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Impact of Nasopharyngeal Swab Types on Detection of *Bordetella pertussis* by PCR and Culture

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A study was designed to assess the performance of various swabs and transport media routinely used to collect specimens submitted for *Bordetella* culture and PCR. Calcium-alginate swabs inhibited the PCR. No inhibition was detected in any PCRs with dacron or rayon swabs. All swab materials performed similarly for recovery of *Bordetella pertussis* in culture. The Amies with charcoal transport system performed poorly for culture. Calcium-alginate swabs are not recommended for PCR-based detection of *B. pertussis*. Dacron and rayon swabs are an excellent choice for both PCR and culture.

Recent articles describing assays of real-time PCR for the detection of *Bordetella* species have not resolved the problem of calcium-alginate inhibition (9). The inhibitory effects of calcium-alginate-tipped nasopharyngeal (NP) swabs have been reported by Wadowsky et al. (10). Even with this knowledge, laboratories offering PCR for the detection of *Bordetella pertussis* continue to accept samples collected with calcium-alginate swabs. Evaluations of pertussis PCR assays compared with culture (5, 9) may be misleading if the samples were collected with calcium-alginate swabs.

It is not likely to be practical to collect two swabs per patient, i.e., one calcium-alginate for culture and one synthetic for PCR, as suggested by Wadowsky et al. (10). Some laboratories may be concerned about the use of synthetic fiber swabs, such as dacron or rayon, for culture. NP specimens are routinely collected on calcium-alginate swabs and submitted to the lab for detection of *B. pertussis* by culture. Early reports suggested that calcium-alginate swabs were superior to dacron, rayon, or cotton wool swabs in their ability to maintain the viability of *B. pertussis* (2). Additional reports have supported the fact that cotton wool swabs perform poorly for isolation of *B. pertussis* (2, 3, 8).

We compared several types of swabs to determine which single material can optimally be used for both culture and PCR. Calcium-alginate-, dacron-, and rayon-tipped NP swabs (COPAN, Corona, Calif.), with and without Regan-Lowe transport medium, were evaluated for their ability to maintain viable *B. pertussis* as well as their effect on detection by PCR. Rayon swabs in the presence of Amies with charcoal transport medium (COPAN) as well as the DNA/RNA Protect system (COPAN) were included in the study.

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Ten clinical isolates of *B. pertussis*, isolated from sporadic cases throughout Utah and Idaho, were utilized in the swab transport evaluation. Primary Children's Medical Center (Salt Lake City, Utah) previously identified the isolates by culture and direct fluorescent-antibody staining. The isolates were suspended in 10% Casamino Acids and held frozen at −80°C until use. The bacteria were grown on Regan-Lowe medium (Becton Dickinson Microbiology Systems, Sparks, Md.) in a humid environment at 36°C. Bacterial suspensions were prepared to match a 0.5 McFarland standard, from which dilutions of $1{:}10^3$, $1{:}10^4$, $1{:}10^5$, $1{:}10^6$, and $1{:}10^7$ were made with phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, Calif.). Aluminum shaft swabs with various types of head material (rayon, dacron, or calcium-alginate) were analyzed. Five microliters of each dilution was inoculated onto each type of swab in triplicate. One swab of each triplicate was held at room temperature for 24 h and analyzed by PCR only. The remaining two swabs were stabbed into Regan-Lowe deep medium (Becton Dickinson Microbiology Systems) and incubated at 36°C for 48 h. One of the inoculated swabs incubated in the agar tubes was analyzed by PCR while the other was set up for culture.

Growth controls were prepared to determine if cultivable organisms were present in the dilutions. Ten samples of each dilution were assessed for each type of swab. Swabs to be cultured were rolled onto a quadrant of a Regan-Lowe plate and then streaked out into the remaining quadrants. The plates were incubated at 36°C and protected from desiccation. Plates were examined daily for 7 days. Colonies were confirmed positive for *B. pertussis* by PCR.

Swabs were prepared for DNA extraction by one of three protocols. The first protocol involved direct DNA extraction in which swabs were immersed in 200 μl of tissue lysis buffer supplied in the QIAamp DNA mini kit (Qiagen, Valencia, Calif.). Swabs were vortexed, wrung out, and discarded. The remaining solution was used for nucleic acid extraction. In the second protocol, swabs were immersed in 1 ml of PBS, incubated for 10 min at room temperature, vortexed, wrung out, and discarded. The remaining suspension was centrifuged for

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TABLE 1. Percentage of *B. pertussis* detected by PCR determined directly from inoculated swabs and controls

Dilution (*n*)	% *B. pertussis* detected with swab type:				
	None (control)	Dacron	Rayon	Calcium-alginate	DNA/RNA Protect system
10^{-3} (10)	100	100	100	50	100
10^{-4} (10)	100	70	80	20[a]	90
10^{-5} (10)	80	10	10	10	50[b]
10^{-6} (10)	0	0	0	0	0

[a] Calcium-alginate versus all other swabs at the 10^{-4} dilution ($P < 0.02$).
[b] DNA/RNA Protect system versus all other swabs at the 10^{-5} dilution ($P < 0.02$).

10 min at 6,000 × *g*, the supernatant was decanted, and the pellet was used for nucleic acid extraction. The third protocol differed from the second by soaking the swabs in 2 ml of PBS for 10 min, using 1 ml of this solution (after vortexing) for centrifugation, and retaining the pellet for extraction.

Nucleic acid was extracted from swab preparations or 5 μl of bacterial dilutions with the QIAamp DNA mini kit according to the manufacturer's instructions, and then it was eluted from the column in 100 μl of TE prime buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). Extracted DNA was directly analyzed by LightCycler PCR without storage. Positive extraction controls were 10-μl aliquots of a 0.5 McFarland standard of *B. pertussis* (ATCC 9797) diluted 1:2,000 with PBS. Control aliquots were stored at −80°C until use.

Primers and probes for *Bordetella* real-time PCR have been described previously (7). Oligonucleotides were synthesized by IT BioChem (Salt Lake City, Utah). All primers and probes were resuspended by using TE prime buffer.

PCR inhibition was assessed by the addition of approximately 50 fg of previously extracted *B. pertussis* DNA. The DNA was applied in 5-μl volumes to each well of microtiter plates, allowed to desiccate, and stored at −20°C until put into use. Once thawed for use, the inhibition control plates were held at room temperature for up to 1 month. An aliquot of each PCR mixture was applied to a well of the inhibition control plate before analysis (described below).

A multiplex real-time PCR assay was used for PCR analysis of *Bordetella* sp. The PCR master mix consisted of 3 mM $MgCl_2$, 1× LightCycler FastStart DNA master hybridization probes (Roche Diagnostics, Indianapolis, Ind.) prepared according to the manufacturer's instructions, 0.5 μM concentrations of each forward primer, 1.0 μM concentrations of each reverse primer, 0.2 μM concentrations of *B. pertussis* anchor and reporter probes, 1.0 μM concentrations of *Bordetella parapertussis* anchor and reporter probes, and 20 U of uracil-*N*-glycosylase (UNG; Applied Biosystems, Foster City, Calif.)/ml. The master mix was prepared to achieve a total PCR volume of 30 μl per sample (24 μl of master mix plus 6 μl of sample DNA or control). Twenty microliters was transferred to a LightCycler capillary tube (Roche Diagnostics). The remaining 10 μl was mixed in a well of the inhibition control plate (described above) prior to being transferred to a capillary tube. All capillary tubes were capped, inserted into a LightCycler carousel, and centrifuged prior to amplification and detection. The cycling parameters began with 1 cycle of 40°C for 4 min (activation of UNG) and 95°C for 9 min (inactivation of UNG and activation of FastStart polymerase). The second part of the cycling protocol was 50 cycles of 95°C for 1 s, 53°C for 8 s, and 72°C for 18 s. The last portion of the protocol was a cooldown step at 40°C for 5 s. Data analysis was performed with LightCycler, version 3.5.3, software. A positive result was defined as showing a visible fluorescence curve and an apparent crossing threshold value by using the second derivative maximum function of the LightCycler software. Positivity rates were established, and statistical analysis was performed with the chi-square test.

Tenfold dilutions of 10 separate *B. pertussis* suspensions (0.5 McFarland) were analyzed. Using dry swabs inoculated with a 10^{-3} dilution of organisms from a 0.5 McFarland suspension, PCR was positive for all inoculated dacron and rayon swabs. However, for calcium-alginate, PCR was only positive for 50% of all samples analyzed at the same dilution (Table 1). This difference was not statistically significant ($P = 0.15$), likely due to the high concentration of organisms. At the 10^{-4} dilution, the differences in detection rates between calcium-alginate swabs and swabs of all other materials were statistically significant (Table 1). In an effort to mimic the recommended transport system for swab samples submitted for culture (3), inoculated swabs were inserted into Regan-Lowe deep transport media and incubated at 36°C for 48 h before testing. Table 2 shows the PCR results of swabs inserted into transport media. Dacron and rayon swabs both resulted in 100% positivity with samples at the 10^{-5} dilutions while calcium-alginate swabs were positive only 20% of the time at the 10^{-5} dilution ($P < 0.02$). Because of our anticipation of bacterial growth during the 48-h incubation, the 10^{-3} dilution was not tested with swabs inserted into Regan-Lowe media. Rayon swabs incubated for 48 h in Amies with charcoal medium showed a lower

TABLE 2. *B. pertussis* PCR and culture positivity rates following incubation for 48 h in Regan-Lowe or Amies with charcoal medium

Dilution (*n*)	% Positive with swab type (medium)[a]:							
	Dacron (RL)		Rayon (RL)		Calcium-alginate (RL)		Rayon (Amies with charcoal)	
	PCR	Culture	PCR	Culture	PCR	Culture	PCR	Culture
10^{-3} (10)	NP	NP	NP	NP	NP	NP	100	20
10^{-4} (10)	100	100	100	100	40	100	80	0
10^{-5} (10)	100	90	100	90	20	60	40	0
10^{-6} (10)	50	40	20	20	0	20	0	NP
10^{-7} (10)	0	0	0	0	0	0	NP	NP

[a] RL, Regan-Lowe deep medium; NP, not performed.

sensitivity of detection by PCR than rayon or dacron swabs incubated for 48 h in Regan-Lowe media (Table 2).

A newer swab transport system for PCR is the DNA/RNA Protect system, consisting of a standard-sized rayon swab with a plastic shaft which is inserted into a tube containing a proprietary moist sponge-like material. PCR positivity for this system was similar to that of the no-swab control (Table 1).

Culture of dacron and rayon swabs after incubation in Regan-Lowe transport media for 48 h prior to plating was just as good or better than with calcium-alginate swabs (Table 2). Both rayon and dacron showed 90% recovery while calcium-alginate showed a 60% recovery at the 10^{-5} dilution. No growth was detected at the 10^{-4} or 10^{-5} dilutions with the Amies with charcoal transport media.

PCR inhibition was only detected when calcium-alginate swabs were used. Inhibition rates were determined by using three separate protocols for sample preparation prior to DNA extraction. The lysis buffer preparation resulted in 100% inhibition with calcium-alginate swabs. When the swabs were soaked in 1 ml of PBS prior to pelleting and extraction, the inhibition rate dropped to 71%. Better performance was seen by soaking the swabs in 2 ml of PBS and pelleting half of the 2-ml suspension prior to extraction, which dropped the inhibition rate to 21%.

Dacron or rayon NP swabs with aluminum wire shafts can be used for PCR for the detection of *B. pertussis*. Also, if the swab is to be plated for culture prior to PCR, dacron or rayon swabs can be used as well. Neither type of synthetic swab, dacron or rayon, is superior to the other according to our studies. They both perform well for PCR and culture. For laboratories aiming to validate a PCR test for *B. pertussis* detection, sample retrieval would be simplified if the same swab submitted in Regan-Lowe media could be used. The swab samples would have been incubated for 48 h prior to plating, after which time the remaining organisms would be available on the swab for PCR.

As PCR becomes more popular for the detection of infectious diseases, attention must be given to collection systems. The DNA/RNA Protect system showed promising results in our study. The nucleic acid preservative allows the bacteria to be centrifuged into a pellet. Although the swabs included in the DNA/RNA Protect system for our study were rayon swabs with standard-sized heads and plastic shafts, NP swabs with aluminum wire shafts can be used with this system as well.

The main component of calcium-alginate swabs responsible for PCR inhibition has been reported to be the alginate, which is a crude extract from seaweed (10). Reports of whether or not components of the aluminum shaft are inhibitory have been conflicting. Wadowsky et al. (10) reported inhibition due to the aluminum shaft, whereas Rasmussen et al. (6) and He et al. (1) had success with aluminum shafts in their PCR assays. We did not see any inhibition with swabs containing aluminum shafts, even after we had soaked the swabs, with 2 in. of aluminum shaft, overnight before extraction and analysis of DNA (unpublished observation). The controversy may be due to differences in swab components provided by manufacturers or to differences in extraction methods.

Unlike Regan-Lowe medium, Amies with charcoal supports growth of *B. pertussis* poorly and therefore should not be used to increase the sensitivity of detection. This finding is in agreement with other reports (3, 8). For PCR detection, our studies show that rayon swabs incubated for 48 h in Amies with charcoal transport medium performed similar to PCRs of dry swabs (Tables 1 and 2). Medium containing animal blood (i.e., Regan-Lowe media) is known to be advantageous to the support of growth of *B. pertussis* (4). Amies medium does not contain blood. To increase the sensitivity of detection by either PCR or culture, we found Regan-Lowe medium to be advantageous.

Calcium-alginate swabs should not be used for the PCR detection of *B. pertussis*. If a calcium-alginate swab is received for PCR detection of *B. pertussis*, washing the swab in 2 ml of PBS reduces the inhibition rate. Based upon our findings, it is advisable to use only dacron or rayon swabs for all NP swab collections for culture and/or PCR of *B. pertussis*.

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