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ESwab System for *Bordetella* Testing

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Abstract

Background: *Bordetella pertussis* and *Bordetella parapertussis* cause pertussis. Laboratory detection methods include culture, direct fluorescence assay (DFA), and PCR. Successful detection requires proper specimen collection and transport. We examined the effectiveness of the ESwab system (liquid Amies transport medium and a flocked nasopharyngeal swab, Copan Diagnostics, Inc.) for maintenance of culture viability of *B. pertussis* and *B. parapertussis*, and preservation of cells for DFA and nuclear material for PCR.

Methods: *B. pertussis* (ATCC 9340 and 8467 and 3 clinical isolates) and *B. parapertussis* (ATCC 15237 strain and 2 clinical isolates) were tested. ESwab inoculation and culture methods were based on CLSI M40 guidelines. Three saline suspensions (10^8, 10^6, and 10^4 CFU/mL) were tested for each *Bordetella* strain or isolate. Each suspension was used to inoculate ESwabs in triplicate. Inoculated ESwabs were stored at 2-8ºC before plating, DFA, and PCR testing. At 0, 24, 48, and 96 h of refrigerated storage Amies medium from the inoculated ESwabs was cultured onto Regan Lowe plates (Becton Dickinson, Sparks, MD), which were then incubated at 37ºC in ambient air for ≥4 days. Colony counts at 24, 48, and 96 h were compared to the 0-h count to determine percent recovery (viability). DFA slides, prepared by centrifuging inoculated liquid Amies and applying the resuspended sediment onto slides, were stained with polyclonal DFA *Bordetella* reagents (Becton Dickinson). Real-time PCR, using primers targeting *B. pertussis IS481* gene and the *B. parapertussis IS1001* gene was performed on 24- and 96-h inoculum in the ESwabs.

Results: *Bordetella* was isolated from all ESwabs after 96 h 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 for the three inoculum densities. DFA detected *Bordetella* in all ESwabs at 10^6 CFU/mL after 96 h. PCR detected *B. pertussis* and *B. parapertussis* in all 24- and 96-h ESwabs, regardless of initial inoculum concentration.

Conclusions: The ESwab maintains sufficient *B. pertussis* and *B. parapertussis* viability to permit detection in culture and preserves cell and DNA integrity for DFA and PCR detection after 96 h 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 experienced a resurgence of pertussis in 2010, most notably in California where 9273 cases were reported; this represents the highest number of cases reported in 63 years and the highest incidence in 52 years. Part of this increase reflects the natural cyclicity of pertussis, which exhibits incidence peaks approximately every 3 to 5 years. Other contributing factors include waning immunity in older children and adults, low rates of booster shots to maintain immunity, and greater awareness among clinicians.

Introduction (cont)

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 nasopharyngeal 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 cells for DFA as well as nuclear material for 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, *B. pertussis* ATCC 9340, and *B. parapertussis* ATCC 15237.

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 seconds, 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 seconds.
   b. 4- or 2-serial 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.

DFA Testing
Four recent clinical isolates (Q1, Q2: *B. pertussis* and Q3, Q4: *B. parapertussis*) were tested using Becton Dickinson *Bordetella* DFA reagents according to manufacturer's instructions.

1. The 24- and 96-h tubes of the 10^4 and 10^6 inoculum suspensions were used with 200 µL and 400 µL of inoculated Amies liquid centrifuged at ~13,400 x*g* to pellet cells. 10 µL of this final suspension was added to the DFA slides.

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

1. Detection of *Bordetella 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. Recovery ranged from 27.2% to 96.3% for *B. pertussis*.
   b. Recovery ranged from 32.7% to 74.5% for *B. parapertussis.*
   c. Percent recovery was similar across the 3 inoculum densities for all isolates tested.
   d. See Figure 1 for typical 0-h vs 96-h culture recovery.
2. DFA was detected in the lowest inoculum (10^4 CFU/mL) after 96 hours for all 4 *Bordetella* tested.
   a. Although detected in 10^4 CFU/mL tubes, only rare cells were observed. Note: the actual organism load was approximately 10^3 CFU/mL due to 1:10 dilution in Amies.
   b. Few to moderate cells were seen with the 10^6 CFU/mL tubes (actual load approximately 10^5 CFU/mL).
   c. Typical DFA results are seen in Figure 2. for *B. pertussis* isolate Q1 at 10^6 CFU/mL.
3. 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) (Table 2).

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

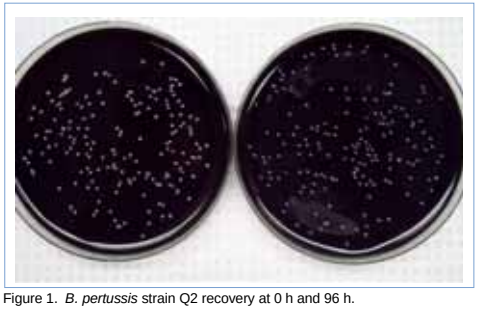

Figure 1. *B. pertussis* strain Q2 recovery at 0 h and 96 h.

Results (cont)

Figure 2. DFA of *B. pertussis* isolate Q1, 10^6 CFU/mL at 96 h.

Table 2. *Bordetella* PCR detection (cycles) from ESwab after 96 h 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	26.0	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

Conclusions

After inoculation with *B. pertussis* or *B. parapertussis* and 96 hours of refrigerated storage, the ESwab with liquid Amies transport system*:*

- maintains sufficient viability to permit detection of both organisms in culture.
- maintains cell integrity for direct fluorescent assays.
- preserves DNA integrity for PCR detection.

Acknowledgments

The authors gratefully acknowledge Copan Diagnostics for supplying the Eswab transport systems and Becton Dickinson for supplying the Regan Lowe agar for this study. We also acknowledge the staff at Quest Diagnostics for supplying *Bordetella* isolates.