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Diagnosis of Community-Acquired Pertussis Infection: Comparison of Both Culture and Fluorescent-Antibody Assays with PCR Detection Using Electrophoresis or Dot Blot Hybridization

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Diagnosis of *Bordetella pertussis* infection has been difficult due to the low sensitivity of culture. PCR tests have been shown to be more sensitive than culture, but the reported sensitivity of PCR is variable. We evaluated PCR product detection by using either agarose gel electrophoresis (PCR-gel) or dot blot hybridization with ^{32}P-labeled oligonucleotide probes, and we compared these methods to both culture and direct fluorescent-antibody (DFA) assays with microscopy for the detection of pertussis. This was done with 225 nasopharyngeal swab specimens collected in community clinic settings. The multiplexed PCR amplified the multiply repeated IS*481 B. pertussis* sequence and a sequence from the human globin gene as a positive control for specimen adequacy. Of 225 specimens, 179 were judged to be adequate for PCR analysis. Among the adequate specimens, 9, 4, and 10 were culture, DFA, and PCR-gel positive, respectively. The sensitivity of PCR-gel versus culture was 89% while the sensitivity of culture versus PCR-gel was 80%. DFA had the lowest sensitivity. Thirty specimens were positive by PCR with dot blot hybridization; no negative control specimens showed a signal above the background. Among the 79 (44%) adequate specimens with clinical data available, the rates of reported cough or persistent cough were similar for persons who were pertussis positive by each assay. The IS*481* PCR, with either electrophoresis or dot blot hybridization, is a sensitive assay; however, at this time it cannot completely replace culture without an overall loss in sensitivity for the detection of pertussis. Further study is required to understand the clinical significance of *B. pertussis* PCR products detected by dot blot hybridization alone.

Bordetella pertussis causes persistent cough in children and adults (22) and potentially life-threatening apnea in infants. Although vaccination has been proven effective in dramatically reducing rates of disease over the last half century (4), increasing rates of adverse events in older children and adults restricted the use of whole-cell vaccines to infants and young children. While use of the acellular pertussis vaccine may reduce reactogenicity from pertussis vaccination, (2) waning protection after the last routine childhood dose of vaccine (13) has resulted in a reservoir of susceptible adolescents and adults capable of developing pertussis infection and passing it on to under- or unvaccinated infants. Although recommendations for the use of the acellular pertussis vaccine in adolescents and young adults have been proposed (3), currently interruption of the spread of infection rests on early diagnosis and prompt treatment of persons with pertussis.

The diagnosis of pertussis has been problematic (17). Although culture of pertussis is highly specific and often considered the diagnostic standard, sensitivity has been reported to be as low as 25 to 50% when judged against serology (9, 10). Direct fluorescent-antibody (DFA) assay has been found to be prone to false-positive results (6), and although serodiagnosis can be sensitive and specific (20), its requirement of a convalescent-phase specimen makes serology of minimal clinical utility.

Several PCR assays for the diagnosis of pertussis infection have been developed, and clinical studies have compared the sensitivity and specificity of these PCR tests to culture, serology, and DFA assay. In general, pertussis PCR has been found to be as or more sensitive than culture, although the reported sensitivity of various pertussis PCR assays versus serologic evidence of infection has ranged from 21 to 61% (11, 19, 21). More recently, real-time PCR assays have also been reported (7, 18). However, it is difficult to compare different studies which used PCR diagnosis, since many studies differ in DNA purification techniques, PCR primers, reaction conditions, and product detection methods. In particular, there have been no studies with clinical specimens that directly compare the most commonly employed product detection techniques of gel electrophoresis (using agarose or polyacrylamide) and dot blot hybridization. Furthermore, few studies have examined the utility of specific PCR diagnostic assays in the context of routine clinical diagnostic testing. Clinical diagnosis with routinely collected specimens is complicated by lower incidence rates, leading to a lower positive predictive value for diagnostic assays than would be encountered in a high-incidence population experiencing a pertussis outbreak.

A PCR assay for *B. pertussis* based on detection of the pertussis-specific multiply repeated IS*481* sequence has been previously described (8, 12, 14). A PCR assay based on this method was first used at the Washington State Department of Health (DOH) in 1996. By 1997, this assay, in conjunction with culture and DFA analysis, was used for the routine detection of *B. pertussis*. We were interested in assessing the comparative

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sensitivities and specificities of this PCR assay with those of culture and DFA assay among the routine clinical respiratory swabs. In addition, since agarose gel electrophoresis detection of PCR products is not sequence specific, we compared this method (PCR-gel) to a highly sensitive and sequence-specific dot blot hybridization assay with a ^{32}P-labeled oligonucleotide probe.

MATERIALS AND METHODS

Specimens. The Washington State DOH receives nasopharyngeal swab specimens (primarily Dacron swabs on a rigid shaft) taken statewide from patients suspected of pertussis infection. All swabs are cultured for *B. pertussis* by being streaked onto Regan-Lowe agar plates and incubating the plates for 3 to 5 days. The DFA assay with microscopy was performed only when clinicians provided a glass microscope slide streaked with the patient's nasopharyngeal sample; fluorescent detection was accomplished with the Accu-MAb DFA conjugate (Cytovax Biotechnologies, Inc., Edmonton, Alberta, Canada).

Between 1 January and 28 February 1998 all clinical swabs sent by private physicians or community or public health clinics to the DOH for pertussis evaluation were stored at −20°C after routine processing. After storage for <2 months at −20°C, the swabs were randomly divided between each of two study site labs (site 1 and site 2) and processed independently for DNA by personnel proficient in molecular biology techniques. DNA aliquots were shared between the sites to allow PCR studies for each swab to be independently performed at each site. PCR-gel product detection was performed at both sites and dot blot hybridization was performed at one site (site 2).

Specimen processing. Swabs were placed in 1.5-ml Eppendorf tubes, and the swab handles were cut with clean scissors distal from the swab itself. Swabs were then solubilized and processed with QiaAmp kits (Qiagen, Inc., Valencia, Calif.) by using buffers included in the kits and following the manufacturer's tissue extraction procedure.

PCR primers and hybridization probes. Standard purity synthetic deoxynucleotide primers were purchased (Gibco-BRL, Inc./Invitrogen Corp., Carlsbad, Calif.) and dissolved to concentrations of 1 mM in distilled water. Primers for amplification of the IS*481 B. pertussis*-specific repeat sequence were BP1 (5′-GATTCAATAGGTTGTATGCATGGTT-3′) and BP2 (5′-AATTGCTGGACCATTTCGAGTCGACG-3′). Amplification of the human globin gene as an internal positive control was performed with primers HG1 (5′-CAACTTCATCCACGTTCACC-3′) and HG2 (5′-GAAGAGCCAAGGACAGGTAC-3′). An oligonucleotide probe, 5′-ACACCCATAAGCATG-3′, was synthesized for specific dot blot detection of amplification products from the *B. pertussis* IS*481* gene. A probe for dot blot detection of amplification products from the human globin gene was also synthesized (5′-AGAGCCATCTATTGC-3′) and used for a limited number of specimens, but dot blot hybridization with the globin probes was not performed systematically for all specimens.

The specificities of primers and probes were evaluated by performing PCRs on 1 ng of genomic DNA isolated from clinical *Bordetella* spp. isolates (*B. pertussis*, *B. parapertussis*, and *B. holmesii*) and from clinical isolates of organisms identified as potential respiratory tract colonizers or pathogens, including *Archanobacterium pyogenes*, *Bacillus fragilis*, *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Moraxella catarrhalis*, *Peptostreptococcus* spp., *Staphylococcus aureus*, and *Streptococcus pyogenes* (group A). The sensitivities of primers and probes were evaluated with serial dilutions of pertussis genomic DNA starting at 40 pg (10 genome equivalents) and diluting to 0.04 pg (0.01 genome equivalents).

As a result of the target being multiply repeated, the IS*481 B. pertussis* PCR assay used in this study is highly sensitive with a detection limit of ~1 to 0.5 genome equivalents by agarose gel electrophoresis and 0.5 to 0.1 genome equivalents by dot blot analysis (data not shown). PCR amplification was detectable only from *B. pertussis* and the closely related *B. holmesii* but not from *B. parapertussis* or other respiratory pathogens and commensals. This finding has been previously reported (15).

PCRs. PCRs were performed in 25-μl volumes with 0.2 mM (each) dATP, dGTP, dCTP, and dUTP, 2.5 μl of *Taq* buffer, 5.0 μl of Qiagen Q-solution, 0.2 μl of *Taq* polymerase (Qiagen, Inc.), and 0.5 μM primer. Uracil DNA glycosylase (0.25 μl) (New England Biolabs, Beverly, MA) was added to each reaction mixture to reduce the risk of contamination with PCR products (16). The reaction mixtures were multiplexed with all four primers (BP1, BP2, HG1, and HG2), and PCR was performed with a Perkin Elmer 6600 (site 1) or an Ericomp thermocycler (site 2) with one 10-min denaturation step at 94°C, 30 cycles of amplification of 1 min at 94°C, 30 s at 60°C, and 45 s at 72°C, and a final 5-min extension at 72°C. PCR analysis was performed independently at each site; the number of repeated runs for a given specimen at each site depended on the consistency of the results obtained for that sample and the amount of specimen remaining. Overall, PCR was performed on each specimen at each site at least once and up to four times, with detection by gel electrophoresis at both sites as well as by dot blot hybridization at site 2. Multiplexed PCRs with high levels of pertussis amplification were repeated with human globin primer pairs alone. A negative-control reaction mixture with water substituted for specimen DNA was included after every fourth clinical specimen reaction mixture. One positive-control reaction mixture with 1 genome equivalent of *B. pertussis* genomic DNA was included for each PCR run.

PCR product detection. (i) Agarose gel. The 154-bp pertussis and 268-bp human globin amplification products were separated on a 1% Metaphor agarose gel (FMC Corporation, Philadelphia, Pa.) in TAE buffer (50× TAE is 242 g of Tris base, 57.1 ml of glacial acetic acid, and 100 ml of 0.5 M EDTA, pH 8.0) with 10 μl of PCR product and 2 μl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) and run at 100 V for 30 min; clinical specimens and negative controls were loaded onto gels in the same order that they were manipulated for PCR. Gels were stained for 15 min in 0.5 mg of ethidium bromide/ml, destained for 30 min in water, and visualized on a UV transilluminator.

(ii) Dot blot hybridization. After PCR-gel analysis, 6 μl of PCR product was denatured with 0.6 μl of denaturing solution (3.6 N NaOH, 1 μl of 1-mg/ml sheared salmon sperm DNA). From this mix, 2 μl was spotted on GeneScreen nylon membranes (New England Nuclear/Perkin Elmer Life Sciences, Boston, Mass.), which were cross-linked on a UV irradiator (Stratagene, La Jolla, Calif.). Prehybridization was done in 0.8 M NaCl, 0.2 M Tris-Cl (pH 8.0), 0.05 M EDTA-Na_2, 1% sodium dodecyl sulfate, and 0.5% nonfat milk. Specific oligonucleotides were 5′ end labeled with [^{32}P]ATP (New England Nuclear/PerkinElmer Life Sciences) and polynucleotide kinase (New England Biolabs, Beverly, Mass.) at 37°C for 30 min, with a final specific activity of 3×10^5 to 5×10^5 counts/min/μg. Hybridization was performed for 2 h at 30°C in prehybridization buffer with 0.1 to 0.3 pM of specific labeled oligonucleotide. Filters were washed three times for 10 min each in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (pH 7.0) with 1% sodium dodecyl sulfate at 34°C and exposed to Kodak XAR-5 film. We assessed detection sensitivity by using 8-h (short) and 24-h (long) exposures. Individual short- or long-exposure dot blot signals were classified as positive if the signal was greater than the signal from PCR-negative controls or classified as negative if the signal was less than that of the controls.

Clinical symptoms. Clinical symptoms reported by the patients were collected from the request slips that accompanied the swabs and entered into the lab result database; the date of symptom onset was generally not available. Variables were created to identify cases in which cough or chronic cough were reported or in which the patient had a positive test for respiratory syncytial virus (RSV) (no details on the type of test were obtained from the request slips).

Data analysis. Results from multiple gel runs at each testing site and short or long exposure dot blot analyses at one testing site were combined into final results as follows. (i) Results were first analyzed by a specific detection method: PCR-gel, dot blot with short exposure, or dot blot with long exposure. A specimen was positive by a specific detection method if >2 PCR runs had been performed and the pertussis amplicon was detected by that method in ≥50% of the runs. If only 2 runs were performed, specimens were classified positive, indeterminate, or negative if pertussis amplicons were present on both, one, or no runs, respectively. If both globin and pertussis PCR results were negative by electrophoresis, the specimen was classified as inadequate. (ii) PCR results from short- and long-exposure dot blots were then combined into a final dot blot result. If the results of the short and long exposures were in agreement, then that result was used as the final dot blot result. If the short exposure result was negative and the long exposure result was positive, the final dot blot result was positive. If the short exposure result was positive and the long exposure result was negative or indeterminate, the final result was indeterminate. In the event of an indeterminate short exposure, the final result would be classified as indeterminate, except if the long exposure were positive, in which case the final result would be positive.

RESULTS

Two hundred twenty-five swabs for pertussis evaluation were received by the Washington State DOH during the study period. Table 1 summarizes the testing results for all 225 speci-

TABLE 1. PCR, culture, and DFA assay results

Test	No. of specimens with result[a]					No. of adequate specimens with result[b]				
	Indeterminate	Positive	Negative	Missing	Total	Indeterminate	Positive	Negative	Missing	Total
Culture	0	10	215	0	225	0	9	170	0	179
DFA	0	4	182	39	186	0	4	150	25	154
Agarose gel										
Pertussis PCR	0	10	215	0	225	0	10	169	0	179
Globin PCR	0	178	47	0	225	0	178	1[c]	0	179
Dot blot										
Short exposure	0	29	196	0	225	0	29	150	0	179
Long exposure	10	29	186	0	225	10	29	140	0	179
Combined result	1	30	194	0	225	1	30	148	0	179

[a] Results are for all specimens.
[b] Globin or pertussis amplicons were detected with multiplexed primers or with a globin primer pair alone.
[c] This specimen was negative for pertussis and globin PCR amplicons by gel electrophoresis but positive for pertussis by dot blot hybridization.

mens tested and for the 179 specimens classified as adequate based on combined PCR-gel and dot blot detection.

PCR-gel. After the swabs were processed for DNA, all specimens were independently analyzed at each study site, with totals of 359 and 456 multiplexed PCRs with gel electrophoresis detection performed at study sites 1 and 2, respectively. A total of 6 of 815 (0.7%) pertussis specimens and 60 of 823 (7.3%) globin specimens gave PCR-gel electrophoresis assay results that were discordant with the majority of test results for that specimen at that site. The rates of discordant results varied by site from 0.2 to 1.1% for pertussis amplification and 7.4 to 13% for globin amplification. The 6 discordant pertussis results were from specimens classified as indeterminate at either site. When the analysis of specimen results by site was compared to the analysis of specimen results from both sites combined, the only difference was that the 6 specimens with indeterminate results in the site-specific analyses were classified as negative (3 specimens) or positive (3 specimens) based on the combined data from both sites.

Seven specimens demonstrated the presence of high levels of pertussis amplification and the absence of globin on multiplex PCR; all seven demonstrated the 268-bp globin amplicon when tested by globin PCR alone. For specimens that had high levels of globin amplification on multiplex PCR, no discrepancies in pertussis results were noted even when PCR was repeated with pertussis primers alone. No contamination was present, as measured by the absence of the 154-bp pertussis band in 203 negative-control specimens.

When results of gel electrophoresis detection at both sites were combined, 10 of 225 specimens were positive for pertussis.

Based on negative electrophoresis results for both pertussis and globin PCR products, 47 of 225 specimens were classified as inadequate for PCR.

PCR with detection by dot blot hybridization. Repeat dot blot assays for pertussis were performed on each specimen two to four times (a mean of three repeats for long exposures and two repeats for short exposures). Six hundred eighty-five PCR assays were tested with short exposures (8 h), and 455 PCR assays were tested with long exposures (24 h). Results differed from the majority of results for the same specimen in 34 of 685 (5%) of the short exposures and in 13 of 455 (2.8%) of the long exposures.

Thirty-one specimens were identified as positive by either short- or long-exposure dot blot analysis. Two specimens positive by long exposure but negative by short exposure were classified as dot blot positive. No specimens had an indeterminate short-exposure result with a positive long-exposure result. When short- and long-exposure results of dot blot hybridization assays were combined, 30 of 225 specimens were positive for the pertussis PCR amplicon. One specimen with a positive short-exposure result and a negative long-exposure result was given a final dot blot classification of indeterminate. The indeterminate specimen was associated with negative-pertussis and positive-globin PCR-gel results with two of four short-exposure dot blots interpreted as positive and both long-exposure results classified as negative. One of the 47 specimens initially classified as inadequate for PCR by gel electrophoresis was positive on two of four short-exposure dot blots and two of four long-exposure dot blots. This specimen was therefore reclassified as positive by pertussis dot blot and therefore adequate for PCR; thus, based on gel and dot blot detection, 179 specimens were adequate for PCR.

Dot blot detection with the globin oligonucleotide probe was only performed on a small number of specimens, including some specimens that were negative by gel detection. The globin hybridization results for those specimens tested did not differ from the PCR-gel results (data not shown), possibly reflecting the difference in copy number for the two genes (2 copies of the globin gene per human haploid cell and 80 copies of the IS*481* insertion sequence per pertussis cell) and characteristics of the PCR and probe hybridization.

Comparison of conventional culture, DFA assay, PCR-gel, and PCR dot blot analysis. Table 2 summarizes the relationships among the overall results obtained from the different testing methods. Among the nine adequate specimens that were culture positive for *B. pertussis*, eight were positive by pertussis PCR (either gel or dot blot detection) and one was negative. Two PCR-gel-positive specimens were negative on culture. Dot blot detection identified 22 positive specimens that were culture negative; 20 of these were negative by PCR-gel. All specimens positive by PCR-gel were also positive by PCR dot blot analysis. Of three adequate specimens in which culture and PCR-gel results were discordant, two (one PCR positive and one culture positive) had a DFA assay performed with negative results for both (data not shown). A fourth specimen with discordant culture and PCR-gel results (culture positive and PCR-gel negative) was found to be inadequate for PCR analysis. The overall sensitivities of specimens adequate

TABLE 2. Comparison of DFA, PCR, and culture assays for specimens considered adequate

Culture result	No. of specimens with result for assay								
	DFA			Pertussis PCR-gel			Pertussis PCR with dot blot hybridization		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	4[a]	5[a]	9[a]	8	1	9	8	1	9
Negative	0[a]	145[a]	145[a]	2	168	170	22	147	169[b]
Total	4	150	154	10	169	179	30	148	178

[a] Culture data for specimens with DFA performed.
[b] A specimen with an indeterminate dot blot result was not included.

for PCR analysis by DFA assay, PCR-gel detection, and PCR dot blot detection were 44, 89, and 89%, respectively, of those for culture detection. The sensitivity of the DFA assay was 44 and 15% of that found for gel and dot blot detection, respectively (data not shown). The detection sensitivity of culture was 80 and 27% of that found for PCR-gel and PCR dot blot detection, respectively.

Clinical correlations. Clinical symptoms were reported with 95 (42%) of the specimens, with 49 (52%) patients reporting a history of cough, 17 (18%) patients reporting a cough with a duration of more than 10 days, and 6 (6%) patients having a history of recent RSV infection (the specimen slips gave no indication of how this result was obtained). Clinical data available were only available for 79 (35%) adequate specimens. Clinical symptoms were reported for 4 (40%), 5 (50%), and 14 (50%) of the specimens that were pertussis culture positive, PCR-gel positive, or PCR dot blot positive, respectively. Rates of cough, chronic cough, and RSV were similar when culture-, PCR-gel-, and PCR dot blot-positive specimens were compared (Table 3). Among the 18 specimens that were negative for pertussis by gel detection of PCR amplicons and positive by dot blot detection, 9 were reported with associated clinical findings; of these, 5 (56%) patients had cough and 2 (22%) patients had persistent cough. Of four specimens that were adequate for PCR and identified as coming from a patient with RSV infection, three were negative for pertussis by all assays and one was positive by PCR dot blot analysis.

DISCUSSION

Clinical diagnosis of pertussis has been plagued by poor sensitivity and/or specificity of diagnostic assays. This has resulted in an overall low rate of successful diagnosis as well as pseudo-outbreaks associated with false-positive test results (F. Lievano, National Immunization Program, Centers for Disease Control and Prevention, personal communication).

In this report we compare the laboratory test results of four different assays for *B. pertussis* with 225 community-acquired clinical specimens. When assessed without regard to the adequacy of the specimen for PCR, the sensitivities of culture with PCR-gel detection as a "gold standard" and PCR-gel detection with culture as a "gold standard" were both 80%. Among specimens identified as adequate for PCR analysis, gel electrophoresis detection of pertussis PCR amplicons had a sensitivity of 89% of that of culture, whereas the sensitivities of culture versus gel and dot blot detection of PCR amplicons were 80 and 27%, respectively. The sensitivity of the DFA assay was 44% of that of culture and PCR with gel detection; the sensitivity of the DFA assay was 15% of that of PCR with dot blot detection. The low sensitivity of DFA is notable since the assay performed here was based on a monoclonal antibody. These data, therefore, reemphasize the insensitivity of this assay compared to either culture or PCR and underscore that it should only be used in conjunction with other assays.

Despite the higher sensitivity of PCR with agarose gel detection than that of culture among specimens deemed adequate for PCR, at least one specimen in our cohort was negative for pertussis by PCR but positive by culture. This is slightly lower than, but consistent with, the results of previous studies that found 2% of specimens were negative for *B. pertussis* culture but positive for pertussis PCR (17). Furthermore, over 20% of all specimens in this study were deemed to be inadequate for PCR analysis. Since at least one of these specimens was culture positive for *B. pertussis*, specimen inade-

TABLE 3. Comparison of pertussis laboratory test results and clinical findings (n = 95)

Clinical symptom and status	No. (%) of specimens with result for assay								
	Culture			PCR-gel			PCR with dot blot hybridization		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Cough									
Present	2 (50)	2 (50)	4	3 (60)	2 (40)	5	8 (57)	6 (43)	14
Absent	47 (52)	44 (48)	91	46 (51)	44 (49)	90	41 (51)	39 (49)	80
Persistent cough									
Present	1 (25)	3 (75)	4	2 (40)	3 (60)	5	4 (29)	10 (71)	14
Absent	16 (18)	75 (82)	91	15 (17)	75 (83)	90	13 (16)	67 (84)	80
RSV									
Present	0 (0)	4 (100)	4	0 (0)	5 (100)	5	1 (7)	13 (93)	14
Absent	6 (7)	85 (93)	91	6 (7)	84 (93)	90	5 (6)	75 (94)	80

quacy can have a significant impact on measured PCR sensitivity and could explain some of the differences in PCR sensitivity reported in the literature. We cannot determine if inadequate specimens arose from compromised specimen collection or inefficient specimen extraction or if streaking the swab for culture reduced the specimen quality for subsequent PCR analysis. Regardless of the explanation, these data suggest that clinical laboratories performing PCR assays for pertussis should consider routinely monitoring specimen adequacy. Furthermore, PCR sensitivity would likely be higher if two swabs are collected simultaneously from each patient (possibly from opposite nares) with one processed for culture and one processed for PCR. These results also suggest that, particularly in the absence of a routine assay for specimen adequacy, at this time PCR should not replace culture in the routine diagnosis of pertussis.

We analyzed our data to assess the reproducibility of the pertussis and globin PCR assays performed at each participating site. The higher variability found with the globin assay is likely due to the high copy number of the IS*481* sequence in the pertussis genome. Although overall test results were generally highly reproducible, significant differences in rates of discrepant results were noted between the sites. We conclude that periodic and standardized determination of PCR assay reproducibility is therefore an important quality control mechanism that should be considered in any clinical laboratory planning to use PCR for clinical diagnosis of pertussis.

We placed a high emphasis on limiting contamination during PCR analysis through the use of barrier (laminar flow hood, filtered pipette tips, and frequent glove changes) and enzymatic (uracil glycosylase) methods. As a result, none of the more than 200 negative-control specimens run during this study were found to be positive by any detection method. In view of this lack of cross-contamination, it is highly significant that pertussis-specific dot blot hybridization detected nearly three times the number of positive specimens as identified by either PCR-gel or culture. These results may represent the enhancement of the sensitivity of PCR to detect organisms at a point when they are no longer culturable (21). Alternatively, the high number of positive PCR results by dot blot detection may suggest the presence of colonizing organisms at a level not detectable by standard culture. Although we did not find any difference in clinical symptoms exhibited by persons testing positive for pertussis by each of our assays, with only 35% of adequate specimens having an associated cursory clinical history, our data are insufficient to assess the relationship of PCR dot blot positivity to clinical symptoms. *Bordetella bronchiseptica* is known to colonize dogs (5), and detection of canine colonization with *B. pertussis* has recently been reported (1). While pertussis colonization in humans would not represent a significant clinical problem to the colonized individual, it could have more-significant public health implications, particularly if colonization did occur and was not affected by vaccination. Larger prospective studies of pertussis PCR with dot blot detection are needed to further understand these findings.

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REFERENCES

1. **Belcher, K. L., D. J. Wise, and H. P. Veit.** 2000. Canine colonization and transmission of Bordetella pertussis. Ann. N. Y. Acad. Sci. **916:**687–690.
2. **Braun, M. M., G. T. Mootrey, M. E. Salive, R. T. Chen, and S. S. Ellenberg.** 2000. Infant immunization with acellular pertussis vaccines in the United States: assessment of the first two years' data from the Vaccine Adverse Event Reporting System (VAERS). Pediatrics **106:**E51.
3. **Campins-Marti, M., H. K. Cheng, K. Forsyth, N. Guiso, S. Halperin, L. Huang, J. Mertsola, G. Oselka, J. Ward, C. H. Wirsing von Konig, and F. Zepp.** 2001. Recommendations are needed for adolescent and adult pertussis immunisation: rationale and strategies for consideration. Vaccine **20:**641–646.
4. **Cherry, J.** 1984. The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a comparative study. Curr. Probl. Pediatr. **14:**1–78.
5. **Coote, J. G.** 2001. Environmental sensing mechanisms in Bordetella. Adv. Microb. Physiol. **44:**141–181.
6. **Ewanowich, C., L. W.-L. Chui, M. Paranchych, M. Peppler, R. Marusyk, and W. Albritton.** 1993. Major outbreak of pertussis in northern Alberta, Canada: analysis of discrepant direct fluorescent-antibody and culture results by using polymerase chain reaction methodology. J. Clin. Microbiol. **31:**1715–1725.
7. **Farrell, D. J., M. McKeon, G. Daggard, M. J. Loeffelholz, C. J. Thompson, and T. K. Mukkur.** 2000. Rapid-cycle PCR method to detect *Bordetella pertussis* that fulfills all consensus recommendations for use of PCR in diagnosis of pertussis. J. Clin. Microbiol. **38:**4499–4502.
8. **Glare, E., J. Paton, R. Premier, A. Lawrence, and I. Nisbet.** 1990. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J. Clin. Microbiol. **28:**1982–1987.
9. **Granstrom, G., B. Wretlind, and M. Granstrom.** 1991. Diagnostic value of clinical and bacteriological findings in pertussis. J. Infect. **22:**17–26.
10. **Halperin, S., R. Bortolussi, and A. Wort.** 1989. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. J. Clin. Microbiol. **27:**752–757.
11. **Heininger, U., G. Schmidt-Schlapfer, J. Cherry, and K. Stehr.** 2000. Clinical validation of a polymerase chain reaction assay for the diagnosis of pertussis by comparison with serology, culture, and symptoms during a large pertussis vaccine efficacy trial. Pediatrics **105:**E31-E36.
12. **Houard, S., C. Hackel, A. Herzog, and A. Bollen.** 1989. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. Res. Microbiol. **140:**477–487.
13. **Lambert, H. J.** 1965. Epidemiology of a small pertussis outbreak in Kent County, Michigan. Public Health Rep. **80:**365–369.
14. **Loeffelholz, M. J., C. J. Thompson, K. S. Long, and M. J. Gilchrist.** 1999. Comparison of PCR, culture, and direct fluorescent-antibody testing for detection of *Bordetella pertussis*. J. Clin. Microbiol. **37:**2872–2876.
15. **Loeffelholz, M. J., C. J. Thompson, K. S. Long, and M. J. Gilchrist.** 2000. Detection of *Bordetella holmesii* using *Bordetella pertussis* IS*481* PCR assay. J. Clin. Microbiol. **38:**467.
16. **Longo, M. C., M. S. Berninger, and J. L. Hartley.** 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene **93:**125–128.
17. **Muller, F.-M. C., J. E. Hoppe, and C.-H. Wirsing von Konig.** 1997. Laboratory diagnosis of pertussis: state of the art in 1997. J. Clin. Microbiol. **35:**2435–2443.
18. **Reischl, U., N. Lehn, G. N. Sanden, and M. J. Loeffelholz.** 2001. Real-time PCR assay targeting IS*481* of *Bordetella pertussis* and molecular basis for detecting *Bordetella holmesii*. J. Clin. Microbiol. **39:**1963–1966.
19. **Schlapfer, G., H. Sen, R. Berger, and M. Just.** 1993. Use of the polymerase chain reaction to detect Bordetella pertussis in patients with mild or atypical symptoms of infection. Eur. J. Clin. Microbiol. Infect. Dis. **12:**459–463.
20. **Steketee, R., D. Burstyn, S. Wassilak, W. J. Adkins, M. Polyak, J. Davis, and C. Manclark.** 1988. A comparison of laboratory and clinical methods for diagnosing pertussis in an outbreak in a facility for the developmentally disabled. J. Infect. Dis. **157:**441–449.
21. **Van der Zee, A., C. Agterberg, M. Peeters, F. Mooi, and J. Schellekens.** 1996. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J. Infect. Dis. **174:**89–96.
22. **Wright, S., K. Edwards, M. Decker, and M. Zeldin.** 1995. Pertussis infection in adults with persistent cough. JAMA **273:**1044–1046.