Testing Times – Diagnostics and POC Testing for RSV

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Conflicts of Interest

• I have received honoraria from GSK, Sanofi, Pfizer, CSL and Janssen for taking part in advisory boards and expert meetings and for acting as a speaker in congresses outside the scope of the work discussed.
Talk Outline

Methodologies Available for RSV Diagnostics

RSV Symptoms

RSV Testing in Infants

RSV Testing in Adults

Awareness of RSV
Introduction: Testing Methodologies for RSV
Testing Methodologies for RSV

Viral Culture (historically gold standard):

- Used Hep-2 / Human Fibroblasts and others
- 35-80% sensitive
- Took 3-9 days for results
- Less used since PCR

Limitations: Loss of cells, time to detection, issues with the cell lines, not practical in the clinical setting...


Testing Methodologies for RSV

• Serology
• Usually based on Antibody testing to RSV specific antigens
• 4-fold rise from baseline is considered Positive (historical cut-off from ELISA)
• Antibodies to Pre/Post-F, Ga/b, N

Adapted from Bianchini S et al. 2020
Testing Methodologies for RSV

- PCR
- Can be specific for RSV A or B
- Multiplex available with other viruses (often a little less sensitive)
- Sequence selection important
Testing Methodologies for RSV

- Point of Care Testing
- Historically rapid-antigen style tests
- Now often PCR based
- Good accuracy when compared to standard PCR

### Diagnostic and economic evaluation of a point-of-care test for respiratory syncytial virus

A. Joy Allen, Andrea Gonzalez-Ciscar, Clare Lendrem, Jana Suklan, Karen Allen, Ashley Bell, Frances Baxter, Stephen Croulley, Louise Fairlie, Danielle Hardy, Louise Johnston, Joanne McKenna, Nicole Richards, Gavin Shovlin, Clare Simmister, Sheila Waugh, Philip Woodsford, Sara Grazadio, Michael Power, A. John Simpson, Prashant Kumar, Katherine Eastham, Malcolm Brodlie

ERJ Open Research Jul 2020, 6 (3) 00018-2020; DOI: 10.1183/23120541.00018-2020
Critical Issue:

It is not possible to diagnose RSV on symptoms alone:
Variation in symptoms in infants / children

Symptoms of RSV infection compared to other common viral pathogens in older adults

Frequency of Symptoms Among Single Virus Infections (Viruses Detected in at Least 30 Cases)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. of Episodes (N)</th>
<th>RSV (N = 39)</th>
<th>Influenza A (N = 98)</th>
<th>Metapneumovirus (N = 31)</th>
<th>Rhinovirus/Enterovirus (N = 75)</th>
<th>Coronavirus (N = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% (95% CI)</td>
<td>n</td>
<td>% (95% CI)</td>
<td>n</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>28</td>
<td>71.8 (55.1–85.0)</td>
<td>76</td>
<td>77.6 (68.0–85.4)</td>
<td>23</td>
<td>74.2 (55.4–88.1)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>25</td>
<td>64.1 (47.2–78.8)</td>
<td>63</td>
<td>64.3 (54.0–73.7)</td>
<td>20</td>
<td>64.5 (46.4–80.8)</td>
</tr>
<tr>
<td>New or worsening cough</td>
<td>36</td>
<td>92.3 (79.1–98.4)</td>
<td>87</td>
<td>88.8 (80.8–94.3)</td>
<td>28</td>
<td>90.3 (74.2–98.0)</td>
</tr>
<tr>
<td>New or worsening dyspnea</td>
<td>20</td>
<td>51.3 (34.8–67.6)</td>
<td>32</td>
<td>32.7 (23.5–42.9)</td>
<td>8</td>
<td>25.0 (11.9–44.6)</td>
</tr>
<tr>
<td>New or worsening sputum production</td>
<td>27</td>
<td>69.2 (52.4–83.0)</td>
<td>49</td>
<td>50.0 (39.7–60.3)</td>
<td>14</td>
<td>45.2 (27.3–64.0)</td>
</tr>
<tr>
<td>New or worsening wheezing</td>
<td>18</td>
<td>46.2 (30.1–62.8)</td>
<td>30</td>
<td>30.6 (21.7–40.7)</td>
<td>3</td>
<td>9.7 (2.9–25.8)</td>
</tr>
<tr>
<td>Fever</td>
<td>22</td>
<td>56.4 (39.6–72.2)</td>
<td>71</td>
<td>72.4 (62.5–81.0)</td>
<td>17</td>
<td>54.8 (36.0–72.7)</td>
</tr>
<tr>
<td>Headache</td>
<td>32</td>
<td>82.1 (66.5–92.5)</td>
<td>72</td>
<td>73.5 (63.6–81.9)</td>
<td>25</td>
<td>80.6 (62.5–92.5)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>31</td>
<td>79.5 (63.5–96.7)</td>
<td>75</td>
<td>76.5 (66.9–84.5)</td>
<td>21</td>
<td>67.6 (48.6–83.3)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>25</td>
<td>64.1 (47.2–78.8)</td>
<td>69</td>
<td>70.4 (60.3–79.2)</td>
<td>19</td>
<td>61.3 (42.2–78.2)</td>
</tr>
<tr>
<td>Feverishness</td>
<td>18</td>
<td>46.2 (30.1–62.8)</td>
<td>58</td>
<td>59.2 (48.8–69.0)</td>
<td>18</td>
<td>58.1 (39.1–75.5)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; RSV, respiratory syncytial virus.

* n, number of subjects in a given category.

Symptoms of RSV infection compared to other common viral pathogens in older adults

RSV is indistinguishable from Flu in community based on symptoms:


Talbot, H. Keipp MD; Belongia, Edward A. MD; Walsh, Edward E. MD; Schaffner, William MD. Respiratory Syncytial Virus in Older Adults: A Hidden Annual Epidemic. Infectious Diseases in Clinical Practice 24(6):p 295-302, November 2016. | DOI: 10.1097/IPC.0000000000000455
RSV Testing in Infants

- More established than testing in adults
- Viral load in infants is generally higher than in adults
- POC testing more sensitive
Testing in infants / children

- Recent meta-analysis of 157 papers
- RT-PCR was the most sensitive paediatric RSV diagnostic test. Adding multiple specimens did not substantially increase RSV detection, but even small proportional increases could result in meaningful changes in burden estimation.
- Adding paired serology testing increased RSV detection by 10%, Nasal Swab by 8%, oropharyngeal swabs by 5%, and NPS by 1%.
- Compared to RT-PCR, direct fluorescence antibody tests, viral culture, and rapid antigen tests were 87%, 76%, and 74% sensitive, respectively.
- Pooled sensitivity of multiplex versus singleplex RT-PCR was 96%

POC vs RT-PCR in infants

Sensitivity varied from 26-100% comparing monoplex PCR to POCT
Multiplex PCR is less sensitive than monoplex in infants

Table 3. Sensitivity of two kinds of ResPlex II kit for common viruses *

<table>
<thead>
<tr>
<th>Virus</th>
<th>ResPlex II Panel</th>
<th>ResPlex II Plus Panel PRE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M+R+</td>
<td>M+R-</td>
</tr>
<tr>
<td>RSV</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>FluA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>hMPV</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PIV3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>PIV1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>hBoV</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

* R, ResPlex II Panel; M, monoplex real-time TaqMan RT-PCR.

Serology in Infants

- If first infection, minimal RSV specific antibodies in serum
- Some antibodies detectable in early infancy from maternal transfer
- All children will have been infected by ~ age of 2
- Evidence of maternal antibodies affording protection
Serology Sensitivity in Infants

Generally, does not add substantially to PCR
Lysate + F-based antibody tests up to 100% sensitive
Difficult in practice as pre-illness serum needed (some studies use cord blood for comparison).

Testing in Adults

• As we have heard there is significant burden of RSV amongst older adults
• There is an issue of clinical governance with regards to diagnostics
• Accurate testing will help inform vaccine roll-out and decision makers
Diagnostic barriers for RSV in Older Adults:

- Clinical diagnosis is not possible due to similarities with other viral diseases
- Lack of incentive to diagnose RSV as no dedicated treatment exists
- Many cases, especially in primary care, currently go undiagnosed
- Relatively high cost of polymerase chain reaction analysis (developing countries specifically)
Practical Issues with Diagnostics

- PCR based diagnostic techniques are however not 100% sensitive in adults.
- There is a propensity for RSV to replicate in the lower airways, therefore nasal swabbing alone might not be sufficient to obtain a diagnosis.
- Multiple methods often used in studies, not practical in the clinical setting
- Older POC testing was not sensitive in adults, newer systems however show promise
- Adults shed considerably less virus than infants ($\leq 10^3$ versus $\leq 10^6$ PFU/ml)

Adapted from Carvajal et al. 2019
Viral Culture Adults

- 3 studies that assessed viral culture versus a reference standard of RT-PCR. The sensitivity ranged between 49% and 86%.

- Diagnosis of RSV infection by culture is considerably more difficult than diagnosis of influenza, with sensitivities ranging from 17% to 39%, compared with serological tests and PCR.
Multiplex is less sensitive than Monoplex in Adults

- Six studies evaluated the performance of a multiplex RT-PCR versus a monoplex platform. Pooled sensitivity was 92% and specificity 99%.

POCT vs PCR

Adults

PMID: 36661222; PMCID: PMC10345483.
POC Testing

• POC Testing in Adults in the Outpatient setting

RSV Testing in Adults: Combining Techniques


Comparing PCR, saliva, sputum and serology

Prospective cohort study of patients aged ≥ 40 years hospitalized for acute respiratory illness (ARI)

NP swab, saliva, and sputum specimens were collected at enrollment. Serology specimens were obtained at acute and convalescent timepoints.

NP swab plus additional specimens, corresponding to a 1.95 times higher rate.

Sensitivities by specimen type were: NP swab 51%, saliva 70%, sputum 72%, and serology 79%.

Comparing PCR, saliva, sputum and serology

Using all 4 specimens there was a 2.60-fold increase compared to NP swab alone
Adding specimen types such as paired serology and sputum to NP/nasal swab RT-PCR increased RSV detection by 50% to 66% on average, respectively.

Suggest using ≥3 specimen types for robust analysis of prevalence in studies.
Hospital Outbreaks

- French geriatric hospital outbreak
- 12 cases in 2 adjacent wards
- Mean age 89 – all had co-morbidities
- 2 passed away
- Genotyping confirmed these cases as an ‘outbreak’

Whole Genome Sequencing

Possible vaccines could drive mutations

Large-scale genomic surveillance needed

Early detection of strains that escape intervention
The Future: Serology in Vaccinated Adults

Critical Issue:

Awareness of RSV in adults is lacking
Older Adult RSV Awareness

- In one study RSV was not considered on admission to A+E in most cases where it was eventually diagnosed. This included high risk patients. Only 36% of admitted patients eventually diagnosed with RSV were swabbed for viral PCR for RSV on admission.

- Among 827 of survey respondents, only 43.3% had ever heard of RSV

Elizabeth M. La, Su Bunniran, Diana Garbinsky, Maria Reynolds, Phil Schwab, Sara Poston & Lauriane Harrington (2024) Respiratory syncytial virus knowledge, attitudes, and perceptions among adults in the United States, Human Vaccines & Immunotherapeutics, 20:1, 2303796, DOI: 10.1080/21645515.2024.2303796
8. A 66-year-old man attends clinic, following a hospital admission for an exacerbation of COPD. Latest spirometry showed FEV₁ 1.65 (55% predicted), 2.66 (79% predicted), ratio 0.62. He wishes to reduce the risk of infection in the future.

What is the single most appropriate vaccination to recommend?

- A Haemophilus influenzae type B vaccine
- B Influenza H5N1 vaccine
- C Pneumococcal polysaccharide vaccine
- D Respiratory syncytial virus vaccine
- E Varicella-zoster virus vaccine

The pneumococcal polysaccharide vaccine protects against 23 strains of pneumococcus. It only needs to be given once, and should be offered to over 65s at risk, including patients with COPD [C].

Respiratory syncytial virus is a common virus, most prevalent between October–March. It affects babies, particularly those that were born prematurely. It is not a significant problem for adults, and there is no vaccine available [D].

Varicella-zoster virus causes chickenpox and shingles. The vaccine is not specifically recommended for patients with COPD but is available to adults 70–80 years in the UK [E].
Take-Home Messages

• No assay has 100% accuracy
• Big differences in sensitivity between adult and infant populations
• Monoplex PCR is sensitive in diagnosing RSV in infants
• Adding extra methods will however increase diagnostics
• For adults, more methods are needed than just PCR
• Awareness of RSV in adults needs to improve
• Consider non-F-antigen based serology for a vaccinated cohort
Thank You!