Rapid Detection of Respiratory Viruses: Why?

• Early diagnosis, proper care, cohorting
  - reduced hospital admission/stay
  - prevention of nosocomial spread
• Appropriate use of targeted antiviral drugs
• Reduction of inappropriate use of antibiotics
• Epidemiological information
Rapid Detection of Respiratory Viruses: When and How?

Which viruses? When?
• influenced by knowledge on etiologic agents
• influenced by the age of the subject population
• influenced by clinical severity
  - URTI
  - LRTI
• influenced by the environment
  - community
  - hospitalized
  - long term care facilities
**Viral etiology of Respiratory Tract Infections**

- In all studies the etiologic diagnosis for a considerable proportion of cases is unknown.
- The discovery of each new virus represents a missing piece of the puzzle of respiratory infections:
  - Tecumseh family study: 1965-1971
    only 25% of cases
    Monto Am J Med 2002; 112: 43
  - CAP in children: 1992
    44% of cases: no rhinovirus detection
    Ieven et al. J Infect Dis. 1996; 173: 1445
  - CAP in children: 1999
    62% of cases: rhinovirus 24%
# Viral Causes of Community-Acquired Pneumonia in Otherwise Healthy Children

<table>
<thead>
<tr>
<th>Common</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>Varicella-zoster virus</td>
</tr>
<tr>
<td>Influenza A and B</td>
<td>Coronavirus</td>
</tr>
<tr>
<td>Parainfluenza viruses 1, 2 and 3</td>
<td>entroviruses (Coxsackie and echo)</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>Rhinoviruses</td>
<td>EBV</td>
</tr>
<tr>
<td>hMPV</td>
<td>Mumps</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex (newborn)</td>
</tr>
</tbody>
</table>

McIntosh K. N.  *Engl J Med* 2002; 346: 419
## Microbial Etiology (%) of Adult Community-Acquired Pneumonia

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Community&lt;sup&gt;a&lt;/sup&gt; (n = 236)</th>
<th>Hospital&lt;sup&gt;b&lt;/sup&gt; (n = 1137)</th>
<th>Intensive Care&lt;sup&gt;c&lt;/sup&gt; (n = 185)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>in UK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>36.0</td>
<td>39.0</td>
<td>21.6</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>10.2</td>
<td>5.2</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>1.3</td>
<td>10.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Viral</td>
<td>13.1</td>
<td>12.8</td>
<td>9.7</td>
</tr>
</tbody>
</table>


| **in Belgium**                    |                              |                                 |                                     |
|-----------------------------------|------------------------------|---------------------------------|                                     |
| *Streptococcus pneumoniae*        | 27.3                         | 35.0                            |                                     |
| *Haemophilus influenzae*          | 2.3                           | 4.8                             |                                     |
| *Mycoplasma pneumoniae*           | 18.2                          | 6.8                             |                                     |
| Viral                             | 12.2                          | 16.7                            |                                     |

Ieven et al. ECCMID 2004

<sup>a</sup>On study [3], <sup>b</sup>Five studies, <sup>c</sup>Four studies
# RSV Infections in Long Term Care Facilities

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Yr</th>
<th>Study method</th>
<th>No. of RSV</th>
<th>Methods of diagnosis</th>
<th>% of cases</th>
<th>Pneum.</th>
<th>Death</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC</td>
<td>1977</td>
<td>Outbreak</td>
<td>15</td>
<td>CF</td>
<td>47</td>
<td>40</td>
<td></td>
<td>Several employees ill</td>
</tr>
<tr>
<td>Mathur et al.</td>
<td>1980</td>
<td>Prospective</td>
<td>8</td>
<td>Culture, CF</td>
<td>25</td>
<td>0</td>
<td></td>
<td>Concurrent influenza A outbreak</td>
</tr>
<tr>
<td>Morales et al.</td>
<td>1983</td>
<td>Prospective</td>
<td>12</td>
<td>Culture, CF</td>
<td>16</td>
<td>5</td>
<td></td>
<td>All RSV cases had LRTI</td>
</tr>
<tr>
<td>Sorvillo et al.</td>
<td>1984</td>
<td>Outbreak</td>
<td>40</td>
<td>Culture, CF</td>
<td>55</td>
<td>20</td>
<td></td>
<td>High rate of radiographic pneumonia</td>
</tr>
<tr>
<td>Agius et al.</td>
<td>1990</td>
<td>Outbreak</td>
<td>52</td>
<td>CF, IFA, WB</td>
<td>42</td>
<td>12</td>
<td></td>
<td>Pharyngitis, gastrointestinal complaints uncommon</td>
</tr>
<tr>
<td>Falsey et al.</td>
<td>1992</td>
<td>Prospective</td>
<td>40</td>
<td>Culture, EIA*</td>
<td>10</td>
<td>5</td>
<td></td>
<td>Clear clustering on floors</td>
</tr>
<tr>
<td>Orr et al.</td>
<td>1996</td>
<td>Prospective</td>
<td>3</td>
<td>CF</td>
<td>33</td>
<td></td>
<td></td>
<td>Only febrile illnesses</td>
</tr>
</tbody>
</table>

---

*CDC, Centers for Disease Control  
*CF, complement fixation serology  
*WB, Western blot  
*greater than fourfold rise in titre required for diagnosis

Respiratory Viruses in the Elderly

- RSV, Influenza and parainfluenza: prospective Belgian study
  - only hospitalized patients: 26 %
  - 26 % Flu > 3.6 % RSV > 1.2 % PIV

- RSV and Influenza: prospective study 1999-2002
  - healthy elderly (n=396): 3.3 % Flu < 9.8 % RSV
  - hospitalized elderly (n=1139): 12.0 % Flu > 9.4% RSV

⇒ RSV is more prevalent, Flu is more severe
Falsey A, ICAAC 2003
Respiratory Virus Infections in Transplant Patients

• Significant viruses in URTI and LRTI in lung transplants:
  - total 27 % of patients: Flu > RSV > PIV

• Significant cause of pneumonia in hematological cancers
  - total 35 % of patients: RSV > Rhino > Influenza > PIV

• Human coronavirus increasingly important: 11 %
Importance of Rhinoviruses in Respiratory Infections

• Cause of pneumonia in hematopoetic stem cell transplant recipients:
  - 8% of cases
  - 11.6% of cases of which 30% more by PCR

• Chronic obstructive pulmonary disease: 39% of viral-proven illnesses

• Respiratory illness in elderly: most prevalent pathogen (53%):
  65% had lower respiratory tract symptoms

• Common cause of bronchitis (25%), acute attacks of asthma (6%) and pneumonia in children (2->10%)
  Papadopoulos N. Ped Resp Rev. 2004; 5: S191-95
human Metapneumovirus (hMPV)

- newly discovered respiratory virus
- reported in the Netherlands, Australia, Canada, UK, France, Finland, USA
- hospitalised adults and children, outpatients elderly
- closely related to avian pneumovirus
- infects ferrets, guinea pigs and primates (not birds)
- widespread seroconversion by 5 years of age, including sera from 1953

V d Hoogen et al, Nature Medicine, 2001
hMPV as Etiologic Agent in RTI

- Nashville, USA: Pediatric clinic
- Study period: 1976-2001: 1127 LRTI
- Patient population: < 5 yrs : 687 NPA
  - LRTI: hMPV : 12%
  - RSV : 15%
  - PFI : 10%
  - Influenza : 5%
  - Adeno : 4%
- URTI hMPV : 15%

Viral Etiologies in RTI: Are they Important?

- General population: 10-13% in LRTI
- In children: up to 40%: mostly RSV, hMPV, rhino and influenza
- Elderly in the community: RSV and Rhinovirus
- Elderly in hospitals: Influenza predominant
- hMPV in 10-15% of LRTI and URTI
- Rhino- and coronaviruses are important in immunocompromised patients and transplant patients
Rapid Detection of Respiratory Viruses: When and How?

How?
- Influenced by specimen type
- Influenced by patient population
- Detection methods
  - Culture
  - Rapid tests: EIA, OIA, immunofluorescence (IF)
  - Serology
  - Amplification tests:
    - Traditional and real-time
    - Single and multiplex
    - Commercial and in-house
Effect of Patient-related Variables on Detection rates of the Influenza A Virus-specific RT-PCR, Virus Isolation, and ELISA

Influence of Patient Population on Virus Detection

- Rapid antigen tests for RSV in adults:
  - RSV positive by any rapid test: 32 % (BD, DFA, Vidas)
  

- DFA-EIA for the detection of influenza A:
  - NP swabs adults: DFA: 90 %
    EIA: 66 %
  - NPA children: DFA = EIA

⇒ Rapid Antigen tests in adults: of limited value!

Influence of Clinical Specimen Types on the Detection of Influenza A and B Viruses by OIA

NA, nasal aspirate; NPS, nasopharyngeal swab; TS, throat swab; SP, sputum;

Serology for the Diagnosis of Viral RTI

- Rarely helpful in diagnosis of acute infection:
  - IgG: only 4 fold rise between acute and late phase serum specimens are informative: denotes past infection
  - IgM may appear late or not at all: 10 to 30% of patients with documented infections remain serologically negative

- Useful in epidemiologic studies
- Useful in vaccine studies

Henrickson K. Ped Infect Dis 2004; 23: S6-10
Tissue Culture for Respiratory Virus Diagnosis

- Rapid culture assays:
  - centrifugation cultures (shell vials): take 4-5 days for positive results; multiple cell lines needed
  - R-Mix shell vials: mixture of mink lung cells and A 549:
    TAT improved: 1.4 d vs 5.2 d for $\oplus$ 60-100% $\oplus$ at day 1
    Barenfanger J Clin Virol 2002; 24: 107

- Important regionally: source for analysing genetic/antigenic change in virus populations
- Important for discovering new unknown viruses (hMPV)
CPE voor Rhinovirus op MRC5 cellen
Antigen-based Rapid Diagnostic Assays Compared with Tissue Culture

- Technically less complex
- Less sensitive than other methods
  - Median sensitivity: e.g. Zstat Flu 69%
  - Directigen Flu A + B 87%, Flu OIA 72%, RSV OIA 88%
  - Quick Vue Flu 79%, Testpack RSV 70%
- Sensitivity depends on specimen type and is different for NPA, nasal wash, throat swab

Henrickson K. Ped Infect Dis J 2004; 23:S6-10
Antigeen detectie door latex agglutinatie
Antigeendetectie door immunochromatografische methodes

**INTERPRETATION OF RESULTS**

**POSITIVE**

**NEGATIVE**

**INVALID**
Immunofluorescence Based Diagnostic Assays

• Individual or pooled monoclonal antibodies: Flu A/B, PU 1-3, RSV, adenovirus

• Sensitivity generally higher than other rapid tests
  - Flu A : 40-90%
  - Flu A+B : 60-90%
  - RSV : 94%
  - PIV : 70-80%
  - Adenovirus : 22-67%

Henrickson K. Ped Infect Dis J 2004; 23:S6-10
Immunofluorescence RSV
## Immunofluorescence

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>Low throughput</td>
</tr>
<tr>
<td>Several simultaneous tests</td>
<td>Limited scope</td>
</tr>
<tr>
<td>Feed back on specimen quality</td>
<td>Depends on specimen quality</td>
</tr>
<tr>
<td>Certainty</td>
<td>Requirement for individual skill</td>
</tr>
<tr>
<td>Clinical significance</td>
<td>Novel viruses</td>
</tr>
<tr>
<td>Robustness</td>
<td>Reagents</td>
</tr>
<tr>
<td>Cost effectiveness</td>
<td></td>
</tr>
</tbody>
</table>

Viral RT-PCRs

- In house PCRs: single - multiplex - real time
- Commercially available
  - Single PCR
    Chemicon Flu A/B, adenovirus, RSV Artus (Germany): Sars coronavirus, DNA technology (Denmark): Flu A
  - Multiplex PCR
    Prodesse: Flu A+B, RSV A+B, Parainfluenza 1,2,3; adenovirus; SARS, OC43 coronavirus, E 229 coronavirus
  - Real time PCR
    Prodesse RSV A, B; adenovirus; flu A,B, parainfluenza 1, 2,3; RSV/Flu/para
### Viral Detection by RT-PCR and by Conventional Culture

<table>
<thead>
<tr>
<th>Detection by RT-PCR</th>
<th>RSV</th>
<th>Influenza A and B</th>
<th>Parainfluenza 1-3</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>44</td>
<td>78</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>543</td>
<td>1</td>
<td>645</td>
</tr>
</tbody>
</table>

Note. The ratio of the no. of specimens positive by RT-PCR to the no. of specimens positive by culture was 2.1:1 for all combined (P<0.0001)

Comparison of Tissue Culture and PCR for the Detection of Rhinoviruses

<table>
<thead>
<tr>
<th>Reference</th>
<th>No specimens</th>
<th>% Positive by TC</th>
<th>% Positive by PCR</th>
<th>Rate PCR +/- TC +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnston S. 1993</td>
<td>292</td>
<td>15.8</td>
<td>50.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Freymuth F. 1997</td>
<td>277</td>
<td>3.9</td>
<td>12.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Arruda E. 1997</td>
<td>346</td>
<td>66.8</td>
<td>81.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Hyypia T. 1998</td>
<td>200</td>
<td>63.5</td>
<td>93.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Andeweg A. C. 1999</td>
<td>1070</td>
<td>6.0</td>
<td>24.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Blomqvist S. 1999</td>
<td>203</td>
<td>25.6</td>
<td>48.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Loens K. 2004</td>
<td>552</td>
<td>3.6</td>
<td>18.1</td>
<td>5.02</td>
</tr>
</tbody>
</table>

* RT-PCR and/or NASBA
# Sensitivity, Specificity, and Positive and Negative Predictive Value of Hexaplex, Tissue Culture, and EIA for RSV A and B and Flu A

<table>
<thead>
<tr>
<th>Virus</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total RSV</td>
<td>Tissue culture</td>
<td>74.3</td>
<td>100</td>
<td>100</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>TestPack RSV</td>
<td>70.3</td>
<td>100</td>
<td>100</td>
<td>92.5</td>
</tr>
<tr>
<td></td>
<td>Hexaplex</td>
<td>98.6</td>
<td>97.4</td>
<td>91.2</td>
<td>99.6</td>
</tr>
<tr>
<td>Flu A</td>
<td>Tissue culture</td>
<td>83.3</td>
<td>100</td>
<td>100</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>Directigen Flu A</td>
<td>69.4</td>
<td>100</td>
<td>100</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>Hexaplex</td>
<td>95.8</td>
<td>98.7</td>
<td>92.0</td>
<td>99.3</td>
</tr>
</tbody>
</table>

NASBA Assays for the Detection of Respiratory Viruses

- RNA amplification method (bioMérieux)
  - ECL detection or molecular beacons
  - kit for extraction, amplification, detection (Nuclisense Basic Kit)
  - primers and probes to be synthesized
- Flu A/B, PI 1-4, RSV, adeno
  - single test or multiplex
- Analytical sensitivity: 0.01-100 TCD 50/ml
- Clinical evaluation ongoing

Loens K et al. J Clin Microbiol. 2003; 41: 1971-76
Comparison of Culture - NASBA and PCR for the Detection of Rhinoviruses

• Specimens tested: 520 NPA from children admitted at the Univ. Hosp. Antwerp
• Methods
  - NASBA: 5’NCR primers + ECL detection
  - RT-PCR: 5’NCR primers + agarose detection
• Results

<table>
<thead>
<tr>
<th></th>
<th>EGS</th>
<th>LCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASBA</td>
<td>sens: 87.2%</td>
<td>82.1 %</td>
</tr>
<tr>
<td></td>
<td>spec: 98.3 %</td>
<td>99.8 %</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>sens: 85.1 %</td>
<td>77.9 %</td>
</tr>
<tr>
<td></td>
<td>spec: 93.4 %</td>
<td>94.5 %</td>
</tr>
</tbody>
</table>

⇒ NASBA is more sensitive than RT-PCR
⇒ by NASBA and/or PCR: 5 x more rhino’s than by culture.

Loens et al. 2004 (unpublished)
Comparison of Conventional or Shell Vial Culturing, In-house Nested PCR, and Real-Time PCR

• RSV detection in 411 NT swabs 1999-2002
  ⇒ Real-time PCR: equally sensitive as nested PCR
  ⇒ Real-time PCR: 70% more sensitive than culture

• RSV detection in 71 NPA 2002-2003
  ⇒ Real-time PCR:
  60% more sensitive than Ag ELISA
  25% more sensitive than nested RT-PCR
Detection of Human Coronavirus (HCoV) by Real-time RT-PCR and/or Nested RT-PCR

• Nasal wash in URTI/LRTI
  ⇒ 16.3% real-time PCR positive versus 11.6% in nested RT-PCR

• Nose and throat swabs in
  ⇒ URTI / LRTI: 6.6% positive in real-time PCR
  ⇒ with pneumonia: 15.4% positive

• BAL
  ⇒ with pneumonia: 18.2% positive

Amplification Methods for the Detection of Viruses

• **Advantages**
  - improved sensitivity versus conventional diagnosis
  - rapid results especially for the real-time formats
  - simultaneous detection of several viruses

• **Disadvantages**
  - TAT compared to direct specimen testing and IF
  - requirement for special infrastructure and equipment
  - requirement for individual skill and expertise
  - some tests are labor-intensive
  - expensive
## Comparison of Available Methods for Virus Diagnosis

<table>
<thead>
<tr>
<th>Method</th>
<th>Approx. Time to diagnosis</th>
<th>Skill factor</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunofluorescence</td>
<td>1 - 3 h</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Enzyme immunoassay</td>
<td>30 min - 1h</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>10 min</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Culture</td>
<td>overnight-weeks</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Nucleic Acid Amplification</td>
<td>2 - 3 h</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>15 min - 1h</td>
<td>High</td>
<td>Medium</td>
</tr>
</tbody>
</table>

Questions

- What is the diagnostic yield of rapid molecular amplification methods to identify viral pathogens in adult patients admitted to the hospital because of lower respiratory tract infection?

- Is the application of such methods in routine diagnostic investigation of these patients cost-effective?

Van Loon AM et al, 2004
Patient population
- Adult patients admitted because of community acquired LRTI and being given antibiotic treatment
- Two diagnostic arms: one with results in 24-48 hrs allowing rapid withdrawal of antibiotic treatment
- University Medical Centre Utrecht, Diakonessenhuis Utrecht
- Period November 2002 – March 2004
- Total number of patients included: \( n = 107 \)

Van Loon AM et al, 2004
Cost-Effectiveness of Real-time PCR to identify respiratory viruses in patients hospitalised with lower respiratory tract infection

PCR applied for:

**Viruses:**
Adenovirus, Human Coronavirus, Influenza virus A/B, Parainfluenza 1-3, RS-virus A/B, Rhinoviruses

**Bacteria:**
*Chl. Pneumoniae, Leg. Pneumophila, Myc. pneumoniae*
Six (5.6%) patients died: 4 with unknown etiology, 2 with a virus infection (RSV, HuCoV).

In 25 (23.4%) patients a virus was the only pathogen detected.

Following PCR results, antimicrobial treatment was modified in 6/55 patients (10.9%): 

- Cessation in 2 patients
- Adaptation in 4 patients (exclusion of atypical pathogens, influenzavirus A)

Van Loon AM et al, 2004
Cost-Effectiveness of Real-time PCR to identify respiratory viruses in patients hospitalised with lower respiratory tract infection: CONCLUSIONS

• Real-time PCR increased the diagnostic yield from 22.4% to 50.4%.
• Antimicrobial treatment was continued in 23/25 (92%) patients in whom only a viral pathogen was detected.
• In the present study real-time PCR was not cost-effective.
• Need for major reduction of cost:
  - Selective use of real-time PCR: limit to Flu (season) and HuCoV, low CRP, absence of infiltrate.
  - Reduction of reagent/equipment cost.
  - New technology: DNA chips.
• Is cessation of antibiotic treatment possible if only a viral pathogen is detected?

Van Loon AM et al, 2004
Rapid Viral Diagnosis in RTI: When to Implement?

- Infections leading to hospital admissions or prolong hospital stay
  - moderate to severe LRTI
  - fever syndrome (e.g. young infants, immunocompromised)
- Stop inappropriate antibiotic use
- Direct antiviral use
- Decrease unnecessary diagnostic studies.
- Epidemiologic surveillance for specific population groups
- Outbreaks in selected populations.
- Research: to complete the missing pieces in the etiologic puzzle.
Rapid Testing for Respiratory Viruses: Conclusions

- Laboratory diagnosis is clinically useful in some but not all cases: guidelines are needed
- There is no single perfect test or approach
- Issues to consider:
  - patient population: adults/children, immune status, in- or outpatients
  - TAT versus sensitivity
  - available resources
Detection of

*Mycoplasma pneumoniae*

and *Chlamydia pneumoniae*
Etiology of CAP in Adult Hospitalized + Non-Hospitalized Patients

- **S. pneumoniae**
- **M. pneumoniae**
- **C. pneumoniae**
- **Legionella species**

<table>
<thead>
<tr>
<th>Study</th>
<th>Geographical Region</th>
<th>Hospitalization Rate</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almirall / Eur Resp J 6: 14, 1993</td>
<td>Spain (105)</td>
<td>50.1% (hospitalized)</td>
<td>C. pneumoniae: not reported</td>
</tr>
<tr>
<td>Blanquer / Thorax 46: 508, 1991</td>
<td>Spain (510)</td>
<td>91.1% (hospitalized)</td>
<td>M. pneumoniae: not reported</td>
</tr>
<tr>
<td>Dunbar / ICAAC 2000 Abst N°595 (% not reported)</td>
<td>Multinational (1600)</td>
<td>S. pneumoniae: not reported</td>
<td></td>
</tr>
<tr>
<td>File / AAC 41: 1965, 1998 (47% hospitalized)</td>
<td>USA (456)</td>
<td>USA (217)</td>
<td>M. pneumoniae: not reported</td>
</tr>
<tr>
<td>Marrie / Am J Med 101: 508, 1996 (5.4% hospitalized)</td>
<td>Canada (149)</td>
<td>S. pneumoniae: not reported</td>
<td></td>
</tr>
<tr>
<td>Ramirez / ICAAC 2000 Abstr N°592 (73% hospitalized)</td>
<td>Multinational (1600)</td>
<td>S. pneumoniae: not reported</td>
<td></td>
</tr>
</tbody>
</table>

- **Spain (105)**
- **USA (456)**
- **USA (217)**
- **Canada (149)**
- **Multinational (1600)**
Mycoplasma pneumoniae: Diagnosis

- Direct examination:
  - Gram stain impossible
  - Immunofluorescence?

- Culture:
  - Slow (2 to 6 weeks)
  - Technical demanding
  - Insensitive (30 to 60%)

- Serology:
  - Late results
  - Specificity?
**Mycoplasma pneumoniae** : Culture versus PCR

<table>
<thead>
<tr>
<th></th>
<th>PCR +</th>
<th>PCR -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture +</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Culture -</td>
<td>15</td>
<td>359</td>
<td>364</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>359</td>
<td>371</td>
</tr>
</tbody>
</table>

Culture:
- **Sensitivity**: $\frac{7}{12} = 58.3\%$
- **Specificity**: $\frac{359}{359} = 100\%$
PCR, Culture and Serology for *M. pneumoniae*

- 92 children with respiratory infection, 74 controls
- diagnostic criteria: culture + and/or CFT $\geq 128$ at 2-3 weeks
  
  CFT: 4x rise

<table>
<thead>
<tr>
<th>Cases</th>
<th>Culture +</th>
<th>PCR+</th>
<th>CFT+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture +</td>
<td>PCR+</td>
<td>CFT+</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Culture +</td>
<td>PCR+</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PCR+</td>
<td>CFT+</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFT+</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>controls</th>
<th>PCR- Culture - CFT -</th>
</tr>
</thead>
</table>

PCR+ : 7/9 sensitivity 77.8 %

combined PCR + CFT : $\uparrow$ sens.

CFT+ : 7/9 sensitivity 77.8 %

IgM IFA : sensitivity 79 % spec. 92 % PPV 50% → not useful

### Mycoplasma pneumoniae

**Sensitivity and Specificity for Techniques Compared to Conventional PCR**

<table>
<thead>
<tr>
<th>Test</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>(no. of samples) (n=106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time PCR</td>
<td>100</td>
<td>100</td>
<td>11.3 (12)</td>
</tr>
<tr>
<td>NASBA</td>
<td>100</td>
<td>100</td>
<td>11.3 (12)</td>
</tr>
<tr>
<td>Serodia (acute-phase sample)</td>
<td>50</td>
<td>100</td>
<td>5.6 (6)</td>
</tr>
<tr>
<td>Serodia (convalescent-phase sample)</td>
<td>66</td>
<td>100</td>
<td>7.5 (8)</td>
</tr>
<tr>
<td>CFT</td>
<td>75</td>
<td>100</td>
<td>8.5 (9)</td>
</tr>
</tbody>
</table>

*See reference 16 for details on conventional PCR.*

Serology for *M. pneumoniae*

- CAP study in Leiden: 11 serological tests evaluated on ± 100 patient samples (34 paired sera)
  - sensitivity of IgM: 7-23% in first 6 days
  - 29-86% after more than 16 days
  - IgG seroconversion or ↑ rise in titre: in 47-63% of PCR positives

  Beersma et al, ECCMID 2004

- CAP + LRTi: study in Antwerp: 4 different tests evaluated on 224 patients (205 paired sera)
  - sensitivity of IgM: 10-31% in first 6 days
  - 20-42% after more than 16 days
  - IgG seroconversion or ↑ rise in titre: 41-68% of positives

  Loens et al, unpublished
Chlamydia pneumoniae: Clinical Manifestations

- Conjunctivitis - trachoma?
- Respiratory infections
  - pneumonia and bronchitis: most frequently recognized
  - also mildly symptomatic: pharyngitis, sinusitis, otitis media, cough often prolonged, asthmatiform bronchitis
  - often in combination with other respiratory agents
- Severe systemic or chronic infection
- Possible role in coronary artery disease
**Chlamydia pneumoniae: Epidemiology by Serologic Surveys**

- **Age distribution**
  - \( \leq 5 \) years: serologic evidence of infection very low
  - 5 - 14 years: annual seroconversion rates of 6% - 9%
  - by age 20 years: 40-50% antibodies

- **Sex distribution**
  - \( \leq 15 \) years: equal seroprevalence
  - adults: seroprevalence higher among men

- **Global distribution**
  - worldwide distribution: higher prevalence in tropical countries
  - both endemic and epidemic
Chlamydia pneumoniae: Diagnosis

- Culture
  - In cell cultures pretreated with metabolic inhibitors: McCoy, Hela 229, Hep2, HL
  - Detection by immunofluorescence with monoclonal antibodies
  - Relatively insensitive: room for improvement

- Antigen detection
  - Microimmunofluorescence
  - Immunocytochemistry

- Serology
  - Microimmunofluorescence: only specific assay but technically demanding and sometimes lack of specificity
C. pneumoniae : Culture versus Serology

- 23% of culture + cases had positive MIF
  > 50% of children < 10 yrs remained MIF negative.

Emre et al., A.P.A.M. 1994; 148: 727

- 35 culture + children with double sera : serologically
  acute infection : 8 (22.9%)
  past infection : 9 (25.7%)
  no infection : 18 (51.4%)

Kutlin et al., J. Infect. Dis. 1998; 177: 720
## Chlamydia pneumoniae: Agreement of Different Serological Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>R</th>
<th>Strength of</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC rELISA</td>
<td>0.234</td>
<td>Fair</td>
<td>109 of 177 (62)</td>
<td>30 of 41 (73)</td>
</tr>
<tr>
<td>LS EIA</td>
<td>0.597</td>
<td>Moderate</td>
<td>143 of 164 (87)</td>
<td>33 of 38 (87)</td>
</tr>
<tr>
<td>RB EIA</td>
<td>0.665</td>
<td>Good</td>
<td>83 of 89 (93)</td>
<td>20 of 23 (87)</td>
</tr>
<tr>
<td>MCp sELISA</td>
<td>0.686</td>
<td>Good</td>
<td>172 of 177 (97)</td>
<td>29 of 41 (71)</td>
</tr>
</tbody>
</table>

### Recommendations for Standardizing *Chlamydia pneumoniae* Diagnostic Assays

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Major recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>- Four of 18 currently published assays met proposed criteria for optimal validation&lt;br&gt;- Each PCR run should include low positive controls (≤ 1 inclusion-forming units), and water controls every fifth extraction</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>- Each tissue block should be tested with 2 Chlamydia antibodies and 2 control antibodies&lt;br&gt;- Each staining run should include 1 positive and 1 negative tissue control, each incubated with the 4 antibodies used on the specimen of interest&lt;br&gt;- Intracytoplasmic staining of macrophages, endothelial cells, or smooth muscle cells in a granula pattern may be considered positive; interpretation of a homogenous staining pattern is controversial</td>
</tr>
</tbody>
</table>

### PCR assays for Detection of *Chlamydia pneumoniae* in Clinical specimens

<table>
<thead>
<tr>
<th>Year of study (reference)</th>
<th>Type of report or assay</th>
<th>Target region</th>
<th>Product size, bp</th>
<th>Method of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published reports regarding assays that meet validation criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campbell (1992)</td>
<td>S + R</td>
<td>Cloned <em>PstI</em> fragment</td>
<td>437</td>
<td>AGE</td>
</tr>
<tr>
<td>Gaydos (1992)</td>
<td>S</td>
<td>16S rRNA gene</td>
<td>463</td>
<td>AGE</td>
</tr>
<tr>
<td>Tong (1993)</td>
<td>N + T</td>
<td>MOMP</td>
<td>outer, 333; inner, 207</td>
<td>AGE</td>
</tr>
</tbody>
</table>

Detection of *M. pneumoniae* and *C. pneumoniae* in Respiratory Specimens

- Molecular techniques are definitely more sensitive than culture

- Still the greatest emphasis on serology despite limited added value in diagnosis of acute infections
  - Even IgM may appear late: mean 27 days.
  - Serology for *C. pneumoniae* < 5 yrs is not useful and > 50% of *C. pneumoniae* infected individuals fail to develop antibodies.

- Rapid diagnosis is useful but not in all cases:
  - guidelines are needed
  - cost-efficiency data are needed