Microbiological Diagnosis of pneumococcal infections

M. Ieven
BVIKM
29.10.2015

Vaccine & Infectious Disease Institute

Universiteit Antwerpen

S. pneumoniae infections

- *S. pneumoniae* is a major cause of pneumonia, meningitis, bacteremia, sinusitis, and otitis media, and it occasionally infects tissues at other sites
- IPD: pneumonia, meningitis, bacteremia and infections of other normally sterile sites
- Worldwide, WHO estimates IPD causes +/- 1.6 million deaths/ year including 1 million children <5yrs

Pneumococcal Disease

- Accurate and reliable detection of *S.pneumoniae* would thus be beneficial for both pneumococcal & nonpneumococcal disease
  - narrow-spectrum agents for *S. pneumoniae*
  - other antibacterials and antiviral agents

- Despite its importance, IPD (particularly pneumococcal pneumonia) can be surprisingly difficult to confirm microbiologically

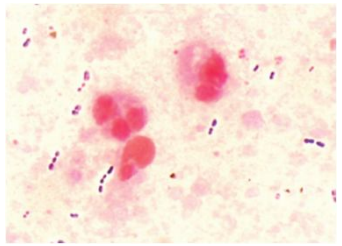
Diagnostic testing for *S.pneumoniae*

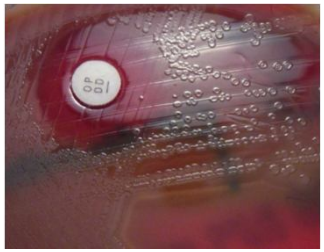
2 fundamental questions to be asked:

- does the test identify *S. pneumoniae* specifically and
- does this detection adequately implicate *S. pneumoniae* as the causative pathogen of disease?

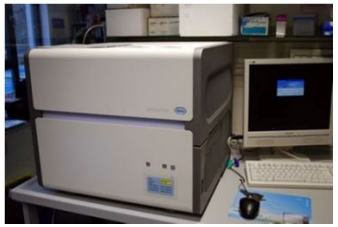
⇨ Distinction between colonisation and infection?

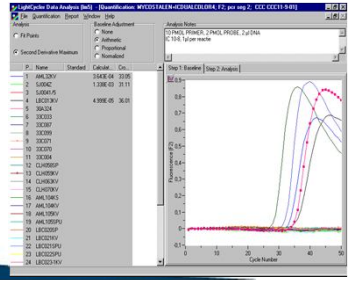
Laboratory Diagnosis of Pneumococcal Disease

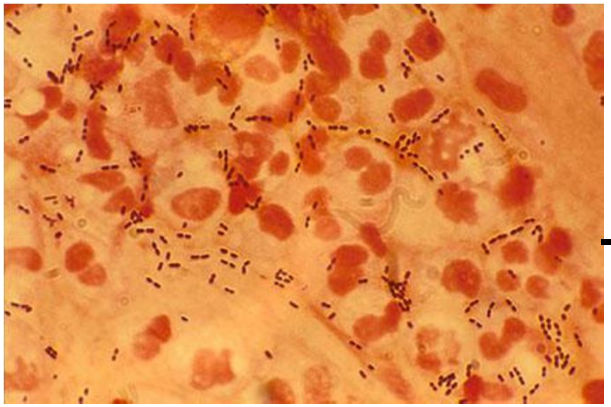




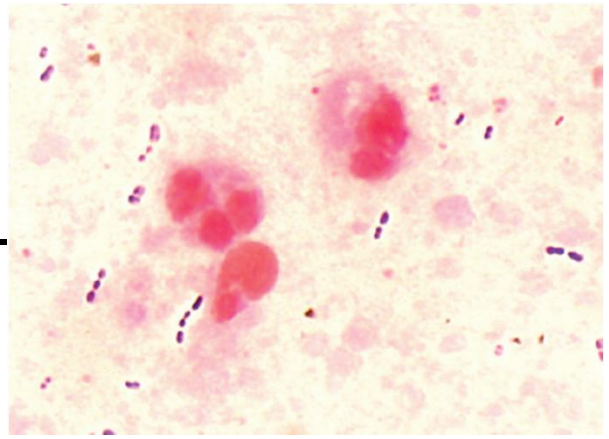
- Microscopy and culture
- Antigen detection assays
  - Urinary antigen test
  - Ag test used on other body fluids
- Nucleic acid amplification tests
  - On normally sterile samples:
    - blood,
    - CSF
    - Pleural fuid
  - On respiratory samples







Streptococcus pneumoniae



- Gram positive cocci in pairs, diplococci
- Causing α-hemolysis of blood agar
- catalase negativity
- optochin susceptibility:
  - But optochin-R pneumococci up to 10%
- bile solubility
  - Discriminatory for optochin-R isolates
- No MALDI-TOF Identification

Eur Respir J 2005; 26: 1138–1180
DOI: 10.1183/09031936.05.00055705

ERS TASK FORCE IN COLLABORATION WITH ESCMID

Guidelines for the management of adult lower respiratory tract infections

**M. Woodhead*, F. Blasi#, S. Ewig¶, G. Huchon+, M. Leven§, A. Ortqvist
T. Schaberg**, A. Torres##, G. van der Heijden¶¶ and T.J.M. Verheij¶¶**

CONTENTS

Guidelines for the management of adult lower respiratory tract infections. *Clinical Microbiology and Infection* 2011; 17: E1-E59

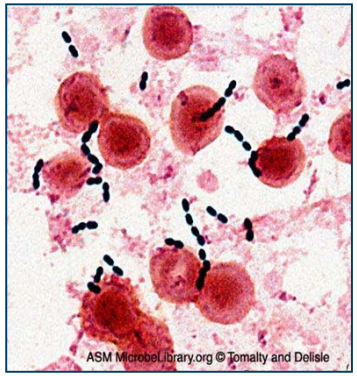
Woodhead M et al Eur Resp J 2005; 26:1138-1180

Woodhead M et al. Clin. Micribiol.Infect. 2011;17, E1-E59

Rapid Sputum Examination by Gram staining has Diagnostic Value

- Sens : 57% increasing to 63% if ≤ 24 hrs antibiotics

Roson B 2000; Butler JC 2003; Musher DM 2004



- 216 pts: 62% sputa with predominant morphotype in 65% Gram + diplococci; sens 68.2%, sp 93.8%

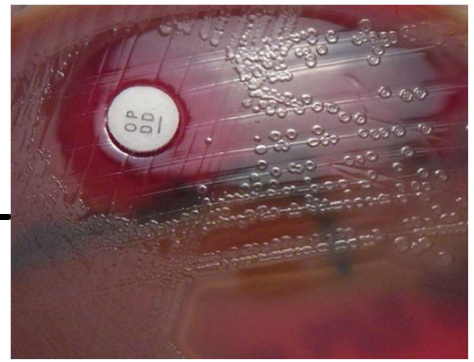
Miyashita N. et al Med Sci Monit 2008; 14:171

- **Old, simple, cheap rapid diagnostic test for etiology of CAP: can be useful in guiding AB treatment in +/- 25%**

- Sputum for routine gram stain and culture, if sputum is purulent and to be correlated with morphotype in gram stain (**A3**)

ERS Guidelines, 2005, Updates, 2011

Culture based detection of *S.pneumoniae*



Advantages:

- low cost and high specificity: 85-95% ⇨ but in adults
- both antibiotic susceptibility and serotype results possible

Difficulties :

- tendency of *S. pneumoniae* to autolyse
- antibiotic treatment prior to sampling
- low prevalence of detectable bacteremia

⇨ False neg

- colonisation vs infection in children?
- difficulty of obtaining good specimen
- description of *S. pseudopneumoniae*

⇨ False pos

Incidence of pneumococcal bacteremia

- rates of pos blood cultures in adults hospitalized with pneumonia are typically only 3%–8%

- **in children even lower rates**

Study	Incidence
Waskerwitz (1981)	5.8%
Dershewitz (1983)	4.3%
Carroll (1983)	10.4%
Bennish (1984)	4.3%
Jaffe (1987)	2.8%
Lee (1998)	1.6%

⇨ **Recommendations on blood cultures?**

Werno A & Murdoch D Med Microbiol 2008; 46:926–32

Value of Blood Culture in the Diagnosis of adult CAP

- Specificity: very high (100 %)
- Sensitivity low:positive in 4-29% of untreated cases; 34 % when initiated within 4 days after first symptoms

Bishara J et al. Eur J Clin Microbiol Infect Dis. 2000;19:926
Butler JC et al J Infect Dis. 2003;187:1422

⇒ **Most sensitive for *S.pneumoniae***

⇒ **But… easy to sample and often the only source of information!**

⇨ Blood cultures before initiation of AB therapy **(A3)**

ERS Guidelines, 2005, Updates, 2011

IDSA GUIDELINES

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

John S. Bradley,[1,a] Carrie L. Byington,[2,a] Samir S. Shah,[3,a] Brian Alverson,[4] Edward R. Carter,[5] Christopher Harrison,[6] Sheldon L. Kaplan,[7] Sharon E. Mace,[8] George H. McCracken Jr,[9] Matthew R. Moore,[10] Shawn D. St Peter,[11] Jana A. Stockwell,[12] and Jack T. Swanson[13]

Bradley J et al *Clin Infect Dis*. 2011;53:e25–e76
Bradley J et al *Clin Infect Dis*. 2011;53:617–630

Blood cultures: recommendations

	Outpatient		Inpatient
Recommendation	**NOT Recommended**	**Recommended**	**Recommended**
Comments	Non-toxic, fully immunized children treated as outpatients	Failure to demonstrate clinical improvement, progressive symptoms, or deterioration after initiation of antibiotic therapy	Requiring hospitalization for moderate-severe bacterial CAP
Strength	**Strong**	**Strong**	**Strong**
Evidence Quality	**Moderate**	**Moderate**	**Low**

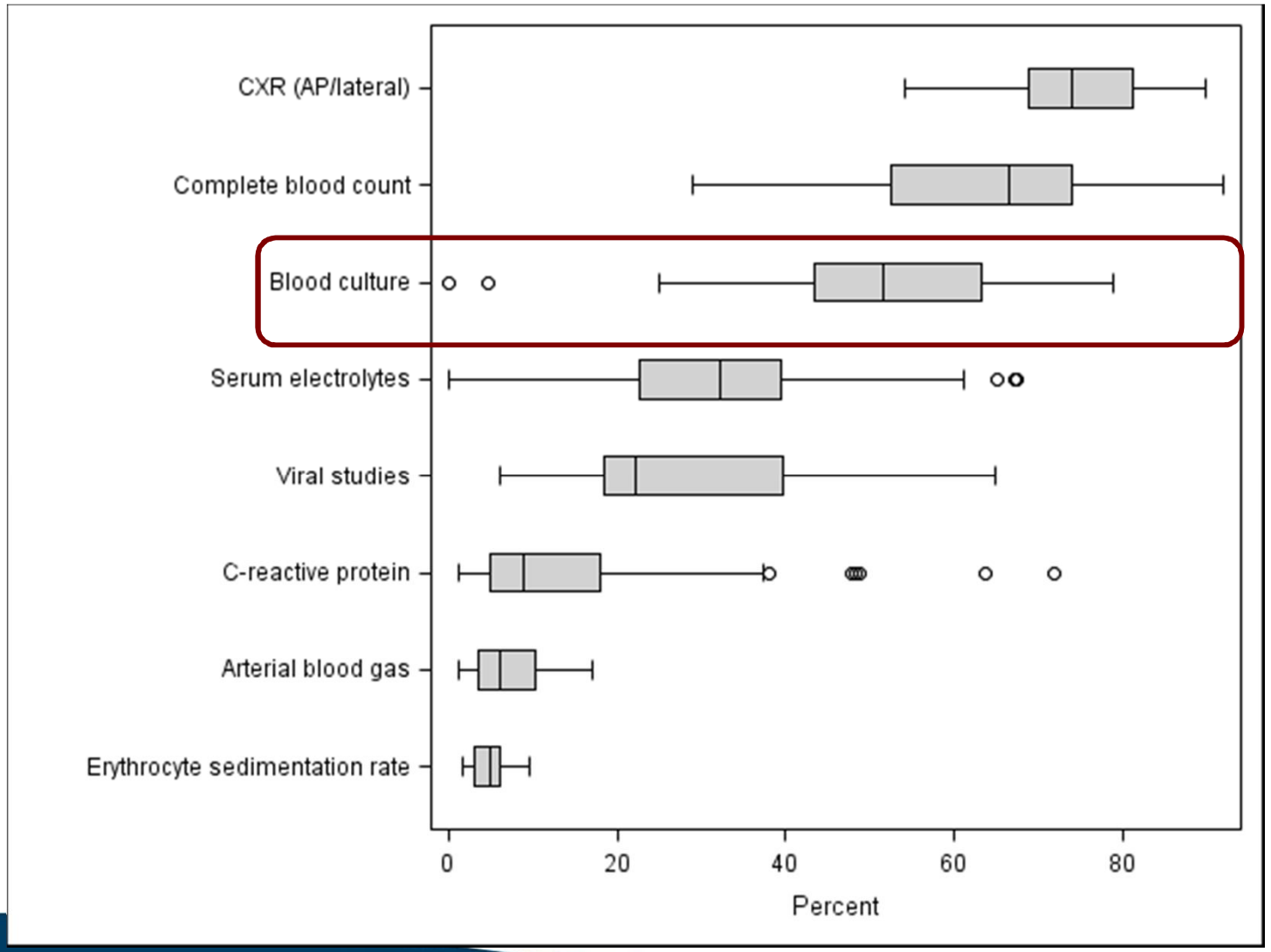
Bradley J et al *Clin Infect Dis*. 2011;53:e25–e76
Bradley J et al *Clin Infect Dis*. 2011;53:617–630

Blood cultures: rationale

- Outpatient
  - Infrequently identifies pathogens (<2%)
  - False-positives more common than true positives at some hospitals
  - Rarely informs outpatient management
- Inpatient
  - Positive in ~3% of uncomplicated pneumonia
  - Positive in ~15% with empyema
  - Allows for culture-directed therapy when positive
  - Provides local epidemiologic data

Bonadio WA. *Pediatr Emerg Care*. 1988; Hickey RW. *Ann Emerg Med*. 1996; Shah SS. Arch *Pediatr Adolesc Med*. 2003; Shah SS. *Pediatr Infect Dis J*. 2011

Diagnostic Testing for pediatric CAP in 47 hospitals



Brogan TV. *Pediatr Infect Dis J*. 2012

Pneumococcal Antigen Detection

A new **Immunochromatographic membrane test** (ICT) has been developed to detect capsular polysaccharide antigens PnC of *S.pneumoniae* in urine samples.

PnC is a common antigen for all pneumococcal serotypes.
ICT has proven very useful in the rapid diagnosis of pneumococcal pneumonia in adults.

Early Diagnosis of Pneumococcal Pneumonia based on Urinary Ag

- **Diagnostic yield increased up to 38.9% using ICT combined with conventional methods**
- The test tends to be **more sensitive** for patients **with** versus those without **bacteremia**

⇩

Sequential approach:

Urinary antigen testing for **high-risk patients** for whom demonstrative results of a **sputum Gram stain are unavailable.**

Roson B et al. Clin Infect Dis 2004; 38: 222

Diagnositc efforts should be directed towards **the most severely affected patients** and the ones with greatest risk of death.

Ortega et al. Scand J Infect Dis 2005

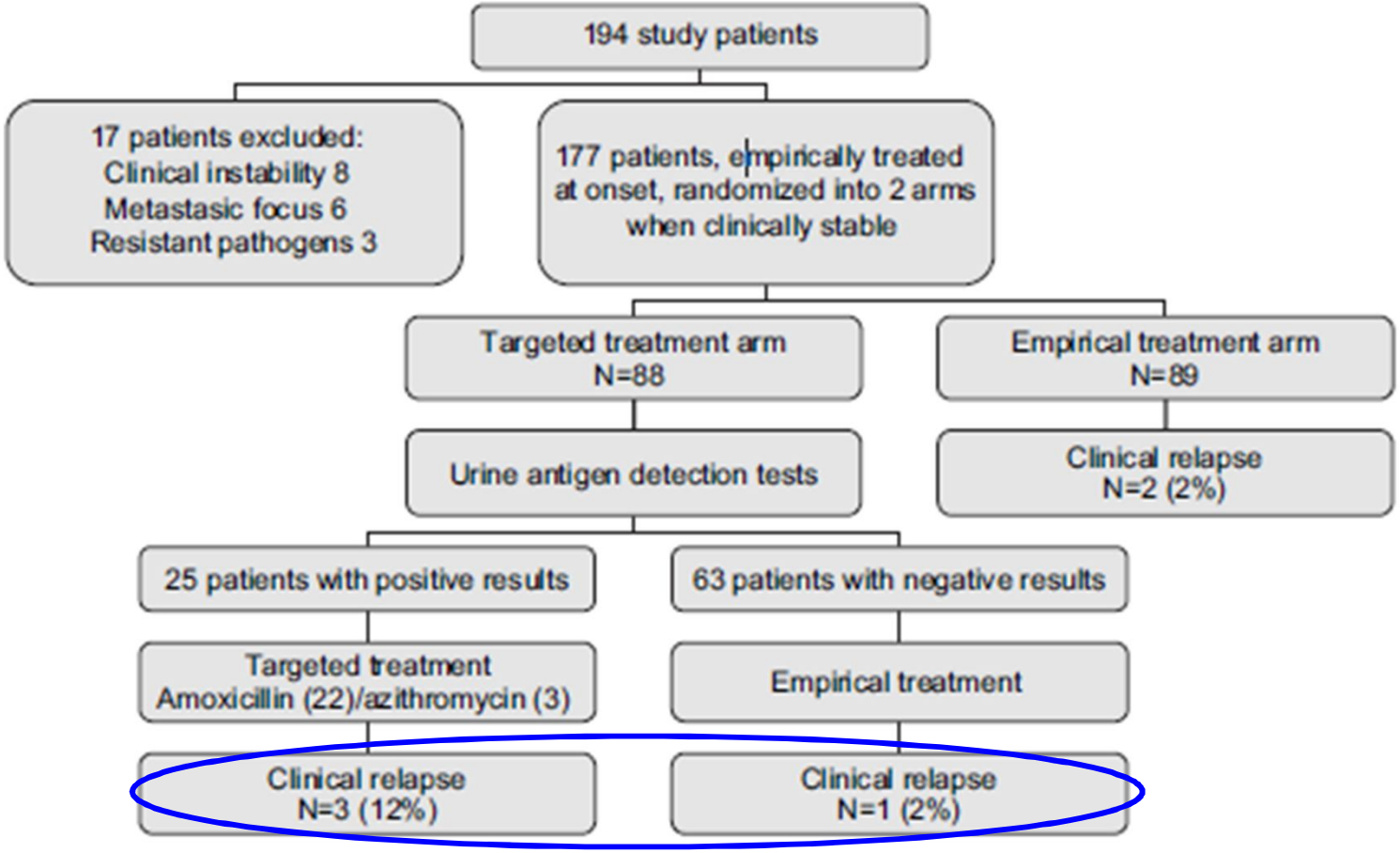
Limited impact of *S. pneumoniae* U Ag test on adjustment of AB treatment

- Case Control study in 2 groups of pneumonia patients randomised:
  - PnAg performed: N= 139: 22/139 pos
  - PnAg not performed: N= 147

	Pn Ag group N= 139	**Control N= 147**	***P* -value**
	Absolute nr (%)	Absolute nr (%)	
No change	51 (37%)	67 (46%)	0.15
Narrowing	66 (47%)	73 (50%)	0.73
Streamlining to peni or amoxi	17 (13%)	13 (9%)	0.44
Stop macrolide	56 (40%)	54 (36%)	0.54

⇨ **Implementation of Ag test: no result on change of AB prescription**

Empirical vs targeted AB in CAP based on results of Pn UAg



Narrowing treatment according to the UAg may be associated with a higher risk of clinical relapse; no outcome or economic benefits

Falguera M et al Thorax 2010; 65: 101-106

S. pneumoniae Urinary Ag test, According to Pneumococcal Colonization Status of Pediatric Patients, with or without Pneumonia

Patients	No. of positive results/ no. of total results (%) among children		*P*
	With pneumococci in nasopharynx	Without pneumococci in nasopharynx	
Children with pneumonia	25/41 (61)	6/47 (13)	.001
Control children with dermatitis of diarrhea	43/80 (54)	25/118 (21)	.001

⇒ Antigen test does not distinguish children with pneumonia from controls without pneumonitis

Dowell SF et al. Clin Infect Dis 2001; 32: 824
Navarro D et al, J Clin Microbiol 2004; 42: 4853

Comparison of the manufacturer's protocol and a protocol modified to increase specificity

Binax NOW method	No. of positive results/no. of total results (%) among: Children with pneumonia	Controls	*P*
Manufacturer's protocol	31/88 (35)	68/198 (34)	NS
Modified protocol[a]	12/88 (14)	19/198 (10)	NS

modified protocol : a pos **reaction within 5 min,** rather than within the full 15 min.

⇨ Specificity somewhat increased by reading within 5 min, but test does not differentiate pneumonia patients from controls

Dowell SF et al. Clin Infect Dis 2001; 32: 824

Binax NOW *S. pneumoniae* Ag on other body fluids

- **on CSF samples** in pneumococcal **meningitis:**
  - Sens: 95%–100% and a spec of 100% : 30% more cases vs culture

Saha SK et al Pediatr Infect Dis J 2005; 24:1093–8
Samra Z et al Diagn Microbiol Infect Dis 2003; 45:237–40

- **pleural fluid** specimens from children and adults with
  - Sensitivity: 71% vs +/- 32% by culture

Ploton C et al Pathol Biol 2006; 54:498–501
Porcel JM et al Chest 2007; 131:1442–7

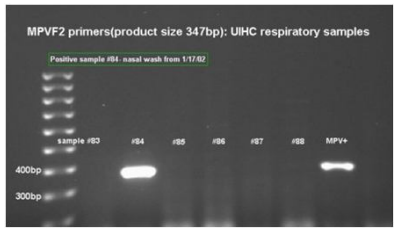
- **BAL samples**:

  sensitivity of 95% and a specificity of 87%

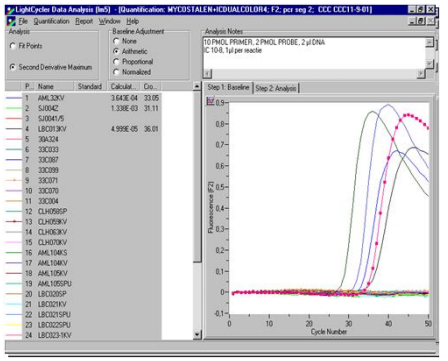
Jacobs JA et al J Clin Microbiol 2005; 43:4037–40

- The NOW test can also provide a rapid provisional identification of *S. pneumoniae in blood cultures* with positive results

Petti CA et al J Clin Microbiol 2005; 43:2510–2



Real-Time in-house NAATs



Ref, year	Assay	targets
Luo Y, 2012	PCR+ agarose GE	*S. pneumoniae*, *H. influenzae* type b, *M. tuberculosis*
Kim W, 2013	Mx PCR+ agarose GE	*S. pneumoniae, S. mitis, S. oralis*
Weinberg G, 2013	RT-Mx PCR, TaqMan array	HAdV, hMPV, PIV1-4, influenza A, influenza B, influenza C, RSV, rhinovirus, HCoV OC43, 229E, NL63, HKU1, enterovirus, *B. pertussis, C. pneumoniae, H. influenza, L. pneumophila, M. pneumoniae, S. pneumoniae, S. pyogenes*
Abdeldaim G , 2008	Quantitative RT-PCR	*S. pneumoniae*

Loens K & Ieven M 2015; review

PCR Using Blood for Diagnosis of Invasive Pneumococcal Disease

Systematic Review and Meta-Analysis

- 29 studies published between 1993 - 2009 included
- Pneumococcal bacteremia for case definition and patients with bacteremia caused by other bacteria as controls:
  - Sens: 57.1%, Spec: 98.6%
- When the controls were patients suspected of having IPD without pneumococcal bacteremia:
  - Sens: 66.4%, Spec: 87.8%
- being a child was associated with low specificity

⇨ **Currently available PCR methods on blood for diagnosis of IPD lack sens and spec needed for clinical practice**

Tomer A et al. J Clin Microbiol 2010; 48: 489–496

Pneumococcal Pneumonia in Children: Diagnosis by R-T PCR on Blood Samples

- 753 children 0–16 yrs with a diagnosis of CAP
- pneumococcal infection in 80/753 (10.6%) of patients by RT-PCR
- culture and RT-PCR simultaneously performed in 292 patients:
  - 45 (15.4%) pos by RT-PCR
  - 11 (3.8%) pos by culture

} ***P*** **< .001**

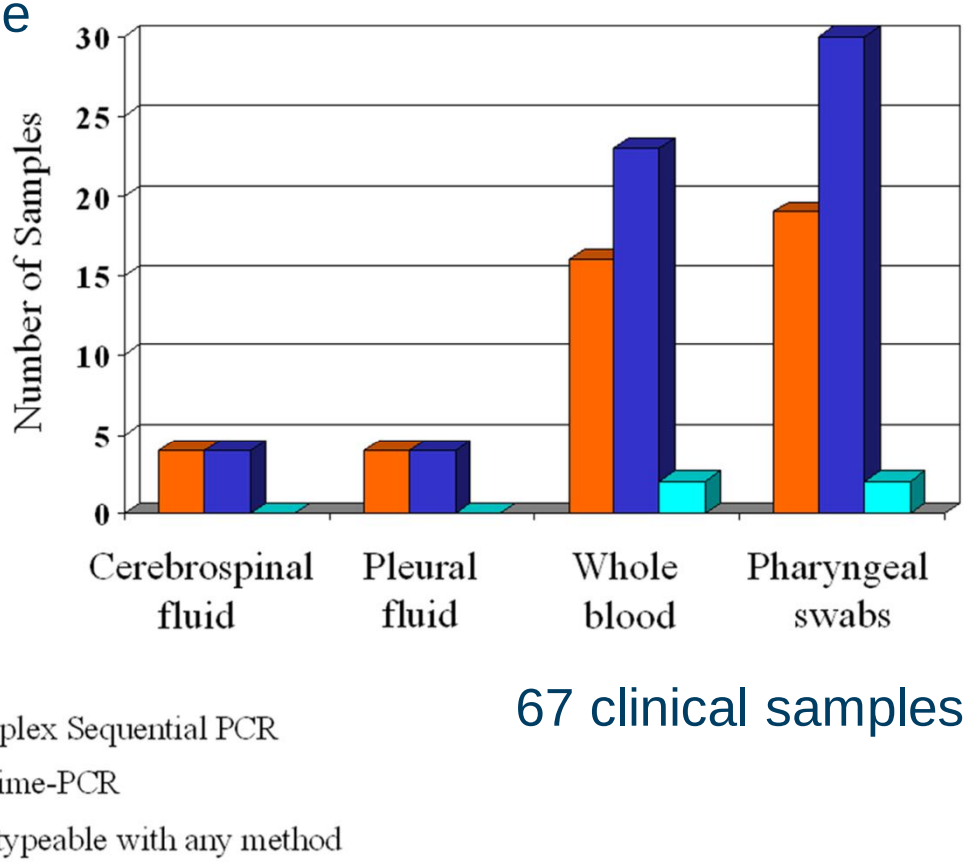
- **RT-PCR: significantly more sensitive than culture in revealing bacteremic pneumonia**

Resti M et al. Clin Infect Dis 2010; 51:1042–1049

RT-PCR and Mx PCR for diagnosis and Serotyping in Children with Culture - PID

All samples PCR positive for *lytA* gene
Serotyping

- On normally sterile fluids
  - RT-PCR : 31/33 (93.9%)
  - MS-PCR : 24/33 (72.7%)
    - *P*= 0.047
- On Npswabs
  - RT-PCR : 30/34 (88.2%)
  - MS-PCR : 19/34 (55.9%)
    - *P*= 0.007



67 clinical samples

⇨ Both MS PCR and RT-PCR useful for pneumococcal serotyping but RT-PCR appears more sensitive

Azzari C et al PLoS ONE 2010; 5:e9282

Added value of *S. pneumoniae* RT-PCR in IPD in blood

cases	confirmed IPD		Probable pneumococcal infection
cases	1-3	4-5	6-10
age	4 – 7 years	10 -11 years	2- 6 years
Clinical diagnosis	pneumonia with pleurisy	pneumonia (with secondary pleurisy)	pneumonia with pleurisy
Blood cultures	sterile	*S.pneumoniae*	sterile
Pleural fluid culture	*S.pneumoniae*	Not done	Sterile (1/5 ND)
Blood real-time PCR	Pos	Neg	**Positive**

Chantreuil J et al J Microbiol Exp 2015, 2: 00040

PCR for detection of IPD in children

- 76 children with **IPD**: PCR for *Ply* and *LytA* gene
  - Sensitivity PCR: 80% , specificity 98%
  - 5 additional cases identified vs standard techniques

Chantreuil J et al J Microbiol Exp 2015, 2: 00040

pneumococcal meningitis: 122 cases

- 87/122 pos by culture: sensitivity 71%
- Among culture neg:
  - 35 pos by *LytA* PCR: sensitivity: 100%
- All 122 pos by ICT : sensitivity:100%

Saha SK et al Pediatr Infect Dis J 2005; 24:1093–8

Value of Pneumococcal Q-PCR

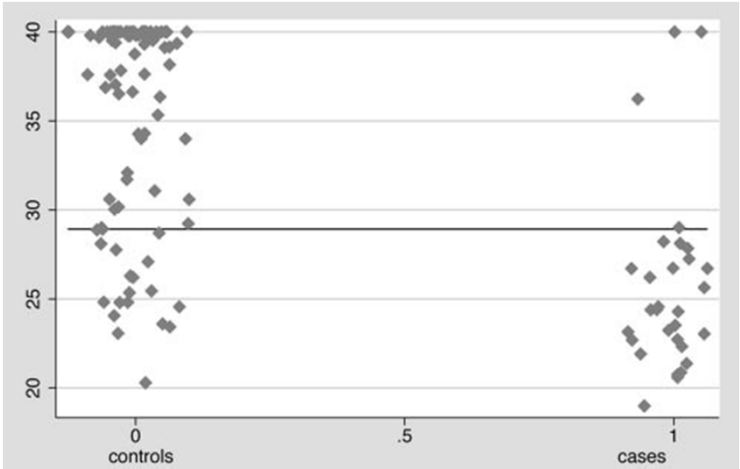
- Children with confirmed meningitis (n = 82) or pneumonia (n = 13) prospectively recruited
- blood and CSF taken for pneumococcal DNA loads
- Median blood and CSF bacterial loads (log DNA copies/mL) were significantly higher in nonsurvivors than in survivors:
  - blood (3.80 vs. 2.97, *P* = 0.003),
  - CSF (8.17 vs. 7.50, *P* = 0.03)

⇨ **High Pneumococcal DNA Loads are Associated With Mortality in Children With IPD**

Carrol ED et al. Pediatr Infect Dis J. 2007 ; 26: 416–422

Quantitative PCR for Diagnosis of *S. pneumoniae* Infection

- First prospective study on Q-PCR
- Based on ROC curve analysis
  - Ct with maximal sensitivity: 28.96
  - Corresponding to +/- $3.7x10^4$ DNA c/ml
  - Sens: 90%; spec: 80%



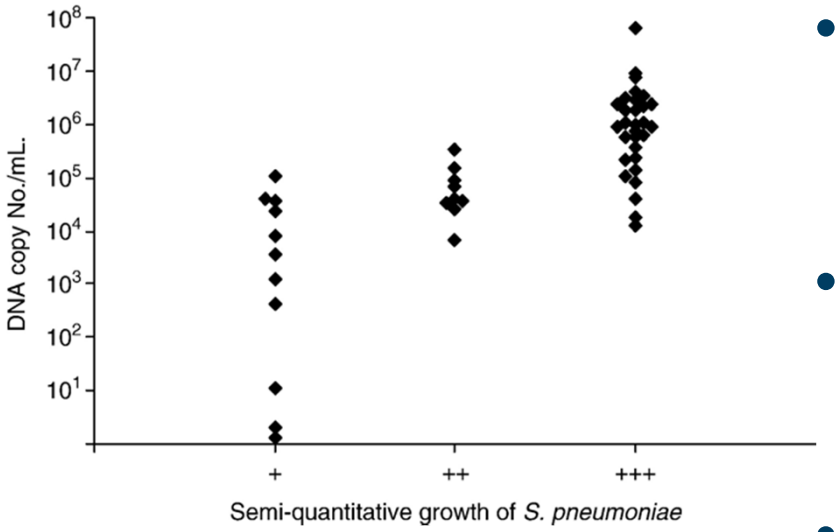
Yang S. et al. J Clin Microbiol 2005, 43: 3221-26

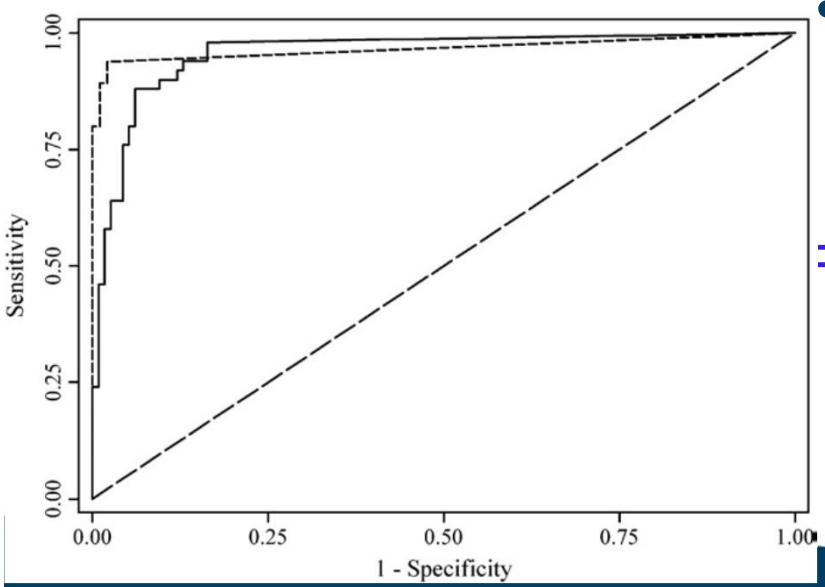
- Significant increase in pathogens: with RQ-PCR (33.5%) vs culture (22.2%) ($p < .05$)
  - RQ-PCR corresp to $>10^5$ CFU/ml

⇒ **Quantitative PCR has favorable accuracy for diagnosis of pneumococcal pneumonia**

Kais M et al Diagn Microbiol Infect Dis J 2006; 55:169-178

Quantitative DNA-based definition of pneumococcal pneumonia





- At detection limit of PCR
  - Sens: 98%
  - Spec: **84%**
- Cut-off 10^4 DNA copies/ml
  - Sens: 84%
  - Spec: **94%**
- Mean Ct value significantly lower for samples with abundant growth

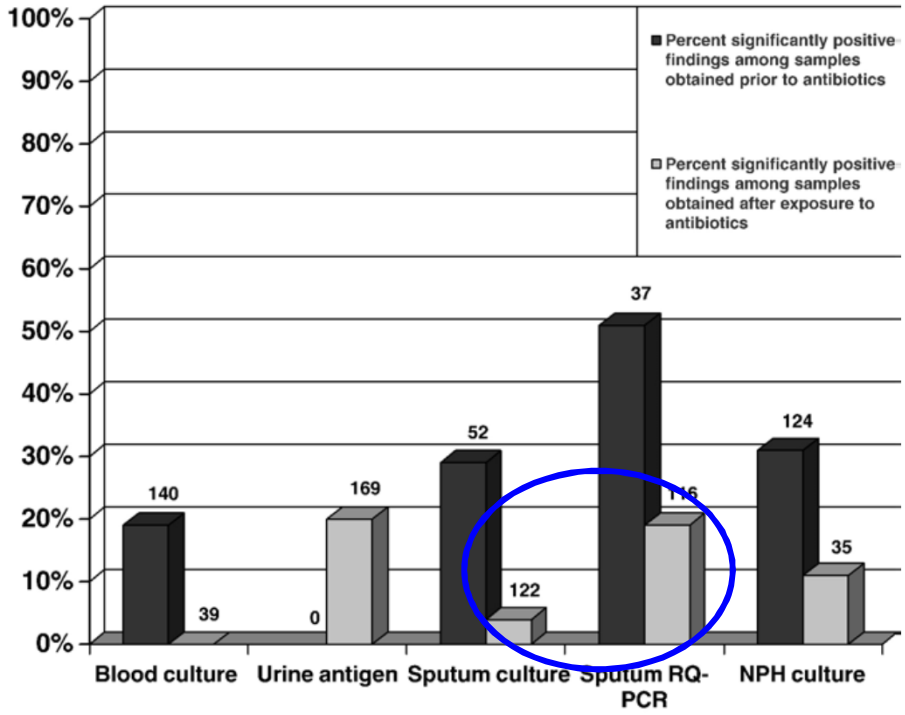
⇒ **Quantitative PCR enables differentiation between pathogenicity and commensalism**

Abdeldaim G et al Diagn Microbiol Infect Dis J 2008; 60: 143-50

Quantitative PCR for Diagnosis of *S. pneumoniae* Infection

70/184 (38%) patients with *S.pneumoniae*

- 15% by blood culture
- 20% by urinary Ag
- 15% culture positive sputa
- 27% by RQ-PCR
  - 82% of these also detected by other methods
  - 50% of these culture -, most of these treated with AB



⇒ RQ-PCR particularly valuable in patients treated with AB

Johansson N et al Diagn Microbiol Infect Dis J 2008; 60: 255-61

Microbiological Diagnosis of pneumococcal infections: Conclusions

- Despite developments in laboratory diagnostics, a **microbiological diagnosis remains difficult in IPD**, particularly for pneumococcal pneumonia
- **Culture-based** methods remain important
- **Antigen based techniques limited to adults but impact of positive tests is limit**
- The role of **nucleic acid amplification tests has yet to be fully clarified** especially of QR-PCR in respiratory samples