Utility of the Copan ESwab transport system for culture of Bordetella pertussis from pediatric nasopharyngeal samples

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Summary

Pertussis is a highly contagious, vaccine-preventable respiratory infection caused by Bordetella pertussis. Diagnosis of pertussis has improved with the use of nucleic acid amplification assays like PCR. However, recommendations from the CDC include the use of culture, particularly in outbreak situations. Historically, rates of recovery of B. pertussis in culture range from 12% to 60% when compared to PCR detection. This study evaluated the utility of the Copan ESwab transport system for culture of B. pertussis from nasopharyngeal (NP) samples collected from 223 pediatric patients (average age: 3.3 years; median age: 1.5 years). A set of paired ESwab-NP samples were collected from each patient, one subjected to real-time PCR for B. pertussis and the other transported to a reference laboratory for culture on Regan-Lowe agar. The average time to PCR after sample collection was 18 hours and all ESwabs were held at 4°C until transported. The average time from collection to initial plating was 50 hours (median, 47 hours).

Results

Of 76 ESwabs transported at ambient temperature, 9 were PCR-positive for B. pertussis, none were positive by culture, and overgrowth with mold or Gram-negative Rods occurred in 21% of cultures. Of 147 ESwabs transported at 4°C, 36 were PCR-positive and, of these, 8 were culture-positive for B. pertussis. For 6 positive cultures, B. pertussis was recovered from ESwabs maintained at 4°C for at least 79 hours and 3 specimens yielded viable B. pertussis for at least 5 days. Overgrowth with mold or Gram-negative Rods occurred in 10% of specimens transported at 4°C. The average PCR crossing threshold (CT) for culture-positive ESwabb samples was 18.3 while that for culture-negative samples was 28.3.

Conclusions

Like other swab transport systems utilized for isolation of B. pertussis, the sensitivity of culture using ESwabs compared to PCR detection is low and recovery may be hampered by overgrowth that could obscure B. pertussis colonies. Studies are underway to determine the recovery rates of B. pertussis from NP samples using paired sets of ESwabs and Regan-Lowe agar deeps subjected to the same transport conditions.

Materials and Methods

Patient NP ESwabs (Copan Diagnostics, Murrieta, CA) were collected in duplicate. One swab was used for PCR and the other sent to a reference laboratory for culture. Samples were divided into four groups as shown in Table 1.

Real Time PCR

Amplification and detection of a 114 base pair segment of the B. pertussis GAP418 sequence was conducted as previously described (Hood et al.) using nucleic acid extracted from 1 mL of E-Swab fluid that was concentrated by centrifugation. The pellet was resuspended in lysis buffer containing proteinase K and heated at 100°C for 2 minutes, 95°C, 30 seconds, 95°C, 60 seconds, and 72°C, 1 minute. Reactions displaying Ct values <35 were considered positive for B. pertussis.

Culture

Samples from Groups 1 – 3 were plated as follows:

- Tubes streaked and 50 µL fluid inoculated to each of two Regan-Lowe plates (BD, Sparks, MD).
- Subcultures repeated daily as above for 5 days.
- Plates incubated 7 days at 35°C in ambient air.
- All plates read daily and results recorded.
- Positive cultures confirmed by Bordetella pertussis fluorescent antibody stain (BD-Difco).

Samples from Group 4 were plated as follows:

- Tubes streaked and 50 µL fluid inoculated to each of two Regan-Lowe plates and streaked for isolation.
- ESwab tubes centrifuged at 5000 rpm for 5 minutes and most of supernatant removed.
- The pellet resuspended and 50 µL inoculated to each of two Regan-Lowe plates and streaked for isolation.
- The Regan-Lowe deep (Hardy Diagnostics) swab inoculated to each of two Regan-Lowe plates, streaked for isolation.
- Plates incubated 7 days at 35°C in ambient air.
- All plates read daily and results recorded.
- Positive cultures confirmed by Bordetella pertussis fluorescent antibody stain (Difco).

Table 1. Description of sample categories

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Type</th>
<th>Culture Status</th>
<th>Transport Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCR positive and PCR negative samples</td>
<td>Stopped and held ambient</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PCR-positive and PCR-negative samples</td>
<td>Stopped and held refrigerated</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PCR-positive samples</td>
<td>Stopped and held refrigerated</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PCR-positive samples</td>
<td>Stopped and held refrigerated</td>
<td></td>
</tr>
</tbody>
</table>

Discussion and Conclusions

The purpose of this study was to determine if ESwabs could be used as an alternative to Regan-Lowe agar in maintaining viability of B. pertussis during transport. As shown previously with other transport media, we observed low B. pertussis culture positivity rates (18%) using ESwab as transport media.

Specimens with lower Ct values (indicative of higher organism concentrations) were significantly more likely to be culture-positive (p<0.001) whereas those with higher Ct values (lower organism concentration) were typically culture-negative. There were no culture positive, PCR negative specimens.

Many of the cultures were contaminated with other gram negative rods or mold, which may have limited recovery of B. pertussis.

The impact of transport temperature on recovery of B. pertussis was difficult to assess since most cultures failed to recover the organism. However, average Ct values may indicate decreased viability under ambient conditions.

Transport temperature did affect the contamination rate, with overgrowth more common from ambient than refrigerated specimens (p<0.05).

The single positive pair of cultures in Group 4, the ESwab and Regan-Lowe transport media yielded B. pertussis colonies in equal quantities. These were subject to identical transport/holding conditions and appear to be equally supportive for B. pertussis. The number of samples examined in this group was limited by diminishing PCR-positives near the end of the study period.

There was no direct correlation between transport time and duration of viable organism recovery. The shortest transport time for a culture positive sample was 31 hours (Ct = 14.5), and the longest was 62 hours (Ct = 31.3). B. pertussis was recovered from the latter specimen more than 6 days after collection. Repeat subcultures from ESwab both remained positive for up to 5 days (average = 3.4 days), except for two samples that were positive on subculture for only 1 day.

Conclusions

Culture-based testing for B. pertussis suffers from poor sensitivity relative to PCR. Successful culture may depend on factors such as shipping temperature and transport time, organism concentration and the presence of contaminating flora. Together, these observations indicate that recovery of viable B. pertussis after specimen transport in ESwab is quite variable.

Because of the small number of samples submitted in both media, further direct comparisons will be required to make a robust assessment of the relative performance of ESwab compared to Regan-Lowe deeps for maintaining B. pertussis viability in transport.

References
