The percentage of PCR sample run failures (requiring repeat testing) with GX, LA and S were 5.71%, 0% and 1.43% respectively.

Conclusions: All assays were rapid and easy to perform. With the limited number of samples we tested, LA appeared to perform the best among the three assays with the highest overall sensitivity and lowest PCR failure rate. A larger evaluation with increased sample size is needed to confirm our findings.

BACKGROUND

Seasonal epidemics of influenza (Flu) and respiratory syncytial virus (RSV) are responsible for yearly epidemics throughout the world. Infection with Influenza A/B causes human disease with significant morbidity and mortality. Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis in infants and is a major contributor to morbidity and mortality especially in premature infants and the elderly. Rapid molecular assays have significantly improved the laboratory detection of Flu and RSV allowing for improved clinical decision making in managing patients and local/institutional outbreaks.

The purpose of this evaluation was to compare the performance characteristics and utility of GX, LA and S using nasopharyngeal (NP) and bronchial alveolar lavage (BAL) specimens.

Methods: A total of seventy samples (fifty NP, sixteen BAL, three proficiency samples from the College of American Pathologists (CAP), and one external Quality Control (QC) sample from a commercial source) were initially tested using the S assay in the routine virology lab. These samples were then frozen at -70°C until further testing by both GX and LA assays. Among these samples, fifty-four were known to be positive for Flu A/B or RSV and sixteen were known to be negative for Flu A, B and RSV viruses.

Samples producing discrepant results were reviewed and retested simultaneously on all three platforms.

Results: Expected results (EXPs) were defined as two or more assays being in agreement or from a known result (positive culture).

Sensitivity and specificity were generated by comparing GX, LA and S to EXPs. LA demonstrated the highest sensitivity among all three assays. The results are as follows:

<table>
<thead>
<tr>
<th></th>
<th>LA vs. EXPs</th>
<th>S vs. EXPs</th>
<th>GX vs. EXPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Flu A</td>
<td>100%</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Flu B</td>
<td>100%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>RSV</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

The purpose of this evaluation was to compare the performance characteristics and utility of GX, LA and S.

RESULTS

1. All three assays correctly identified the CAP specimens
2. Over 95% agreement was achieved between the three assays.

Table 1. Accuracy (% agreement)

<table>
<thead>
<tr>
<th></th>
<th>LA vs. S</th>
<th>GX vs. S</th>
<th>LA and GX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A</td>
<td>97%</td>
<td>94%</td>
<td>91%</td>
</tr>
<tr>
<td>Flu B</td>
<td>95%</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td>RSV</td>
<td>100%</td>
<td>97%</td>
<td>97%</td>
</tr>
</tbody>
</table>

3. Overall, LA demonstrated the highest sensitivity among the three assays

Table 2. Sensitivity and Specificity

<table>
<thead>
<tr>
<th></th>
<th>LA vs. EXPs</th>
<th>S vs. EXPs</th>
<th>GX vs. EXPs</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>RSV</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

One NP was positive for Flu A by LA only. Review indicated that the patient had recently tested positive for Flu A from a specimen collected earlier in their clinical disease;

Two BALs were positive for Flu A by both S and LA, but not GX;

One BAL was positive for both Flu B and RSV by S and LA, but only positive for Flu B by GX;

One external QC specimen was known positive for RSV and was positive for RSV. Both neat and 1:1000 dilution of the specimen were tested by all three assays. Only LA detected both viruses from neat and diluted specimens. S detected RSV only from neat and 1:1000 diluted specimen. GX detected both viruses from the neat sample, but only RSV from the diluted sample.

5. LA had no failed PCR run s during the evaluation.

Table 4. PCR Failure Rate

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>LA</th>
<th>GX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>1.43%</td>
<td>0%</td>
<td>5.71%</td>
</tr>
</tbody>
</table>

Discussion and Conclusion

All assays are FDA cleared;
All assays are rapid and easy to perform;
The on-board PCR times for GX, LA and S are 60 minutes, 113 minutes, and 75 minutes respectively;
Reagents for S must be stored at ~20°C, while reagents for LA and X are stored at room temperature;5. S will give an error warning if there is no sample added, while LA and X will give false negative results if there is no sample added.

With the limited number of samples we tested, LA appeared to perform the best among the three assays with the highest overall sensitivity and lowest PCR failure rate. A larger evaluation with increased sample size is needed to confirm our findings.

ACKNOWLEDGEMENT

Xpert Flu/RSV XC kits were kindly provided for evaluation by Cepheid Innovation. Aries Flu A/B & RSV kits were kindly provided for evaluation by Luminex Corporation.