Comparison of RSV RNA Recovery by PCR Using Different Methods of Specimen Preservation

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Background

- Respiratory infections—and in particular those caused by respiratory syncytial virus (RSV)—are one of the most common illnesses in children.
- Prompt diagnosis of RSV-A and RSV-B can help guide therapy and prevent unnecessary antibiotic use.
- The technique for collection of nasal saline washes was first described by Hall and Douglas in 1975 and is the current standard for collection of nasal samples to test for RSV (Figure 1).
- While this method is suitable for onsite PCR antigen testing, it is cumbersome as samples must be frozen to −70°C and transferred to DNAse-free containers.
- Swabs may be an easier method of collection, however it is known that cotton and alginate swabs are unable to release adequate viral material into storage medium for analyses.
- Flocked swabs (Copan Innovation, Murrieta, CA) may be superior to traditional swabs at releasing viral material (Figure 2).
- Further, universal transport medium (UTM-RT, Copan Innovation) may be a superior storage medium since it can be kept at room temperature.

Methods

Subjects: all infants and toddlers up to 18 months of age presenting to the ED with a clinical diagnosis of bronchiolitis in whom the clinician felt RSV antigen testing was warranted.
- Nasal washings and nasal swabs were obtained using standardized kits (Figure 1).
- Samples were immediately refrigerated at 4°C until they were aliquoted.
- RNA was extracted using the QiAamp Viral RNA purification kit (Qiagen, Valencia, CA).
- Extracted RNA was tested for RSV-A and RSV-B using two separate one-step reverse transcription of PCR assays.

Nasal washings and nasal swabs were obtained using standardized kits (Figure 3). The nostril that had a saline nasal wash and the nostril that had a nasal swab was randomized. Each sample was divided into a 500-µL sample with 500 µL of UTM-RT, which was then frozen at −20°C. The remaining of the nasal washing was aliquoted into DNAse-free transport containers and frozen at −70°C.
- RNA was extracted using the QiAamp Viral RNA purification kit (Qiagen, Valencia, CA).
- Extracted RNA was tested for RSV-A and RSV-B using two separate one-step reverse transcription of PCR assays.
- The presence of RSV-A or -B by PCR in any two of the nasal washings in the unpreserved saline, nasal washings in UTM-RT, or in the flocked swabs in UTM-RT from a patient was assumed to be a true positive.
- The absence of RSV-A or -B by PCR in all specimens from a patient was assumed to be a true negative.
- Sensitivity, specificity, and the area under the curve were calculated using STATA 9.2 (StataCorp, College Station, TX).

Results

- 137 subjects completed the protocol (age distribution shown in Figure 4).
- 35 were positive for RSV-A in at least two samples; 102 were either negative for RSV-A or were only positive in one sample.
- 4 were positive for RSV-B in at least two samples; 38 were either negative for RSV-B or were only positive in one sample.
- Sensitivity was higher for RSV-A and -B in UTM-RT and flocked swabs than unpreserved saline.
- UTM-RT and flocked swabs are superior to unpreserved saline for detecting RSV (Table 1).
- There is no difference between nasal washings in UTM-RT and flocked swabs in UTM-RT for detecting RSV.

Table 1: Sensitivity, specificity, and area under the curve for detection of RSV-A and RSV-B

<table>
<thead>
<tr>
<th>Method</th>
<th>RSV-A</th>
<th>RSV-B</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sensitivity (95% CI)</td>
<td>Specificity (95% CI)</td>
</tr>
<tr>
<td>Nasal washings in UTM-RT</td>
<td>0.96 (91-100)</td>
<td>0.93 (86-100)</td>
</tr>
<tr>
<td>Flocked swabs in UTM-RT</td>
<td>0.94 (91-100)</td>
<td>0.91 (86-100)</td>
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Table 2: Hypothesis testing

<table>
<thead>
<tr>
<th>Null Hypothesis</th>
<th>p Value</th>
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<tbody>
<tr>
<td>No difference between unpreserved saline and UTM-RT</td>
<td>0.41 (reject null)</td>
</tr>
<tr>
<td>No difference between unpreserved saline and flocked swabs</td>
<td>0.01 (reject null)</td>
</tr>
<tr>
<td>No difference between no freezing in UTM-RT and flocked swabs</td>
<td>0.85 (accept null)</td>
</tr>
</tbody>
</table>

Discussion

Limitations:
- Single-center study.
- Difficult to define the gold standard.
- Viral cultures as a gold standard are limited by the fragility of the RSV RNA.
- Instead, we chose two positives as the true presence of the virus which we believe to be reasonable given the accuracy of PCR.

Implications:
- Swabs and UTM-RT may be more important in the clinical setting as PCR becomes more widespread.
- They are an easy method for viral discovery projects and for research involving respiratory viruses.

Future Research:
- It remains to be seen if viral RNA is sufficiently preserved in UTM-RT at room temperature.
- It is also not known whether the preservation of RNA of other respiratory viruses is improved in UTM-RT and using flocked swabs for collection.

Conclusion

Despite requiring dilution, using UTM-RT for storage of nasal washings and flocked swabs for collection of nasal samples may improve the sensitivity of detecting RSV-A and RSV-B by PCR when compared with unpreserved nasal washings.

We would like to acknowledge Copan Diagnostics for providing us with the UTM-RT and flocked swabs. They were not involved in the sample collection, assays, or data analysis and had no control over the reporting of the results.