A Comparison of the FilmArray, xTAG and Viral Culture for the Detection of Respiratory Viruses

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Introduction

Multiplex reverse transcriptase respiratory viral PCR has been shown to be more sensitive than standard respiratory virus culture, direct fluorescent antigen and direct ELISA antigen detection methods (1,2). Viral culture is labor intensive, detects some viruses poorly (e.g. Rhinovirus, Coronavirus), and requires 3 – 5 days to detect most agents. As a result, the positive results are generally not available in an early clinical decision making time frame. Direct fluorescent antibody (DFA) and chromogenic immunocassays are rapid enough to support real time clinical decisions, but DFA is highly labor intensive and chromographic immunocassays are relatively insensitive. The FilmArray multiplex respiratory viral panel uses a poch system that contains all reagents for the identification of 18 respiratory viruses and 3 bacterial respiratory pathogens within 1 hour after inoculation of a patient sample, potentially obviating both labor and turnaround time issues. We compared the performance of the FilmArray with the FDA approved Luminex xTAG multiplex panel and traditional viral culture for 200 retrospective clinical respiratory virus culture samples.

Methods

Patient Samples

Patient specimens sent to the Shands at the University of Florida Hospital Clinical Virology laboratory between October, 2006 and May, 2010 were frozen at -70°C after standard viral culture was performed. There were 139 upper respiratory samples (NP swabs, N=181; throat cultures, N=25; miscellaneous, N=14), and 52 lower respiratory tract specimens (BAL, N=46, bronchial brushings, N=2; endotracheal aspirates, N=11, and one autopsy lung).

Viral Culture and Antigen Detection

One hundred eight specimens were cultured using standard tube cultures and sputum containing human diploid fibroblasts, Monkey kidney cells, and A 549 cells. Sputum were shelled on days 3 and 5 using the Q-light Diagnostics (Camelot, CA) 7-way fluorescent antibody screen, and further identified with specific antibida if positive. Five samples were tested by direct antigen testing only (3 Influenza A and 2 RSV). Filtrate samples were tested by PCR/PCR.

Multiplex Respiratory Virus PCR

The FilmArray detects the following agents: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1N1 swine-origin variant, Influenza B, Respiratory Syncytial Virus, human Metapneumovirus, Coronavirus NL63, Coronavirus OC43, Coronavirus 229E, Coronavirus HKU1, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Bocavirus, Rhinovirus/Enterovirus, Bordetella pertussis, Bacteria pneumococcal and Chlamydia pneumoniae. The xTAG detects Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, human Metapneumovirus, Adenovirus A2, Parainfluenza 1, Parainfluenza 3 and Rhinovirus. Both assays were performed according to the manufacturer’s instructions, following training by the respective companies. Nucleic acid extraction was done with a QIAamp video (QIAGEN) kit for a 5 minute sample. The remaining extracted nucleic acid and aliquots of the original frozen specimen were stored at -70°C for further testing.

Table 1

<table>
<thead>
<tr>
<th>FA +</th>
<th>xTAG +</th>
<th>FA + xTAG +</th>
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<tbody>
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<td>10</td>
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Table 2

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<th>FA +</th>
<th>FA + xTAG +</th>
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<td>32</td>
<td>187</td>
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<tr>
<td>7</td>
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Table 3

<table>
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<tr>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>4</td>
<td>57</td>
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</table>

Figure 1

This Figure shows the relationship between the FilmArray cycle threshold (Ct) and xTAG Mean Fluorescence Intensity (MFI).

Conclusions

1. Both the FilmArray and the xTAG significantly detected significantly more viruses than standard culture and rapid methods, mostly Rhinovirus/Enterovirus, but also RSV in the case of the FilmArray. Pabbaraju et al. (3) also noted a greater number of positive RSV results using an in house PCR that found the xTAG (85 vs. 9 of which RSV was a single positive result).

2. Most of the discordant RSVs had low titers in the FilmArray, suggesting sensitivity is more likely to explain the results than sequence differences.

3. The FilmArray is far easier to use than the xTAG (literally 3 – 5 minutes hands-on time vs 2 -3 hours); and provides results in 1 hour vs 5 ½ - 6 hours. However, as a single unit test, multiple instruments or instruments with batch capacity will need to be developed.

References and Acknowledgement


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