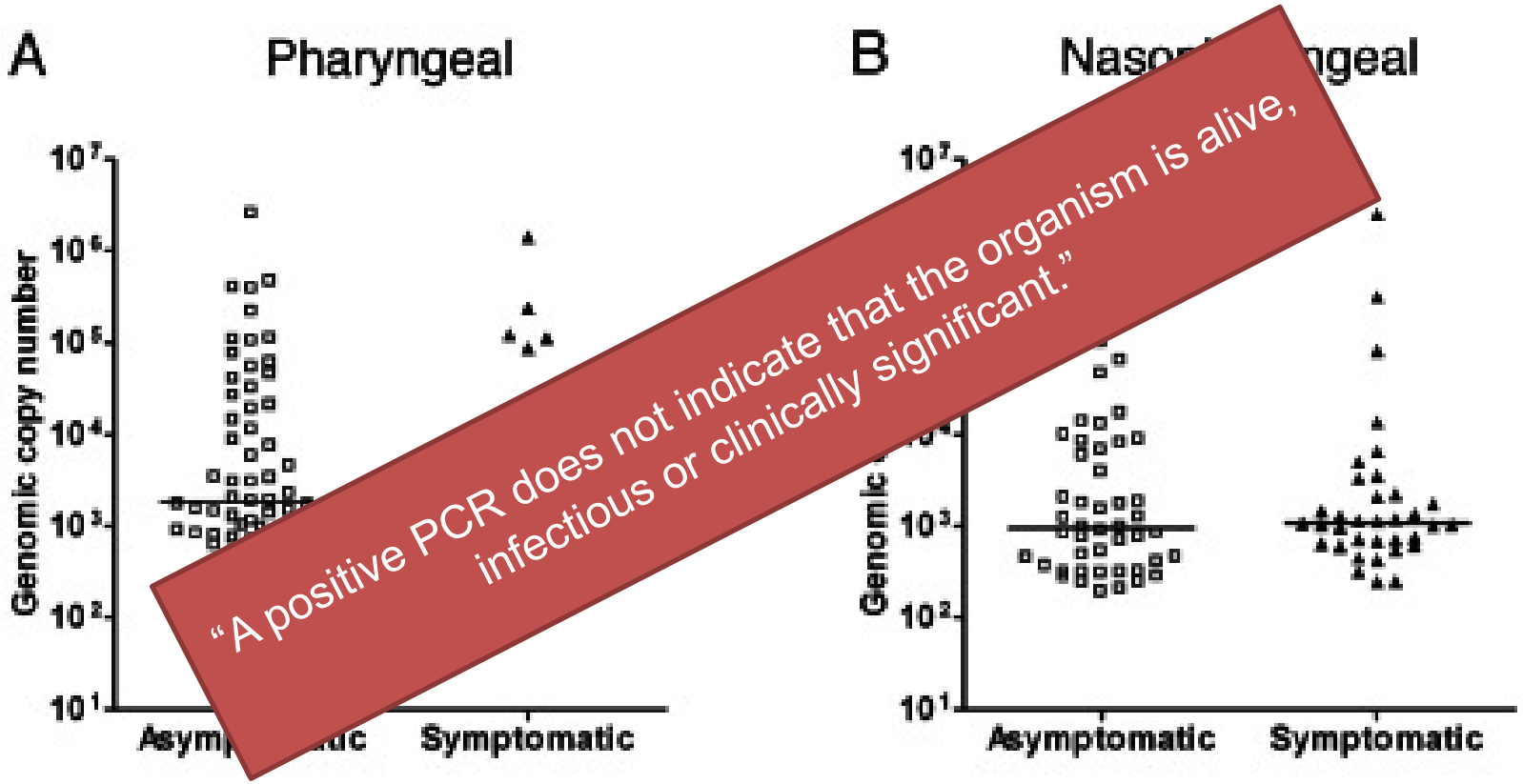
Saah AJS and Hoover DR. Ann Int Med. 1997; 126(1):91-94

Sample quality



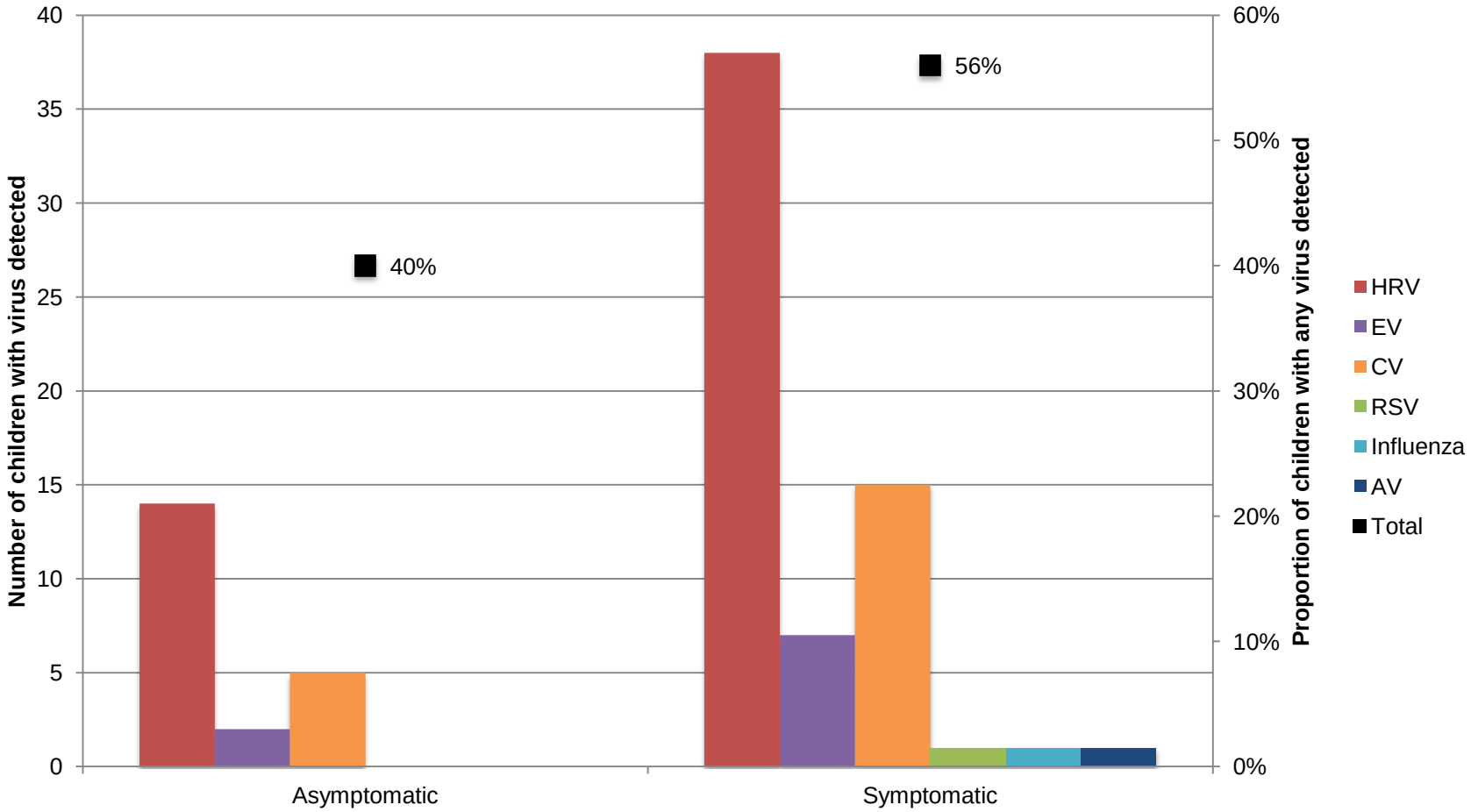
- Technical problems → positive run control: if negative → invalid
- Sample quality??
- Inhibition??

Mycoplasma pneumoniae



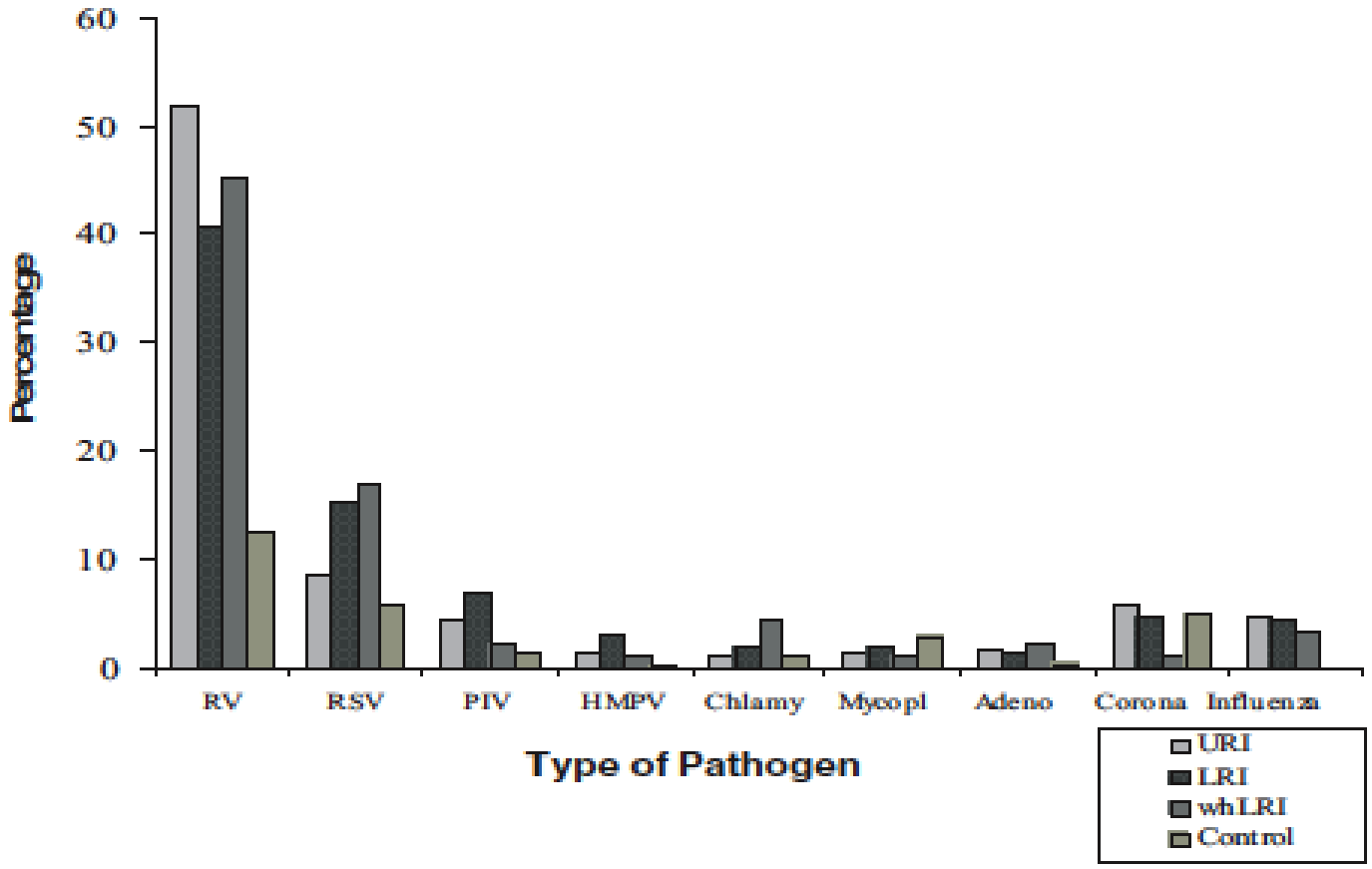
Spuesens EB et al. PLoS Med. 2013; 10(5): e10011444.

Respiratory viruses



van der Zalm MM et al. J Pediatr. 2009; 154: 396-400.

Many viruses can be associated with URTI and LRTI



Kusel MMH et al. Pediatr Infect Dis J. 2006; 25 (8): 680-686.

Sampling site is important

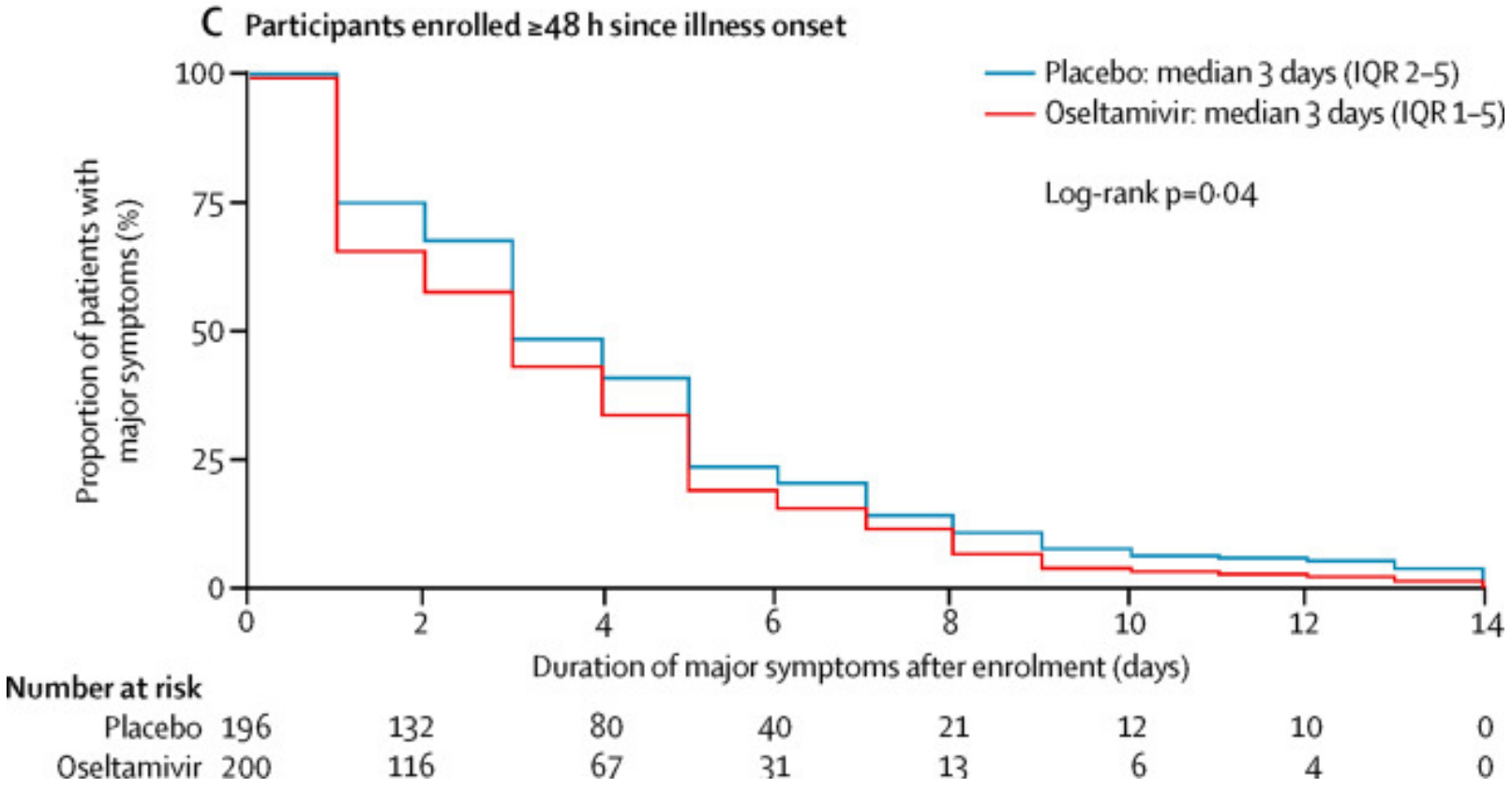
N positive NPA: 52

N positive BAL: 38

		Specificity	
		High	low
Sensitivity	high		HRV
	low	AV, BoV, CoV	

Wurzel DF et al. J Clin Virol. 2013; 58(4): 683-688.

Very modest impact of influenza treatment on symptoms



Fry AM et al. Lancet ID. 2014; 14(2): 109-118.

No impact on antibiotic use in the ED

Analysis 1.1. Comparison 1 Antibiotics use, Outcome 1 Antibiotics prescribed in ED.

Review: Rapid viral diagnosis for acute febrile respiratory illness in children in the Emergency Department

Comparison: 1 Antibiotics use

Outcome: 1 Antibiotics prescribed in ED

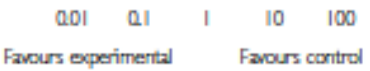
Study or subgroup	Rapid viral testing n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
Bonner 2003	34/193	53/198		25.8 %	0.66 [0.45, 0.96]
Doan 2009	16/89	23/110		13.6 %	0.86 [0.48, 1.53]
Iyer 2006	54/345	59/355		30.6 %	0.94 [0.67, 1.32]
Poehling 2006	43/134	48/166		30.0 %	1.11 [0.79, 1.56]
Total (95% CI)	**761**	**829**		**100.0 %**	**0.89 [0.71, 1.12]**

Total events: 147 (Rapid viral testing), 183 (Control)

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 4.11$, df = 3 (P = 0.25); I^2 =27%

Test for overall effect: Z = 0.99 (P = 0.32)

Test for subgroup differences: Not applicable



Doan Q et al. Cochrane Database of Systematic Reviews. 2012; 5: CD006452

No impact on inpatient management

	Intervention group	Control group	P value
Hospital admission, n(%)	223 (75%)	211 (74%)	.825
Time in hospital, mean (range), d	3.7 (1-18)	4.0 (1-15)	.170
Antibiotic therapy started, n(%)	124 (42%)	78 (27%)	.000
Duration of antibiotic therapy if initiated, mean (range), d	6.5 (1-14)	7.0 (2-21)	.490

Wishaupt JO et al. Pediatrics. 2011; 128(5): e1113-1120.

Impact on cohorting is questionable

	Conservative isolation scenario: screen all, cohort isolate RADT-positive patients, cubicle isolate patients with negative RADT		Intermediate isolation scenario: screen all, cohort isolate RADT-positive patients, cubicle isolate patients with negative RADT only if they belong to the high-risk group		Liberal isolation scenario: screen all, cohort isolate RADT-positive patients, no cubicle isolation	
	40% Median (95% CI)	65%	40% Median (95% CI)	65%	40% Median (95% CI)	65%
Total patients isolated	100	100	50 (39–63)	66 (55–77)	25 (16–34)	41 (30–52)
Cohort isolation	25 (16–34)	41 (30–52)	25 (16–34)	41 (30–52)	25 (16–34)	41 (30–52)
Cubicle isolation	75 (66–84)	59 (48–70)	25 (15–37)	25 (15–36)	0	0
Number of nonisolated RSV-positive patients	0	0	5 (1–11)	8 (2–16)	15 (8–23)	24 (15–34)

All numbers are calculated per 100 children below 2 years of age hospitalized with acute respiratory tract infections.
CI = confidence interval, RADT = rapid antigen detection test.

Pfeil J et al. Medicine. 2014; 93(25): e144.

Is there even a viral/bacterial LRTI distinction?

Smith CM et al. Am J Respir Crit Care Med. 2014;190:196-207

How do you think multiplex PCR should be used?

- Systematically in all patients admitted to hospital
- Systematically in all patients – we deserve to know what is causing it!
- A stepwise or selective approach is best
- Should not be used – knowing what pathogens can be detected e.g. from URT does not impact management

Table 3 Summary of potential benefits of POCT for respiratory viruses along with complicating factors.

Potential benefit of POCT	Complicating factors
Reduced unnecessary antibiotic use	Co-infection with bacteria is common
Improved antiviral use[a]	Ongoing controversy over efficacy of neuraminidase inhibitors
Improved isolation facility use	Increased detection of viruses may stretch limited resources
Reduced economic cost	Health economic modelling assumption are largely speculative

[a] Currently only neuraminidase inhibitors for influenza.

When are molecular assays indicated?

- Consider patient-population to be tested
- Children, elderly?Immunocompromised? CF? NICU? Chronic lung disease?
- Institutional outbreaks

- Purpose of testing
- Individual patient diagnosis; Outbreak diagnosis
- Starting or stopping therapy
- Monitoring vaccine or drug resistance

- Competing diagnoses (diagnosis and therapeutics)

What should I consider when using viral multiplex ?

- Time from onset to testing ->first 3 days of symptoms

- Choice of specimen

- NP swabs/aspirates (inpatients)
- Flocked-nasal mid-turbinate swabs for self collection (outpatients)
- Sputa, BAL, blood, stool -> may be relevant for specific patient-populations and viruses (additional validation of extraction methods and NATs)

- Specimen adequacy-> quality indicators

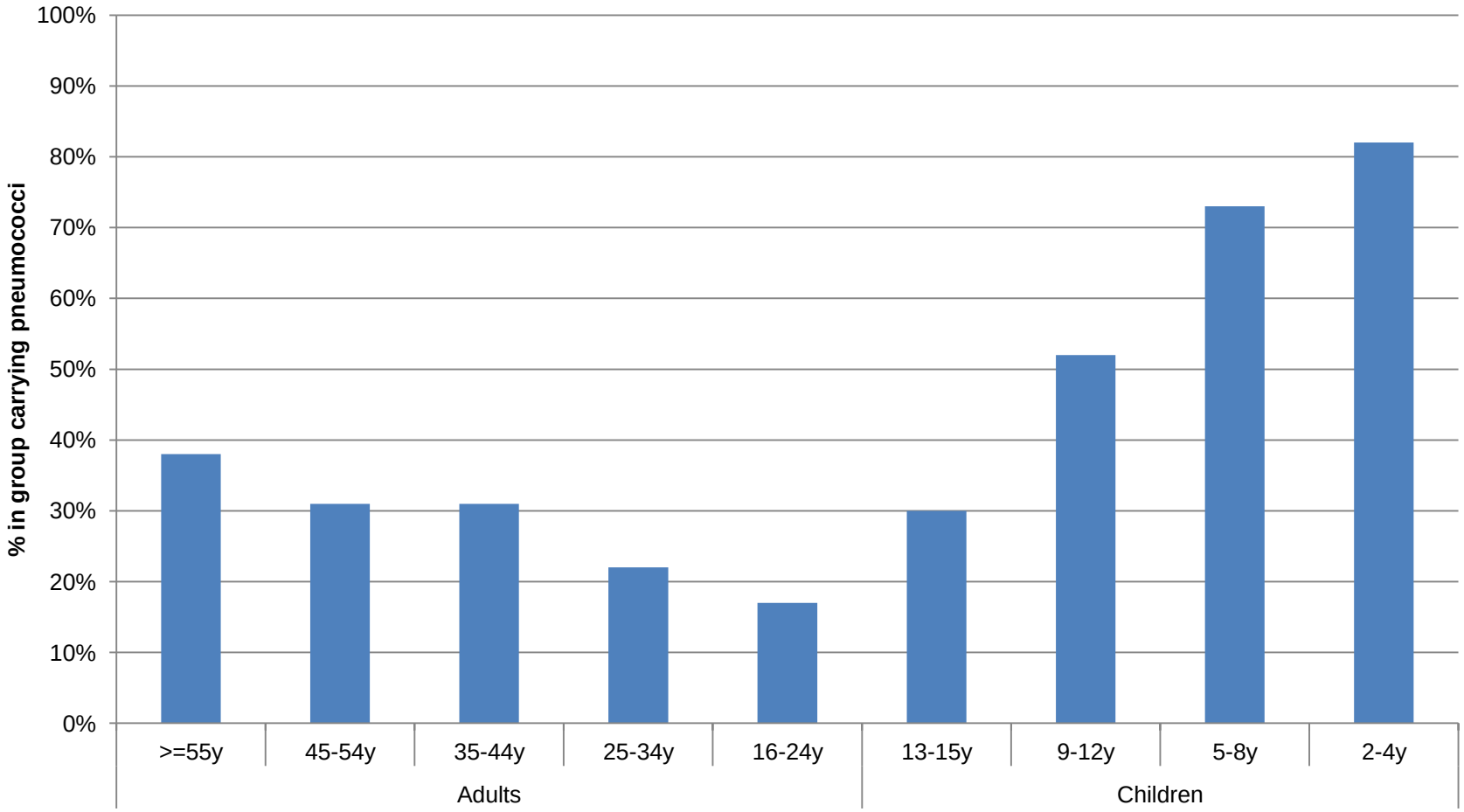
Use of multiplex at University Hospital Lausanne

- Complete multiplex panel for fragile patients : newborns, immunocompromised, patients in the ICU, patinets with chronic pneumopathies (CF, asthma..)

- Restricted panel to RSV and FLU during winter (GenExpert, CHUV)

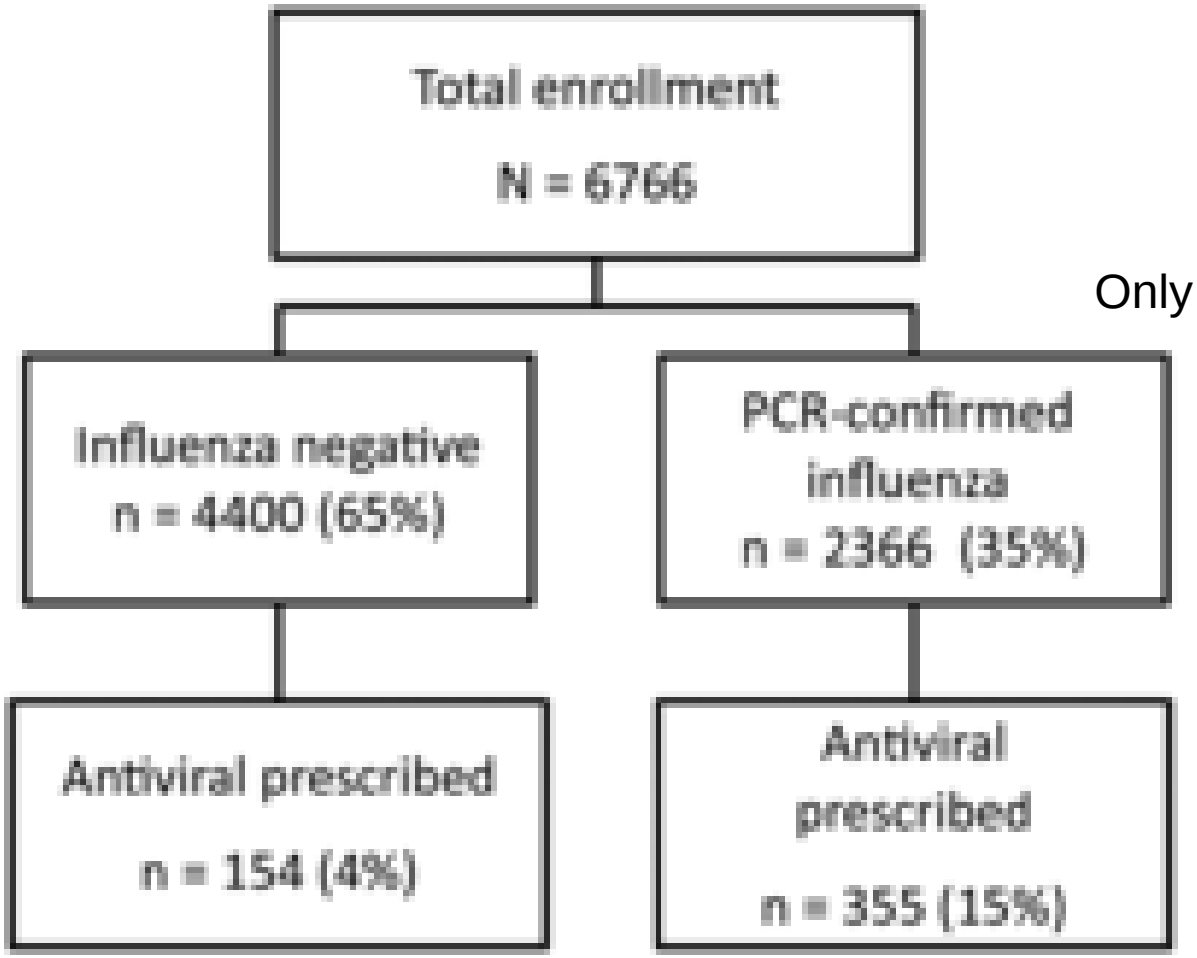
- Other seasons only RHV/EV

High colonization levels in children



Mackenzie GA et al. BMC ID. 2010; 10: 304.

Antiviral use in adult patients with ILI/ARI



Only 1021 <= 2d symptoms

Havers F et al. CID. 2014; 59(6): 774-782.