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Multitarget PCR for Diagnosis of Pertussis and Its Clinical Implications▽

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PCR has greatly facilitated pertussis diagnosis due to the speed, sensitivity, and specificity of this assay compared to other detection methods. Various single-target PCR assays are currently utilized, but none is universally considered to be the "gold standard." Our aim was to assess the use of multitarget versus single-target PCR for the diagnosis of pertussis in clinical samples. PCR assays targeting insertion sequence IS*481* (IS), pertussis toxin *ptxA* promoter region (PT), and outer membrane porin (PO), or *recA* (RA) were evaluated in respiratory specimens collected from 4,442 patients with suspected pertussis. The diagnosis of pertussis was confirmed in 309 (6.96%) patients by the 3-target IS-PT-PO/RA PCR versus 247 (5.56%) by the conventional single-target IS ($P = 0.007$). Compared to single-target IS, the three-target combination increased the proportion of positive specimens by 1.25-fold, and two-target combinations increased the proportion of positive specimens by 1.10- to 1.24-fold. In addition, nine cases of *B. parapertussis* infection were also confirmed by using the discriminative features of this multitarget PCR. Of the 89 culture-proven pertussis cases, 17 (19.1%) and 5 of the 16 patients (31.3%) admitted to intensive care unit would have been missed had only the single-target IS PCR been applied. Patients with mild disease ($P = 0.004$) and shorter hospitalization ($P = 0.006$) were less likely to have positive cultures. This consensus generating real-time PCR approach permits a sensitive detection, as well as an accurate species identification of the causative *Bordetella* pathogens for the timely management of patients.

The timely and reliable diagnosis of pertussis, a highly contagious respiratory tract infection caused by *Bordetella pertussis* and, less frequently, by *B. parapertussis*, is crucial in instituting specific therapy and preventing transmission of disease (16, 19, 28, 40). Clinical manifestations, such as prolonged cough, have been used to define disease, but it is now clear that these definitions have not captured culture-positive, symptomatic patients with a shorter duration of cough (3, 7, 13, 31). A variety of diagnostic methods have been developed for the detection of pertussis disease, but all have limitations in terms of sensitivity, specificity, and practicality (9, 10). Recovery of the organism by culture or direct fluorescent antibody (DFA) methods is highly specific, but the sensitivity is low and the results are not rapidly available (2, 3, 16, 29, 40). Serologic studies, although not practical for a rapid diagnosis, have been used to measure immunoglobulin G antibodies to pertussis toxin successfully in outbreak investigations involving adolescents and adults, as well as vaccine trials (6, 25, 34). Detection of pertussis-specific antibodies in serum has never been widely accepted in clinical settings, since serologic results are not rapidly available, may be confusing early in the disease course especially in infancy, and cannot always differentiate host immunities acquired after infection or vaccination (1, 3, 16, 35).

PCR assays have substantially facilitated the diagnosis of pertussis. PCR assays can be applied directly to specimens, can detect just a few or even nonviable *Bordetella* organisms, provide results rapidly, and perform well in infants. These assays have been shown to be more sensitive than culture, with sensitivity and specificity rates up to 61 and 88%, respectively (1, 2, 9–12, 14, 16, 19, 24, 32, 35, 40).

Although PCR for the detection of pertussis was introduced in 1989, standardization of methods has been problematic (1, 3, 11, 14, 19, 24, 32, 34). PCR methods by far amplify a single gene sequence, usually within the insertion sequence IS*481* (11, 18, 24, 32, 34, 35). Unfortunately, both false-positive and false-negative results have been reported (14, 24, 37). Pseudo outbreaks have been reported as a result of improper laboratory handling and suboptimal testing procedures (24, 29). No specific recommendation using more than one pertussis genetic target for laboratory diagnosis has been made by regulatory agencies in the United States or in Europe (EUpertstrain [34]), although several laboratories, including ours, have previously suggested the use of two-target PCR for pertussis diagnosis (14, 32). We present here the retrospective results of two- and three-target PCR compared to the detection of a single target, including the conventional IS*481* PCR, as well as the correlation of these with culture results, and clinical variables.

MATERIALS AND METHODS

Specimen collection and preparation. All specimens obtained from patients with suspected pertussis from King County, Washington, that were referred to the Microbiology Laboratory at Children's Hospital and Regional Medical Center (CHRMC), Seattle, WA, between January 2002 and December 2005 were studied. Cultures were performed only on PCR-positive and PCR-indeterminate specimens. Two dacron or rayon nasopharyngeal swabs were generally obtained; one was set aside in Regan-Lowe transport medium for culture pending the PCR results, and the other was stored at −20°C in a sterile tube with no transport additives. The latter swab was processed by adding 1 ml of sterile saline, followed

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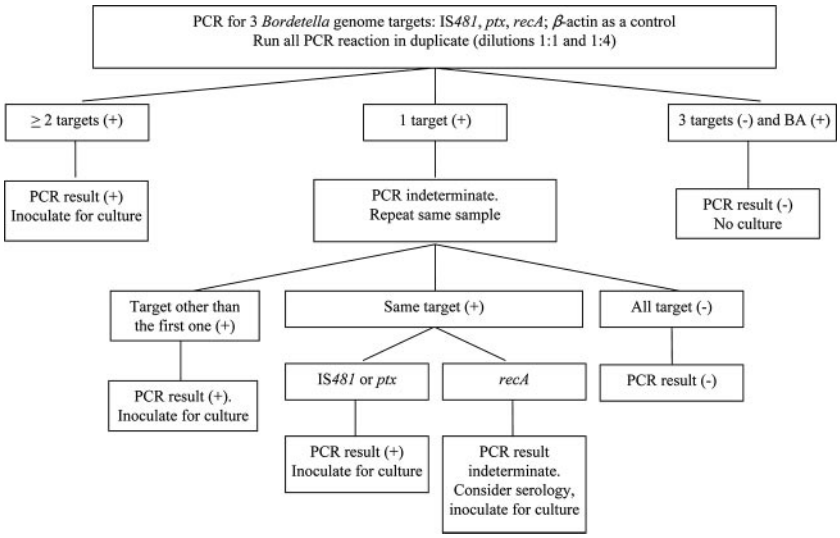

FIG. 1. Flow diagram of evaluating three-target PCR for the diagnosis of pertussis when β-actin was tested positive. In cases of β-actin negative result, the retaining unheated specimen was inoculated for culture.

by 30 s of vigorous vortex mixing. Saline suspensions were transferred to a sterile 1.5-ml Eppendorf tube and centrifuged at 16,110 × *g* for 5 min, and the pellet suspended in 100 μl of molecular-grade water (Fischer Scientific, Fairlawn, NJ) and then heated at 95°C for 5 min and cooled to 4°C. Amplification in-run positive controls consisted of fresh-grown organisms diluted to 1:10 and 1:100 from 0.5 McFarland suspensions of *B. pertussis* ATCC 9340 and *B. parapertussis* ATCC 15237. The PCR sensitivity for IS, PT, and PO/RA was validated at the lower detection limit of ~20 CFU. The final inoculum of the heated *B. pertussis* ATCC 9340 control material using its far-end dilution would ensure a genome copy number of as few as 50 per reaction in each test run. Molecular-grade water was used for the negative controls, and human β-actin marker was used to control for specimen quality and PCR inhibition.

Culture. If a positive or indeterminate PCR assay was noted, the second swab (or the only swab retained after a saline wash) was inoculated onto a Regan-Lowe agar plate and incubated in a humidity chamber at 35°C for a maximum of 15 days. The plates were examined on a daily basis for colonies typical of *B. pertussis*. Colonies were further evaluated by Gram stain for bacterial morphology, and *B. pertussis* was confirmed by direct fluorescent antibody assay (Difco Laboratories, Detroit, MI).

Real-time amplification. The real-time PCR assays were performed by using fluorescence resonance energy transfer SYBR green chemistry that measured the fluorescent SYBR green signal increase as a result of growing amplicon concentration. The amplicons were analyzed at the end of the 45th cycle for their specific melting-point temperatures. The commercial master mix iQ SYBR green Supermix (Bio-Rad, Hercules, CA) was used according to the manufacturer's recommendations. Uracil-*N*-glycosylase was incorporated into the master mix for the amplicon carryover contamination control. The master mix was made fresh daily with primer concentrations at a final concentration of 0.1 μM. The master mix (36 μl) was transferred into premapped wells in a 96-well plate; 4-μl samples (an undiluted 1:1 and the 1:4 dilution) were then each inoculated into two sets of four reactions. A total of eight wells (IS, PT, PO/RA, and BA) were used for each sample. The thermocycling conditions were 20°C for 5 min and 95°C for 2 min, followed by 45 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 30 s, followed in turn by 5 min at 72°C as the extension step before a final melting-peak analysis in an iCycler (Bio-Rad) instrument. The specific melting-peak temperatures with ≤±0.5°C were accepted.

PCR interpretation. The interpretation criteria are depicted in Fig. 1. The PCR result was reported as "positive" when ≥2 positive targets or a single reproducible IS, PO, or PT target was positive; as "negative" when no target was positive or a single target was initially positive but not reproducible; and as indeterminate when RA was the only reproducible target detected or BA was negative for specimen concentrations of 1:1, 1:4, and 1:8 upon repeat. With the indeterminate results, the original specimen was cultured for *Bordetella* spp., and the physician was contacted to suggest recollection of the clinical specimen.

Clinical variables. Clinical data were collected from the medical records of children receiving care at only CHRMC, Seattle, WA, following approval by the Institutional Human Subject Review Board. Clinical variables included gender and age, immunization history and contact with a confirmed case or an individual with prolonged cough illness, characteristics and duration of coughing, severe manifestations (including apnea and cyanosis), fever, administration of antibiotics prior to sampling, and duration of hospitalization.

Statistics. Statistical analysis was based on contingency tables, including two-sided Fisher exact test and odds ratio (OR) calculations, and on calculation of the proportions and of the lower and upper limits of the 95% confidence interval (95% CI) with correction for continuity. Additional analyses included nonparametric evaluations, including the Kruskal-Wallis and two-tailed Mann-Whitney tests. A P value of <0.05 was considered to be significant.

RESULTS

Population. A single sample from each of 4,442 patients with a clinical suspicion of pertussis was investigated. In 309 (6.96%) samples, pertussis was detected based on the diagnostic algorithm presented in Fig. 1. The patients diagnosed with pertussis were between the ages of 18 days to 84 years (median, 2.50 years); 157 were male, and 152 were female. Pertussis was detected more often during the late summer and autumn months, with 98 (31.7%) cases clustering in the 3-month period from August to October. Nine cases were attributed to *B. parapertussis*, all occurring in November, December, and January but in different years. A total of 70 patients were hospitalized at CHRMC, 42 outpatients aged less than 15 years were seen in the emergency department or clinics, and 197 were referral patients whose swabs were sent to our laboratory for pertussis evaluation. The median ages of hospitalized patients was 0.25 years (range, 0.06 to 13.8 years), of outpatients was 0.70 years (range, 0.05 to 14.1 years), and of referral patients was 12.5 years (range, 0.05 to 84 years), a difference that was significant ($P < 0.0001$).

PCR primers designed for specific targets. The primers used in the present study are shown in Table 1. The PCR assay was designed to detect three independent targets in the *Bordetella* genome: chromosomal repeated insertion sequence IS*481* (IS), the polymorphic pertussis toxin *ptxA* promoter region (PT), and the *recA* (RA) gene coding region (3, 15–18, 20–24, 26–

TABLE 1. Primers used as PCR targets in this study

Primer type and name	Sequence	Amplicon length (bp)
IS*481* (IS)		182
IS-F	GATTCAATAGGTTGTATGCATGGTTC	
IS-R	TTCAGGCACACAAACTTGATGGGCG	
ptx promoter (PT)		189
PT-F1	CCAACGCGCATGCGTGCAGATTCG	
PT-F2	CCAACGCGTATGCGTGCGGATGCG	
PT-R1	CTCTGCGTTTTGATGGTGCCTATT	
PT-R2	CTCTGCGTTTCGGTGGTGCCTATT	
Outer membrane porin (PO)		148
PO-F	GGCCGGGCTCCTTGAGTGAACTGG	
PO-R	GTTGGTAAGTTGCAACATCCTGTCC	
recA gene (RA)		204
RA-F	CGCGCTCAAGTTCTATTCCTCG	
RA-R	TTGCACGCCCAGGTCGATGATTTC	
Human β-actin (BA)		331
BA-F	AAAGACCTGTACGCCAACACAGTGCTGTCTGG	
BA-R	CGTCATACTCCTGCTTGCTGATCCACATCTGC	

30); during the first period of the study, the outer membrane porin gene (PO) was used before the RA sequences became available. The sequence IS*481* is present at a rate of approximately 200 copies in the genome of *B. pertussis* but is also found in *B. holmesii*, an uncommon respiratory tract colonizer, and possibly in other species of *Bordetella* such as *B. bronchiseptica* (11, 14, 19, 31, 34). Taking advantage of intra- and interspecies polymorphism, primers for PT amplification were designed to cover both *B. pertussis* and *B. parapertussis* but not *B. bronchiseptica*. Differentiation between *B. pertussis* and *B. parapertussis* was achieved by distinct melting peaks at 89 and 91°C, respectively.

The *recA* gene was used as a common target for detecting *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*. The RA primer pair was chosen based on multisequence alignment of the *recA* coding regions (Fig. 2) from *B. pertussis* (NC_002929), *B. parapertussis* (NC_002928), *B. bronchiseptica* (NC_002927), *B. holmesii* (AF399664), *B. hinzii* (AY124331), and *B. avium* (AY124330). Amplification of β-actin (BA) was designed as a control for specimen quality and for false-negative results due to *Taq* polymerase inhibition in unextracted human specimen materials or inadequate samples.

PCR findings. Pertussis was confirmed in 309 of 4,442 (6.96%) specimens by the three-target PCR and in 247 (5.56%) by the conventional single-target IS PCR ($P = 0.007$). The three-target PCR detected pertussis in 62 more samples (25.1% [95% CI = 20 to 31%]) than single-target IS PCR. Single targets IS, PT, PO, and RA demonstrated detection rates (95% CI) of 0.80 (0.75 to 0.84), 0.76 (0.71 to 0.81), 0.80 (0.73 to 0.86), and 0.68 (0.59 to 0.75), respectively, compared to the three-target PCR algorithm (Table 2). Compared to the conventional single-target IS PCR, the three-target combination IS-PT-PO/RA increased detection by 1.25-fold, and the two-target combinations IS-PT, IS-PO/RA, and PT-PO/RA increased detection by 1.24-, 1.21-, and 1.10-fold, respectively. Single-target reproducible positive results were generated from 47 of the 309 (15%) pertussis-positive specimens (or 1% of the total 4,442 specimens) with 34 IS only, 9 PT only, and 4 PO only, respectively. Nonreproducible single-target positive results were obtained for 223 specimens (i.e., 5% of the total number of specimens, excluding the 47 [1%] samples giving reproducible results by single-target PCR), suggesting potential carryover contamination, nonspecific amplification, or pathogen levels below the detection limit. All nine samples attributed to *B. parapertussis* were positive for PT sequences with a melting point of 91°C and negative for IS. Four of these were tested by PO as a third target, and all were found negative: the remaining five were tested with RA, and all were found to be positive. Nine specimens testing positive only for RA and 32 BA-negative specimens were considered indeterminate.

No relationship between patient gender and PCR results

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BP .NC_002929 CGCGCTCAAGTTCTATTCCTCG.....GAAATCATCGACCTGGGCGTGCAA
BPP.NC_002928 ----------------------.....-------------------------
BB .NC_002927 ----------------------.....-------------------------
BHI.AY124331  T--------------------C.....--G------------------C?
BHO.AF399664  T--A-G----------G-T--T.....--G-----------------AGCT
BAV.AY124330  --A-G------T-----T--C.....----------T-??????????
```

FIG. 2. Sequence alignment of the *recA* coding region of pathogen-specific primers. The dotted lines represent 156-bp intervening sequences; the dashes represent bases identical to those of the three-pathogen consensus; the question marks represent unknown nucleotides. BP, *B. pertussis*; BPP, *B. parapertussis*; BB, *B. bronchiseptica*; BHI, *B. hinzii*; BHO, *B. holmesii*; BAV, *B. avium*.

TABLE 2. Diagnostic sensitivity of various PCR targets in 309 *B. pertussis*-positive samples compared to the detection of three-target PCR algorithms

Target	No. (%)			
	All patients	Inpatients	Outpatients	Referrals
Positive specimens	309	70	42	197
Single target				
IS	247 (79.9)	58 (82.9)	35 (83.3)	154 (78.2)
PT	236 (76.4)	56 (80.0)	32 (76.2)	148 (75.1)
PO/RA	228 (74.5)	63 (90.0)	31 (73.8)	134 (69.1)
Two-target combinations				
IS and/or PT	305 (98.7)	69 (98.6)	42 (100)	194 (98.5)
IS and/or PO/RA	300 (97.1)	70 (100)	42 (100)	188 (95.4)
PT and/or PO/RA	271 (88.6)	67 (95.7)	38 (90.5)	166 (84.3)

was observed. Age was not correlated with rates of detection of IS or PO, but detection of PT and RA was more frequent in younger than in older patients ($P = 0.003$ and $P = 0.014$; median ages of 1.84 versus 7.36 and 2.77 versus 12.9, respectively). No relationship between inpatients, outpatients, or referral patients and the detection of IS, PT, or RA was documented, but PO was detected more frequently in hospitalized patients than in outpatients ($P = 0.031$) or referral patients ($P < 0.0001$).

PCR as a predictor of culture results. A total of 93 (29.2%) *Bordetella* isolates were grown from the 318 PCR-positive specimens. Eighty-nine strains were *B. pertussis*, and four were *B. parapertussis*. Patients with a positive culture had a younger median age than those with a negative culture (median ages of 0.67 versus 3.24 years [$P = 0.003$]), a strong indication of potential lack or incompletion of vaccination. Those with positive cultures were more frequently inpatients compared to referral patients ($P < 0.0001$, OR 3.19 [95% CI = 1.80 to 5.67]). Detection of IS, PT, PO, or RA predicted culture growth with sensitivities (95% CI) of 0.82 (0.72 to 0.89), 0.90 (0.82 to 0.95), 0.93 (0.83 to 0.97), and 0.91 (0.70 to 0.98), respectively. Combinations of IS and either PT or PO/RA predicted growth by culture with a sensitivity of 1.0 (95% CI = 0.95 to 1.0). Of the 89 samples from which pertussis was detected by culture, 17 (19%) were not detected by the single-target IS PCR, including 3 of the 10 culture-positive (noticeably 5 in total 16 PCR-positive) in the intensive care unit (ICU) patients. Isolates were retrospectively tested for the presence of IS, PT, and RA, and all targets were detected in all isolates after culture. Among the 32 indeterminate BA-negative specimens, 1 grew *B. pertussis*, but no growth was observed among the 9 indeterminate RA specimens.

The overall culture sensitivity of the *B. pertussis* PCR-positive cases was 28.8% (89 of 309). The sensitivity of culture increased when the child was hospitalized (34 of 70 [49%]) and ICU (10 of 16 [63%]). The increasing number of positive PCR targets also predicted a higher rate of positive pertussis cultures, since 43.3% (61 of 141) of the three-target PCR positive specimens yielded positive growth, compared to 27.4% (24 of 111) of the two-target positive specimens and 8.5% (4 of 47) the single-target positive specimens.

Clinical variables and positivity of PCR and culture. Clinical variables and the PCR and/or culture results were studied in the 112 children receiving care at CHRMC. Seventy (62.5%) children were hospitalized, of whom sixteen (22.9%) were admitted to the ICU. No association was noted between any single positive PCR target (IS, PT, or PO/RA) any of the following variables: a positive culture result, duration of cough, the presence of cough paroxysms or fever, or a history of having received antibiotics prior to sampling. Patients with mild disease as defined by the absence of apnea or cyanosis were more likely to have negative cultures ($P = 0.004$) or require shorter periods of hospitalization (median, 2 versus 4 days, $P = 0.006$).

DISCUSSION

PCR assays have greatly facilitated pertussis diagnosis, but problems with this assay persist: sensitivity is not yet as high as with serologic tests, false-positive results remain a problem, and differentiation between *Bordetella* species is not usually feasible with the single target assays (24, 28). Furthermore, no single-target PCR assay is universally considered to be a "gold standard" for pertussis diagnosis. Our findings were obtained on samples collected from a large cohort of patients of all ages and disease severity and confirm that individual targets may not perform consistently in all patient groups and demonstrate higher detection rates with multitarget PCR. For analytical accuracy, the use of multitarget PCR can minimize the incidence of the "pseudo-outbreaks" given that the potential error of any single-target approach may be recognized or counterbalanced by the use of additional target(s). Because of the increased sensitivity and the analytical precision permitted by this approach, culturing all clinical samples for *B. pertussis* becomes unnecessary.

The choice of target is critical for the specificity of any PCR assay, and combinations of primers may allow for the simultaneous detection of, and discrimination between, *Bordetella* species in the same assay (8, 18, 21, 27, 32, 33). Pertussis detection based on the single-target IS would have missed nearly 20% (17 of 89) culture-positive pertussis cases, including 3 of the 10 culture-positive ICU patients, or 5 of the 16 PCR-positive ICU patients. Because combinations of IS with either PT or RA provided significantly enhanced diagnostic sensitivity, we believe testing of clinical specimens for more than one pertussis target should be routinely conducted in clinical laboratories.

PT-PCR has been used to confirm the diagnosis of *B. pertussis* and *B. parapertussis* by IS-PCR (11, 14, 17, 30). Although *ptxA* is present as a single-copy gene, and IS is present in multiple copies, detection of PT has been reported to be equivalent to that of IS (14, 36). The present study did not find substantial differences between the detection of PT and IS for pertussis diagnosis: of the 309 PCR positive specimens, the detection rate was 76% by PT alone versus 80% by IS alone. This does not reflect the marked difference in their genome copy numbers at the 1:200 ratio (31). Nevertheless, the IS single-target reproducible positive cases ($n = 34$) outnumbered the PT ($n = 9$) and PO ($n = 4$) positive cases, suggesting the copy number theory. Limited by the availability of patient information for further analysis, we suspect that the multicopy IS may be more sensitive during early onset of illness when the

organism load was low but the genome integrity was high. Despite its high copy number, IS*481* is considered a nonessential genomic element. We therefore speculate that the underperformance of IS*481* overall may be a result of nonrandom genome degradation during the course of host-pathogen interaction further into the disease.

PT was efficient in differentiating between *B. pertussis* and *B. parapertussis* using melting-point analysis. A comparable assay using IS*1001* in a dual-target system also provides such bases for species distinction (36). The detection of PO by PCR has been suggested to allow an accurate approach to the diagnosis of pertussis (14). We replaced the detection of the noncoding PO sequence with RA, a stable coding region, following the availability of the three whole-genome sequencing data in 2003 (31). RA target was specifically chosen in the *recA* protein coding region where phylogenetic divergence of the three pathogens of interest (*B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*) from the other *Bordetella* species can be found. Although not as many *recA* copies as IS*481* copies exist in the *B. pertussis* genome (14, 19, 39), RA was a reliable target, performed better than PO in diagnosing *B. parapertussis* infection, and provided both a biologically stable marker for diagnosis and an analytical control for species confirmation.

All three targets were retrospectively detected in all culture-grown *B. pertussis* organisms in our study. Thus, the presence of a major sequence polymorphism in the targeted regions was unlikely, and the unequal detection of targets seemed to reflect a varying degree of the integrity of the organisms during disease. In other real-time PCR studies, fewer threshold cycles ($C_T < 25$) have been used to predict positive cultures (38). In the present study, an increasing number of positive targets detected was increasingly predictive of a culture being positive. This is important in clinical practice, since positive cultures are more frequently associated with specimens from the much younger patients who were hospitalized.

The cost savings can be considerable when fluorescence resonance energy transfer techniques using CYBR green and melting-point analysis are compared to the commercially available single- or dual-target probe assay systems. Elimination of the DNA extraction step provided additional cost savings and additional specimen protection from crossover contamination. In the present study, only 32 of the 4,442 specimens (0.007%) tested were β-actin negative, indicating potentially poor sampling or PCR inhibition. DNA extraction therefore can be safely replaced by heat lysis after a rigorous saline wash. Further, employing a number of consensus-generating reactions per sample is far more effective than relying on a single stringent reaction. In our experience, an overall 6% rate of repeat testing due to those single-target positive results suggests that the analytical factors intrinsic to this type of molecular assay can be better controlled by multitarget reference parameters. In multitarget analysis, each independent reaction serves as an in-run control for potential false-positive or false-negative results. Thus, from both analytical and operational standpoints, substantial labor and time savings can be achieved by reducing repeat testing due to ambiguous results.

A limitation in our study was the lack of cultures and serological testing in all patients. Specimens that are culture positive and PCR negative have been reported, although only rarely (19, 35), and in our study only one PCR-negative specimen (BA negative) was culture positive, presumably due to polymerization inhibition. Despite the advantages of PCR for the identification of *Bordetella* species, culture techniques remain important for epidemiologic analysis or antibiotic susceptibility (4, 19, 20). In our study, however, only 30% of PCR-confirmed cases were culture positive, a finding in accordance with previous reports (35), and PCR identified many pertussis cases that would have escaped diagnosis by culture alone.

A better understanding of genomic targets of *Bordetella* has provided insight into new approaches for the detection of pertussis (34). Earlier studies have proposed the use of two PCR targets for pertussis diagnosis (9, 11), and we have refined our previous experience (32) to further enhance the reliable PCR detection of a pathogen that is becoming increasingly important in the clinical setting. We demonstrated here the application of the three-target PCR approach under routine diagnostic conditions in a hospital laboratory. Our findings suggest that multitarget PCR, beginning with IS*481*, increases sensitivity, discriminates false-positive and false-negative results, and allows for a specific identification of the causative *Bordetella* organism. Our findings further suggest that two-target approaches may also be worth considering. It is well known that pertussis is under-recognized, both in the community and in hospitalized patients; in the latter setting, missed diagnoses may lead to nosocomial outbreaks (1, 3, 5, 9, 10, 29, 34). The increasing use of vaccination in older children, adolescents, and adults may ultimately decrease the incidence of pertussis, but advances in diagnostic assays that contribute to early and reliable identification will enable treatment to be initiated and infection control measures to be implemented.

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