Stability of *Bordetella pertussis* and *Bordetella parapertussis* in the ESwab Transport System for Culture and PCR

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Revised Abstract

Objectives
Pertussis (whooping cough) is caused by *Bordetella pertussis* and *Bordetella parapertussis*, with several states in the USA reporting an increased incidence. Laboratory detection methods include culture, direct fluorescence assay, and PCR methods; successful culture and PCR detection require proper specimen collection and transport. This study examined the effectiveness of the ESwab system (consisting of liquid Amies transport medium and a flocked nasopharyngeal swab, Copan Diagnostics, Inc.) for maintenance of viability of *B. pertussis* and *B. parapertussis* for culture and preservation of nuclear material for detection by PCR.

Methods
Eight *Bordetella* isolates were tested, including 5 *B. pertussis* isolates (ATCC 9340 and 8467 strains, and 3 recent clinical isolates) and 3 *B. parapertussis* isolates (ATCC 15237 strain and 2 recent clinical isolates). Test methods were based on CLSI M40 guidelines. Three *Bordetella* saline suspensions (10^8, 10^6, and 10^4 CFU/mL) were prepared. Each suspension was used to inoculate ESwabs in triplicate. The inoculated ESwabs were stored refrigerated (2-8ºC) for up to 96 hours prior to plating. Bacteria from the ESwabs were cultured on Regan Lowe agar plates at 0, 24, 48, and 96 hours of refrigerated storage post-inoculation; plates were then incubated at 3-7ºC in ambient air for a minimum of 4 days. The numbers of colonies at 24, 48, and 96 hours were compared to the 0 hour count to determine percent recovery (viability). Real-time PCR, using primers targeting the *IS481* gene of *B. pertussis* and the *IS1001* gene of *B. parapertussis*, was performed on 24-hour and 96-hour inoculum in the ESwab.

Results
Bordetella was isolated from all of the ESwabs after 96 hours of refrigerated storage. Percent recovery ranged from 27.2% to 96.3% for *B. pertussis*, and from 32.7% to 74.5% for *B. parapertussis*, and was similar across the three inoculum densities for all isolates tested. PCR detected *B. pertussis* and *B. parapertussis* in all of the 24- and 96-hour ESwabs, regardless of initial inoculum concentration (10^4 or 10^6).

Conclusion
The ESwab maintains sufficient viability of *B. pertussis* and *B. parapertussis* to permit detection in bacteria cultures and preserves DNA integrity for PCR detection, even after 96 hours of refrigerated storage.

Introduction

Infection with *Bordetella pertussis*, the bacterium that causes pertussis (whooping cough), is spread by person-to-person transmission via aerosolized respiratory droplets or by direct contact with respiratory secretions. Pertussis manifests with mild upper respiratory tract symptoms that begin 7–10 days (range 6–21 days) after exposure, followed by a severe lingering cough that becomes paroxysmal and can last for weeks or even months. Coughing paroxysms vary in frequency and are often followed by vomiting. A similar, milder disease is caused by *B. parapertussis*.

The United States is currently experiencing a resurgence of pertussis, most notably in California where a total of 1,337 cases were reported during the period of January 1 to June 30, 2010. This represents a 418% increase from the same period in 2009. Part of this increase may reflect the natural cycle of pertussis, which exhibits incidence peaks approximately every 5 years; 2010 was at the peak of the cycle. Other contributing factors may include a lower degree of immunity from the current DTaP vaccine relative to the older DTP vaccine, waning immunity in older children and adults, and low rates of booster shots to maintain immunity.

According to current CDC guidelines, laboratory methods for confirmation of pertussis cases meeting the clinical case definition include culture and PCR. Specimens for culture and PCR are often collected from the nasophayngeal region by swab. Other swab systems may not maintain viability of *Bordetella* without added charcoal, and charcoal swabs may not be acceptable for PCR. This study examined the effectiveness of the ESwab system (consisting of liquid Amies transport medium and a flocked nasopharyngeal swab, Copan Diagnostics, Inc.) for maintenance of viability of *B. pertussis* and *B. parapertussis* for culture and preservation of nuclear material for detection by PCR.

Methods

A total of 8 strains of *Bordetella* were tested. Five strains (3 *B. pertussis* and 2 *B. parapertussis*) were recent clinical isolates obtained from Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) or Focus Diagnostics. Three quality-control strains were tested including *B. pertussis* ATCC 8467 (Hardy Diagnostics), *B. pertussis* ATCC 9340 (Focus stock strain), and *B. parapertussis* ATCC 15237 (Focus stock strain).

Bacteria viability
All tests for bacterial viability were modified from the quantitative elution method described in CLSI M40 A. Modifications included:

1. Inoculum suspensions of 10^8, 10^6, and 10^4 CFU/mL were prepared and used for testing.
2. ESwabs were rolled into the inoculum, allowed to absorb for 10-15 sec, and then placed into 1 mL of liquid Amies transport medium.
3. The 10^8 and 10^6 CFU/mL ESwabs were processed as follows:
   a. The ESwab transport tube was considered the initial dilution tube (total of 10^7 CFU/mL) and was vortexed in the transport tube for 10-15 sec.
   b. 10-fold dilutions were prepared in 0.9 mL of sterile saline to reach a final concentration of 10^3 CFU/mL.
4. The 10^4 CFU/mL ESwab remained undiluted.

The average CFU for each incubation time was recorded and compared to the average CFU at 0 h. A percent viability (average CFUs at a particular incubation time compared to the CFUs at 0 h) was obtained for each isolate.

PCR Testing
Performed on all isolates at 0 hr and 96 hr with the 10^6 and 10^4 CFU/mL ESwabs. The 10^8 swabs were not tested.

1. Detection of *B. pertussis* and *B. parapertussis* by real-time PCR was accomplished using an in-house developed assay.
2. Nucleic acids were isolated from patient specimens using the Roche MagNA Pure LC system.
3. Real-time PCR was accomplished using primers and probes designed to hybridize to sequences found in *IS481* and *IS1001*.

Results

1. *Bordetella* was isolated from all of the ESwabs after 96 hours of refrigerated storage (Table 1).
   a. Percent recovery for *B. pertussis* ranged from 27.2% to 96.3%.
   b. Percent recovery for *B. parapertussis* ranged from 32.7% to 74.5%.
   c. Percent recovery was similar across the 3 inoculum densities for all isolates tested.
   d. See Figure 1 for typical 0 hr vs 96 hour culture recovery.
2. PCR detected *B. pertussis* and *B. parapertussis* in all of the 24- and 96-hour ESwabs, regardless of initial inoculum concentration (10^4 or 10^6). The 10^8 CFU/mL ESwabs were not tested. Table 2.

Table 1. Recovery of *Bordetella* strains from ESwab after 96 hr refrigeration

Inoc.	0 hr	96 hr	% Recovery	Inoc.	0 hr	96 hr	% Recovery
***B. parapertussis* ATCC 15237**				***B. pertussis* strain Q2**			
10^8	71	53	73.9	10^8	329	250	76.0
10^6	57	38	67.3	10^6	208	160	76.9
10^4	53	40	74.5	10^4	196	76	38.8
***B. parapertussis* strain Q3**				***B. pertussis* strain Q1**			
10^8	223	123	55.1	10^8	328	316	96.3
10^6	222	141	63.5	10^6	318	214	68.3
10^4	251	82	32.7	10^4	248	189	76.2
***B. parapertussis* strain #20**				***B. pertussis* Focus Isolate**			
10^8	107	68	64.5	10^8	96	35	36.6
10^6	152	104	68.4	10^6	41	29	69.5
10^4	136	80	58.8	10^4	46	30	63.7
***B. pertussis* ATCC 9340**				***B. pertussis* ATCC 8467**			
10^8	56	37	66.7	10^8	71	32	42.4
10^6	50	39	77.5	10^6	71	28	39.2
10^4	47	33	70.0	10^4	90	25	27.2

Values are CFU/mL recovered at the final dilution.

Results (cont)

Table 2. *Bordetella* PCR detection (cycles) from ESwab after 96 hr refrigeration

	Eswab				Eswab		
Inoc.	#1	#2	#3	Inoc.	#1	#2	#3
***B. parapertussis* ATCC 15237**				***B. pertussis* strain Q2**			
10^6	26.2	25.9	25.5	10^6	23.7	23.7	23.6
10^4	32.1	33.9	33.0	10^4	30.7	31.1	30.9
***B. parapertussis* strain Q3**				***B. pertussis* strain Q1**			
10^6	25.0	25.2	NT	10^6	22.7	22.7	23.0
10^4	26.0	26.00	25.6	10^4	26.0	25.9	26.3
***B. parapertussis* strain #20**				***B. pertussis* -Focus Isolate**			
10^6	24.2	23.8	23.8	10^6	21.4	20.5	21.2
10^4	29.8	29.0	30.0	10^4	27.9	27.8	27.8
***B. pertussis* ATCC 9340**				***B. pertussis* ATCC 8467**			
10^6	20.8	20.6	20.7	10^6	23.1	22.9	22.6
10^4	26.0	26.1	26.2	10^4	26.2	25.9	25.5

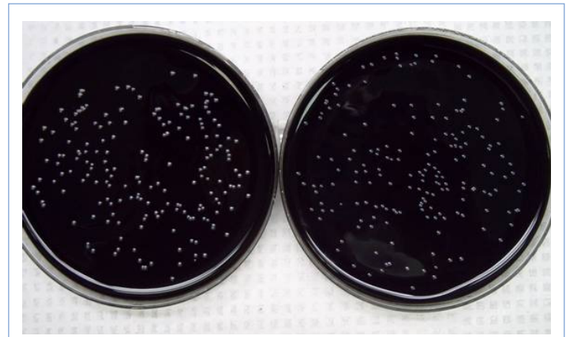

Figure 1. *B. pertussis* strain Q2 recovery after sample refrigeration at (2ºC to 8ºC) for 0 hr (left) and 96 hr (right).

Conclusions

- The ESwab in Amies liquid maintains sufficient viability of *B. pertussis* and *B. parapertussis* to permit detection in cultures after 96 hours of refrigerated storage.
- The ESwab in Amies liquid preserves DNA integrity for PCR detection after 96 hours of refrigerated storage.

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