"Infezioni delle basse vie respiratorie (LRTI): aspetti clinici e diagnosi microbiologica “

I percorsi diagnostici microbiologici nel paziente immunocompetente

Rita De Rosa
S.C. Microbiologia Clinica e Virologia
Azienda Ospedaliera Pordenone

Pordenone, 14 dicembre 2012
Il tipo di patogeni coinvolti nelle LRTI è ampio e con varie sensibilità agli antibiotici e costituisce una grande sfida per la diagnostica microbiologica.

K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009,
There have been a large number of publications looking at the possibility of predicting the aetiological agent from the clinical features at presentation; however, while certain symptoms and signs are more common with specific pathogens, none allow accurate differentiation.

There are no characteristic features on the chest radiograph in CAP that allow confident prediction of the causative organism.

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009
1. Identification of pathogens and antibiotic sensitivity patterns permits selection of optimal antibiotic regimens.

2. Targeted and narrow-spectrum antibiotic therapy limits drug costs, the threat of antibiotic resistance and adverse drug reactions such as C difficile-associated diarrhoea.

3. Specific pathogens have public health or infection control significance, including legionella, psittacosis, C burnetii, influenza A and multiresistant organisms.

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009
Microbiological investigations allow monitoring of the spectrum of pathogens causing CAP over time.

Without the accumulated information available from these culture results, trends in antibiotic resistance are more difficult to track, and empirical antibiotic recommendations are less likely to be accurate.

IDSA/ATS Guidelines for CAP in Adults • CID 2007:44 (Suppl 2) • S27

Local recommendations for empiric antibiotic therapy
The Importance of Initial Empiric Antibiotic Selection

- Adequate init. antibiotic
- Inadequate init. antibiotic

(p < .05)

- Kollef MH and Ward S. Chest 1998;113:412-20
Almost all of the major decisions regarding management of CAP, including diagnostic and treatment issues, revolve around the initial assessment of severity.

IDSA/ATS Guidelines for CAP in Adults • CID 2007:44 (Suppl 2) • S27
Score per determinare gravità delle CAP

➢ PSI
  • Molto popolare
  • Predittivo della mortalità
  • Necessari più di 20 parametri
  • Difficile da applicare fuori dall’ospedale

➢ CRB 65 (confusione, atti Respiratori > 30/min, ipotensione, età > 65)
  • Più utile e comodo in comunità
CRB65 severity score:
1 point for each feature present:
- Confusion
- Respiratory rate ≥ 30/min
- Blood pressure (SBP < 90 or DBP ≤ 60mmHg)
- Age ≥ 65 years

Treat according to clinical judgement and CRB65 severity score

0 → Low severity
1-2 → Moderate severity
3-4 → High severity

Likely suitable for home treatment
Consider hospital referral
Urgent hospital admission

Empirical antibiotics if life-threatening (see section 8.8)

CURB 65 score
NOT VALIDATE IN IMMUNOCOMPPROMISED

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009
CAP severity assessment should be based in three key points:

- a pneumonia-specific score,
- biomarkers,
- and clinical judgment.

J.M. Pereira. Semin Respir Crit Care Med. 2012 Jun;33(3)
The value of procalcitonin level in community-acquired pneumonia in the ED

Jeong Ho Park MD, Jung Hee Wee MD, Seung Pill Choi MD, PhD*, Sang Hoon Oh MD
appropriatezza

It has always been recognized that the use of a diagnostic test is an intervention. A diagnostic test should be requested only when a question is being posed and when there is evidence that the result will provide an answer to the question.

Pneumonia in immunocompetent adults\textsuperscript{1-8, b}

Clinical history / Patient group

Mild pneumonia

- Mild pneumonia admitted to hospital due to other reasons e.g. co-morbidities
  - Not pyrexic
  - Pyrexic (T>38°C)

Sample type

- Blood
- Sputum
- Blood
- Respiratory sample for virus and atypical bacteria detection\textsuperscript{2}
- Urine
- Sputum\textsuperscript{2}
- BAL\textsuperscript{6, 7}
- Pleural fluid

Primary testing

Blood culture (B 57)
- Mycoplasma pneumoniae\textsuperscript{11}

Microscopy, Culture and Sensitivity (B 57)
- Legionella pneumoniae\textsuperscript{11}
- Mycoplasma pneumoniae\textsuperscript{11}

Sputum culture (B 57)
- Mycoplasma pneumoniae\textsuperscript{11}

Urine
- Respiratory virus PCR screen\textsuperscript{4} (Q 60)
- Urine antigen test for Legionella pneumoniae serogroup 1 and Streptococcus pneumoniae (B 47)

Microscopy, Culture and Sensitivity (B 57)
- Mycoplasma pneumoniae\textsuperscript{11}
- Legionella pneumoniae (B 47)
- Mycobacterium sp (B 40)

Microscopy, Culture and Sensitivity (B 57, B 28)
- Mycobacterium sp

Secondary testing

No microbiological investigation required

- Chlamydia pneumoniae
- Chlamydia sp\textsuperscript{12}

- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- Chlamydia sp\textsuperscript{12}

- Mycobacterium sp

- Legionella pneumoniae (B 47)
- Legionella pneumoniae (B 47)
- Legionella pneumoniae (B 47)
- Mycobacterium sp

Legend:
- = PCR
- = Serology
- = Culture
- = EIA
- = IF
- = PCR or IF
- = PCR or culture
General investigations are not necessary for the majority of patients with CAP who are managed in the community.

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009
For patients with low severity CAP the extent of microbiological investigations should be guided by clinical factors (age, comorbid illness, severity indicators), epidemiological factors and prior antibiotic therapy. [A]

British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009
Mild pneumonia admitted to hospital due to other reasons eg co-morbidities

Clinical history / Patient group

Mild pneumonia

Sample type

Primary testing

Secondary testing

No microbiological investigation required

Sputum

Blood

Microscopy, Culture and Sensitivity

Blood culture
The collection of diagnostic samples (respiratory, urine and blood) should be carried out **before the administration of antimicrobials** in order to increase the likelihood of a microbiological diagnosis but initiation of treatment should not be delayed in severe cases.
Gram stains on sputum specimens may be used for determining the quality of the specimen. It may not be appropriate to identify organisms if gross contamination with oropharyngeal flora is evident. The main limitation is the difficulty to obtain good-quality, purulent sputum. Many pneumonia patients do not produce sputum, particularly older patients.
If the patient is immuno-competent, report poor quality or salivary specimens.
Gram Microscopy

Gram stain can also be used to predict the likely pathogens by their characteristic appearance.

Care must be taken in interpreting a Gram-stained sputum smear as the use of antimicrobials may render organisms, which are visible in the smear, non-viable.

All aspects of specimen appearance, Gram stain and culture together with the clinical condition of the patient need to be considered.
Culture remains a cornerstone of the diagnostic techniques, as it can provide information about antibiotic susceptibility.

The result of microbiologic investigations in the clinical setting of critically ill patients led to a change in therapy in approximately 40% of cases (and in 75% of patients in whom the etiology was identified).
A sterile culture from the lower respiratory tract of an intubated patient, in the absence of a recent change in antibiotic therapy, is strong evidence that pneumonia is not present, and an extrapulmonary site of infection should be considered.

ATS/IDSA. Am J Respir Care Med. 2005.
Samples of lower respiratory tract secretions should be obtained from all patients with suspected HAP, and should be collected before antibiotic changes. Samples can include an endotracheal aspirate, bronchoalveolar lavage sample, or protected specimen brush sample (Level II).

The specificity of bronchoscopy for the diagnosis of LRTI is not high because of contamination with the upper airway flora.

Diagnostic accuracy is improved by the use of a protected specimen brush (PSB) and BAL.

K. Loens. JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009,
The **diagnostic threshold** to discriminate infection from colonization **varies** with the technique used.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brush (PSB)</td>
<td>$\geq 10^3$</td>
<td>33 – 100%</td>
<td>50 – 100%</td>
</tr>
<tr>
<td>BAL</td>
<td>$\geq 10^4$</td>
<td>42 – 93%</td>
<td>45 – 100%</td>
</tr>
<tr>
<td>Endotracheal aspirate</td>
<td>$\geq 10^6$</td>
<td>38-82%</td>
<td>72-85%</td>
</tr>
</tbody>
</table>

More representative samples than the PSB, which samples only a single bronchial segment.

- ATS/IDSA. Am J Respir Care Med. 2005
### Clinical Management: Quantitative Cultures of Endotracheal Aspirates

<table>
<thead>
<tr>
<th>Feature or Organism</th>
<th>Management</th>
<th>Patients Who Received Invasive Management (n = 209)</th>
<th>Patients Who Received Clinical Management (n = 204)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative culture, n (%)</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Monomicrobial pneumonia, n (%)</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total number of pathogens, n (%)</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cocc, n (%)</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pneumonia</th>
<th>Neisseria species</th>
<th>Moraxella catarrhalis</th>
<th>Proteus species</th>
<th>Citrobacter species</th>
<th>Klebsiella species</th>
<th>Enterobacter species</th>
<th>Alcaligenes xylosoxidans</th>
<th>Stenotrophomonas maltophilia</th>
<th>Pseudomonas aeruginosa</th>
<th><em>Escherichia coli</em></th>
<th>Acinetobacter baumannii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coci, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>20 (16.5)</td>
<td>19 (15.7)</td>
<td>17 (13.8)</td>
<td>28 (22.0)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
<td>32 (25.8)</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
<td>17 (13.8)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
<td>27 (22.3)</td>
</tr>
<tr>
<td>Proteus species</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Citrobacter species</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Alcaligenes xylosoxidans</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
<td>5 (4.1)</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
<td>14 (11.4)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
<td>38 (30.4)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
<td>25 (20.4)</td>
</tr>
</tbody>
</table>

a strategy based on quantitative bronchoscopic specimen cultures has beneficial effects: improved early survival, fewer antibiotic failures, and less antibiotic use.
Blood cultures

- **Two blood cultures** should be obtained as early as possible in the disease and before any antibiotic treatment is started.
- A direct **correlation** was found between the **severity** of pneumonia and blood culture positivity rate.
- **S. pneumoniae** is identified in approximately 60% of positive blood cultures and **Haemophilus influenzae** in various percentages from 2 to 13%.

Multiplex PCR

The SeptiFast test is more sensitive in the detection of relevant blood pathogens in VAP than the blood culture.

VALUE OF LIGHTCYCLER SEPTIFAST® IN DETECTION OF VENTILATOR-ASSOCIATED PNEUMONIA
S. pneumoniae

Controllo
Rapid immunochromatographic test detecting the group C polysaccharide cell wall antigen common to all pneumococcal strains in urine and other biological fluids.

It can give positive results in healthy children with carriage of pneumococci and of other closely related Streptococcus species.

Thus, it is necessary to use this test in conjunction with other diagnostic modalities.

Sensitivity: 77%–88%
Specificity: 67%–100%

A. J. Blaschke. CID 2011:52 (Suppl 4)
Persistence of *S. pneumoniae* urinary antigen excretion after pneumococcal pneumonia

![Graph showing persistence of *S. pneumoniae* urinary antigen excretion](image)

Fig. 1 Distribution of positive (black) and negative (white) results of *S. pneumoniae* urinary antigen detection in NCU samples collected during the study period.

Investigations for legionella pneumonia are recommended for all patients with high severity CAP, for other patients with specific risk factors and for all patients with CAP during outbreaks.

This assay principally detects infection with *L. pneumophila* serogroup 1.

Antigenuria can be detected as early as 1 day after onset of symptoms and persists for days to weeks.
**Legionella PCR** combined with urinary antigen testing is likely to be the best initial testing strategy that will detect all *Legionella* species and provide results within a time frame that will affect clinical management.

D. R. Murdoch. MEDICAL MICROBIOLOGY • CID 2003:36
Atypical pathogens

- *Mycoplasma pneumoniae:* younger patients, prior antibiotics, less multisystem involvement.
- *Chlamydia pneumoniae:* longer duration of symptoms before hospital admission, headache
- *M. pneumoniae,* and to a lesser extent *C. pneumoniae,* may precipitate an attack of asthma or exacerbate existing asthma.
- Some patients who have recently had *M. pneumoniae* CAP develop post-CAP asthma which may be permanent.

Atypical pathogens

• The outpatient setting is the area where atypical pathogens are quantitatively more important than their typical CAP counterparts.
• The atypical pneumonias require a different therapeutic approach than that for typical CAPs.
• The treatment of *M. pneumoniae* and *C. pneumoniae* CAP is important, not because of the severity of the illness, but if for no other reason, to decrease communicability and to decrease post-CAP asthma

Atypical pathogens

Given the high sensitivity and specificity of nucleic acid amplification techniques (NAATs), NAATs are the preferred diagnostic procedures for the diagnosis of *M. pneumoniae* and *C. pneumoniae*

The best specimen for *M. pneumoniae* and *C. pneumoniae* detection are nasopharyngeal aspirate or nasopharyngeal swab since inhibitors in sputum occur frequently and may be difficult to eliminate

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.
No clinical algorithm exists to discern clearly the cause of pneumonia.

Respiratory viruses usually follow seasonal patterns of activity and are most likely to cause pneumonia during those times.
The knowledge of which virus or viruses is/are present and who has had close contact may guide for prophylaxis or use of antiviral agents.

- If a child with chronic cardiac disease has an increased risk of catching RSV, then palivizumab can be administered to prevent more severe complications from RSV infection.

- The treatment of the influenza virus infection increase survival in persons who are at high risk for complications and mortality during influenza.

It is clear that rapid and accurate diagnosis is central to such therapy decisions.

Optimal samples in viral pneumonia

- In children, for detection by PCR of respiratory viruses nasopharyngeal aspirates are generally deemed the specimen of choice because both nasal and nasopharyngeal mucus samples are gathered.
- In adults, transnasal nasopharyngeal flocked swabs also have high virus detection rates.
- Lower-respiratory specimens have obvious advantages for establishing the cause of pneumonia because they come from the site of infection.

UTM-RT medium are well suited for the detection of respiratory viruses by PCR.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Sample ranking</th>
<th>Method</th>
<th>Age (yr)</th>
<th>Total no. of specimens/ no. of patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum &gt; TW &gt; NPS &gt; OPS</td>
<td>PCR</td>
<td></td>
<td>20-93</td>
<td>552/144</td>
<td>31</td>
</tr>
<tr>
<td>OPS &gt; NPS</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>132/66</td>
<td>43</td>
</tr>
<tr>
<td>OPS &gt; BAL &gt; sputum</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>325/197</td>
<td>49</td>
</tr>
<tr>
<td>Sputum &gt; OPS</td>
<td>Gene-probe test</td>
<td></td>
<td>&gt;18</td>
<td>160</td>
<td>57</td>
</tr>
<tr>
<td>Sputum &gt; NPA</td>
<td>Ag-EIA</td>
<td></td>
<td>&gt;18</td>
<td>102/51</td>
<td>56</td>
</tr>
<tr>
<td>Sputum &gt; OPS</td>
<td>Culture, PCR, NASBA</td>
<td></td>
<td>NSp</td>
<td>302/180</td>
<td>61, 62</td>
</tr>
<tr>
<td>NPS &gt; OPS</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>63</td>
<td>69</td>
</tr>
<tr>
<td>Sputum &gt; NPA → OPS</td>
<td>PCR</td>
<td></td>
<td>22-29</td>
<td>96/32</td>
<td>79</td>
</tr>
<tr>
<td>OPS &gt; NPA</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>102</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPS &gt; TS</td>
<td>Culture, PCR</td>
<td></td>
<td>3-12</td>
<td>260</td>
<td>10</td>
</tr>
<tr>
<td>Sputum &gt; NPS &gt; OPS</td>
<td>PCR, culture</td>
<td></td>
<td>3-79</td>
<td>319/129</td>
<td>12</td>
</tr>
<tr>
<td>OPS &gt; NPS</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>132/66</td>
<td>43</td>
</tr>
<tr>
<td>Sputum &gt; NPA &gt; OPS</td>
<td>PCR</td>
<td></td>
<td>NSp</td>
<td>105/35</td>
<td>58</td>
</tr>
<tr>
<td>NPS &gt; OPS &gt; sputum</td>
<td>PCR</td>
<td></td>
<td>20-93</td>
<td>468/156</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS &gt; NPA</td>
<td>DFA</td>
<td></td>
<td>0-5</td>
<td>910/455</td>
<td>1</td>
</tr>
<tr>
<td>NPA &gt; NPS</td>
<td>PCR</td>
<td></td>
<td>0-18</td>
<td>338/169</td>
<td>19</td>
</tr>
<tr>
<td>NPA &gt; NPS</td>
<td>PCR</td>
<td></td>
<td>0-18</td>
<td>338/169</td>
<td>19</td>
</tr>
<tr>
<td>NPA &gt; NPS</td>
<td>PCR</td>
<td></td>
<td>0-16</td>
<td>250/125</td>
<td>38</td>
</tr>
<tr>
<td>NPA &gt; NPS</td>
<td>DFA</td>
<td></td>
<td>0-2</td>
<td>366/183</td>
<td>64</td>
</tr>
<tr>
<td>NPA &gt; NPS</td>
<td>Culture, Ag-EIA, FAT</td>
<td></td>
<td>0-18</td>
<td>242/121</td>
<td>67</td>
</tr>
<tr>
<td>Adenovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS &gt; NPA</td>
<td>DFA</td>
<td></td>
<td>0-5</td>
<td>910/455</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza virus 1, 2, or 3</td>
<td>PFS &gt; NPA</td>
<td>DFA</td>
<td>0-5</td>
<td>910/455</td>
<td>1</td>
</tr>
<tr>
<td>Picornaviruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum &gt; NS &gt; OPS</td>
<td>Culture</td>
<td></td>
<td>5-15</td>
<td>66/22</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS &gt; NPA</td>
<td>DFA</td>
<td></td>
<td>0-5</td>
<td>910/455</td>
<td>1</td>
</tr>
<tr>
<td>NPS &gt; NS &gt; NPA</td>
<td>Quidel quickvue</td>
<td></td>
<td>0-18</td>
<td>366/122</td>
<td>2</td>
</tr>
<tr>
<td>NPF5 &gt; NPA</td>
<td>PCR</td>
<td></td>
<td>0-18</td>
<td>338/169</td>
<td>19</td>
</tr>
<tr>
<td>NPA &gt; NPF5</td>
<td>DFA</td>
<td></td>
<td>0-18</td>
<td>338/169</td>
<td>19</td>
</tr>
<tr>
<td>Sputum &gt; NA &gt; NPS &gt; OPS</td>
<td>FLU OIA test</td>
<td>Culture</td>
<td>0-76</td>
<td>403/184</td>
<td>24</td>
</tr>
<tr>
<td>NA &gt; sputum &gt; NPS &gt; OPS</td>
<td>Culture</td>
<td></td>
<td>0-76</td>
<td>403/184</td>
<td>24</td>
</tr>
<tr>
<td>NPS &gt; NPA &gt; OPS</td>
<td>PCR, Directigen Flu A+B</td>
<td></td>
<td>61-97</td>
<td>85/47</td>
<td>44</td>
</tr>
<tr>
<td>NPS &gt; OPS</td>
<td>Binax Now, Directigen Flu A+B, DIF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All viruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPA &gt; NS &gt; OPS</td>
<td>PCR</td>
<td></td>
<td>0-16</td>
<td>221/178</td>
<td>46</td>
</tr>
<tr>
<td>NPS &gt; NA &gt; unpreserved saline</td>
<td>PCR</td>
<td></td>
<td>0-1.5</td>
<td>543/181</td>
<td>105</td>
</tr>
<tr>
<td>NPA &gt; NS</td>
<td>PCR, culture</td>
<td></td>
<td>≤5</td>
<td>950/475</td>
<td>97</td>
</tr>
</tbody>
</table>

* BAL, bronchoalveolar lavage; DIF, direct immunofluorescence; DFA, direct fluorescent antibody assay; Ag-EIA, antigen enzyme immunoassay; IFA, indirect fluorescent antibody test; FAT, fluorescent antibody test; NA, nasal aspirate; NASBA, nucleic acid sequence-based amplification; NPA, nasopharyngeal aspirate; NPF5, nasopharyngeal flocked swab; NPS, nasal flocked swab; NPS, nasopharyngeal swab; NW, nasal wash; NSp, not specified; NS, nasal swab; OPS, oropharyngeal swab; PFS, purinase flocked swab; TW, throat wash. (Adapted from reference 111 with permission of the publisher.)
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Adenovirus A/B/C/D/E
Coronavirus 229E/NL63

Coronavirus OC43
Rhinovirus A/B/C
Influenza A virus
RSV A
RSV B

Multiplex PCR
Single gene target PCR

Bocavirus 1/2/3/4
Influenza B virus
Metapneumovirus
Parainfluenza virus 4
Enterovirus
<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>CT</th>
<th>EndFl</th>
<th>Analyte Result</th>
<th>Probe Check Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A</td>
<td>33.4</td>
<td>222.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>2009 H1N1</td>
<td>0.0</td>
<td>16.0</td>
<td>NEG</td>
<td>PASS</td>
</tr>
<tr>
<td>Flu B</td>
<td>0.0</td>
<td>2.0</td>
<td>NEG</td>
<td>PASS</td>
</tr>
<tr>
<td>SPC</td>
<td>23.1</td>
<td>425.0</td>
<td>NA</td>
<td>PASS</td>
</tr>
</tbody>
</table>
Assay Name: Xpert Flu G2

Test Result:
- Flu A NEGATIVE
- 2009 H1N1 NOT DETECTED
- Flu B POSITIVE

For In Vitro Diagnostics Use Only.
Detection of several viruses

- For childhood pneumonia, **two or three viruses** have been detected in 10–20% of children.
- Specifically, human bocavirus is detected frequently in association with other respiratory viruses.
- Detection of a virus in the nasopharynx could represent coincidental upper-respiratory infection or a pneumonia pathogen?
- In one study, **viral co-infection** was associated with more severe pneumonia than with monoinfection. Rates of admission were looked at
## Bacterial co-infection in influenza A H1N1 Pneumonia

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of patients</th>
<th>Blood culture</th>
<th>Sputum culture</th>
<th>Urinary antigen</th>
<th>BAL/BAS</th>
<th>Pleural effusion culture</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 42)</td>
<td>(n = 38)</td>
<td>(n = 16)</td>
<td>(n = 39)</td>
<td>(n = 9)</td>
<td>(n = 3)</td>
<td>(n = 30)</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>26 (62)</td>
<td>3 (7.8)</td>
<td>7 (43.7)</td>
<td>24 (61.5)</td>
<td>3 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>1 (2)</td>
<td>--</td>
<td>1 (6.3)</td>
<td>--</td>
<td>--</td>
<td>1 (33.3)</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>2 (5)</td>
<td>--</td>
<td>2 (12.5)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>3 (7)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>1 (2)</td>
<td>--</td>
<td>1 (6.3)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>C. burnetti</td>
<td>1 (2)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1 (3.3)</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>1 (2)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1 (11.1)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>6 (14)</td>
<td>--</td>
<td>4 (25.0)</td>
<td>--</td>
<td>5 (55.5)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>1 (2)</td>
<td>1 (2.6)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

---

TAT diagnosi polmonite

Giorno 1
- GRAM 1 h
- Antigen test 1/2 h
- Sepsimolecolare 4 h
- PCRmultiplex 15 Virus 5 h
- Mycoplasma, Chlamydophila, Legionella 3 h
- Emocoltura 3-4 gg

Giorno 2
- Es colturale 1-2 gg

Giorno 3
- Antibiogramma 1-2 gg

Giorno 4
- PCRmultiplex 15 Virus
BUON NATALE 2012 E FELICE 2013