ORIGINAL ARTICLE

Evaluation of amplification targets for the specific detection of *Bordetella pertussis* using real-time polymerase chain reaction

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BACKGROUND: *Bordetella pertussis* infections continue to be a major public health challenge in Canada. Polymerase chain reaction (PCR) assays to detect *B pertussis* are typically based on the multicopy insertion sequence IS*481*, which offers high sensitivity but lacks species specificity.

METHODS: A novel *B pertussis* real-time PCR assay based on the porin gene was tested in parallel with several previously published assays that target genes such as IS*481*, *ptx*-promoter, pertactin and a putative thialase. The assays were evaluated using a reference panel of common respiratory bacteria including different *Bordetella* species and 107 clinical nasopharyngeal specimens. Discrepant results were confirmed by sequencing the PCR products.

RESULTS: Analytical sensitivity was highest for the assay targeting the IS*481* element; however, the assay lacked specificity for *B pertussis* in the reference panel and in the clinical samples. False-positive results were also observed with assays targeting the *ptx*-promoter and pertactin genes. A PCR assay based on the thialase gene was highly specific but failed to detect all reference strains of *B pertussis*. However, a novel assay targeting the porin gene demonstrated high specificity for *B pertussis* both in the reference panel and in clinical samples and, based on sequence-confirmed results, correctly predicted all *B pertussis*-positive cases in clinical samples. According to Probit regression analysis, the 95% detection limit of the new assay was 4 colony forming units/reaction.

CONCLUSION: A novel porin assay for *B pertussis* demonstrated superior performance and may be useful for improved molecular detection of *B pertussis* in clinical specimens.

Key Words: Bordetella pertussis; IS481 *element; Pertactin gene; Porin gene;* Ptx*-promoter; Real-time PCR*

L'évaluation des cibles d'amplification pour déceler le *Bordetella pertussis* au moyen de la méthode de réaction en chaîne de la polymérase en temps réel

HISTORIQUE : Les infections à *Bordetella pertussis* continuent d'être un important problème de santé publique au Canada. Les méthodes de réaction en chaîne de la polymérase (PCR) pour déceler le *B pertussis* sont habituellement fondées sur la séquence d'insertion multicopie IS*481*, dont la sensibilité élevée, mais dont la spécificité d'espèce est inexistante.

MÉTHODOLOGIE : Une nouvelle méthode PCR en temps réel du *B pertussis* fondée sur le gène de porine a été mise à l'essai parallèlement à plusieurs méthodes déjà publiées qui ciblent des gènes comme l'IS*481*, le promoteur de *ptx*, la pertactine et une thialase éventuelle. Les méthodes ont été évaluées à l'aide d'un groupe de référence de bactéries respiratoires communes, y compris diverses espèces de *Bordetella* et 107 échantillons nasopharyngés cliniques. Les résultats contradictoires ont été confirmés par séquençage des produits de PCR.

RÉSULTATS : La méthode visant l'élément IS*481* avait la sensibilité analytique la plus élevée, mais manquait de spécificité pour le *B pertussis* dans le groupe de référence et les échantillons cliniques. Les méthodes ciblant les gènes du promoteur de *ptx* et de la perctatine ont également donné des résultats faux positifs. Une méthode de PCR fondée sur le gène thialase était hautement spécifique, mais ne décelait pas toutes les souches de référence du *B pertussis*. Cependant, une nouvelle méthode ciblant le gène de porine a démontré une forte spécificité au *B pertussis*, à la fois dans le groupe de référence et dans les échantillons cliniques et, d'après les résultats confirmés par séquençage, prédit correctement tous les cas positifs au *B pertussis* dans les échantillons cliniques. D'après l'analyse de régression Probit, la limite de détection de 95 % de la nouvelle méthode était de quatre unités formant colonies par réaction.

CONCLUSION : Une nouvelle méthode faisant appel à la porine pour déceler le *B pertussis* donne un rendement supérieur et peut être utile pour améliorer la détection moléculaire du *B pertussis* dans des échantillons cliniques.

Despite high vaccine coverage, the number of reported cases of pertussis (whooping cough) has increased in recent years in the United States and Canada (1,2). Whooping cough is caused by the most widely known *Bordetella* species, *Bordetella pertussis*. For diagnosis, polymerase chain reaction (PCR)-based methods to detect *B pertussis* in nasopharyngeal swabs or aspirates from patients with suspected pertussis are now commonly used due to their improved sensitivity and decreased turnaround time compared with bacterial culture (3). Currently, the most common PCR target is the multicopy insertion sequence IS*481*, which is repeated more than 200 times in the *B pertussis* genome (4). IS*481* PCR assays are widely used because of their high sensitivity. However, these assays lack specificity because the IS*481* sequence is also present in the genomes of other *Bordetella* species (eg, *Bordetella bronchiseptica* and *Bordetella holmesii*) that are not generally associated with serious disease or widespread outbreaks (3,5-8). For example, a recent multicentre study conducted in 19 countries by the European surveillance network for vaccine-preventable diseases and the European Centre for Disease Prevention and Control show that misidentification of *Bordetella parapertussis* and *B holmesii* as *B pertussis* are common occurrences when using IS*481*-based PCR assays (9). These findings led to the conclusion that a positive sample from an IS*481* assay indicates only the presence of *Bordetella* DNA and not the specific presence of *B pertussis* DNA (9). The ability to detect *B pertussis* with high specificity is important, not only because it allows for targeted therapy, but also because it could circumvent the implementation of infection control and public health measures, costly

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TABLE 1
Description of primers and probes used in the present study

Assay	Target gene	Genome position*	Primer/probe	Sequence (5′–3′)	Working concentration	Reference
IS*481*	*Bordetella pertussis* insertion sequence IS481	58491-58511†	Forward	CCGAACCGGATTTGAGAAAC	330 nM	11
		58413-58432†	Reverse	TAGGAAGGTCAATCGGGCAT	330 nM	
		58435-58456†	Probe‡	CCGGCCGGATGAACACCCATAA	200 nM	
OMP	*B pertussis/B parapertussis* gene for a porin protein	868896-868918†	Forward 1§	ATGCTTATGGGTGTTCATCCGGC	240 nM	15
		3658866-3658884¶	Forward 2§	TGAGGTCGGGCGAATCGTC	240 nM	
		869040-869066	Reverse§	TTGTTGGTAAGTTGCAACATCCTGTCC	240 nM	
BPTP	*B pertussis* pertussis toxin gene, promoter region	3988078-3988098	Forward§	TTCGTCGTACAAAACCCTCGA	330 nM	12
		3988122-3988141	Reverse§	GTTCATGCCGTGTTGGATTG	330 nM	
		3988101-3988114	Probe**	CTTCCGTACATCCC	200 nM	
PRN	*B pertussis* pertactin gene	1098189-1098206	Forward	TGCCGACTGGAACAACCA	300 nM	14
		1098243-1098261	Reverse	GTCGGAGCCCTGGATATGG	300 nM	
		1098211-1098232	Probe§	ATCGTCAAGACCGGTGAGCGCC	66 nM	
BP283	Putative thiolase gene (BP0026)	30021-30040	Forward	CAGGCACAGCACGTATTGCG	330 nM	13
		30104-30126	Reverse	GACGATTACCAGCGAGATTACGA	330 nM	
		30065-30088	Probe‡	CCGCCATCGCAACCGTCGCATTCA	200 nM	
PT-P	*B pertussis* pertussis toxin gene, promoter region	3988032-3988050	Forward	CCATCCCGCATACGTGTTG	330 nM	Present study
		3988108-3988127	Reverse	GGATTGCAGTAGCGGGATGT	330 nM	
		3988080-3988098	Probe**	CGTCGTACAAAACCCTCGA	200 nM	
POR	*B pertussis* gene for a porin protein (BPTD_0837)	868978-869001†	Forward§	TGAACCATGCATACAACCTATTGA	330 nM	Present study
		869026-869046	Reverse§	CCTGTCCCCTTAATCCGGAAT	330 nM	
		869003-869024	Probe‡	TCTTCACAGTTAGCCCGCGCGC	200 nM	

**With respect to* Bordetella pertussis *Tohama I chromosome, complete genome (Accession No. NC_002929.2), unless otherwise specified; †Repeated many times (one position shown); ‡5′-end labelled with 6-carboxyfluorescein (FAM) and 3′-end labelled with Black Hole Quencher 1 (BHQ1); §Primers also used for sequencing; ¶With respect to Bordetella parapertussis 12822 chromosome, complete genome (Accession No. NC_002928.3); **5′-end labelled with 6-carboxyfluorescein (FAM) and 3′-end labelled with MGB non-fluorescence quencher. BPTP* Bordetella parapertussis *toxin promoter; OMP Outer membrane porin protein; POR Porin; PRN Pertactin; PT-P Pertussis toxin promoter*

interventions that are generally unnecessary for nonpertussis *Bordetella* strains. Moreover, proper identification of pertussis cases enables more accurate assessment and enumeration of cases of vaccine failure – an issue of considerable public health importance.

Due to concerns about the false-positive results from IS*481* PCR assays produced by nonpertussis *Bordetella* species, suggestions have been made to use multitarget PCR assays for definitive diagnosis of pertussis (10,11). These strategies, however, significantly increase workload, cost and turnaround time, and often involve complex interpretation rules before the results can be reported. A single-target PCR, carefully designed to specifically and exclusively amplify *B pertussis* DNA would, therefore, be preferable to current assays based on IS*481* or multiple targets. Several real-time PCR assays designed to amplify non-IS*481* single-gene targets have been described for the specific detection of *B pertussis* (12-14). In the present study, we evaluated the performance of these PCR assays in parallel with a novel real-time PCR assay that targets the *B pertussis* porin gene and the widely used IS*481*-based PCR assay.

METHODS

Bacterial strains and culture

The reference panel was comprised of *B pertussis* (American Type Culture Collection [ATCC] BAA-589, 9340 and 9797, and a clinical strain isolated at BC Children's Hospital [Vancouver, British Columbia] from a patient nasopharyngeal aspirate), *B parapertussis* (ATCC 15237 and a clinical strain isolated at the BC Centre for Disease Control [Vancouver, British Columbia]), *B holmesii* (ATCC 51541), *B bronchiseptica* (ATCC BAA-588), *Haemophilus influenzae* (ATCC 10211), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13882), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus agalactiae* (ATCC 13813), *Streptococcus mitis* (ATCC 6249), *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19615), *Corynebacterium pseudodiphtheriticum* (ATCC 10700), *Mycoplasma pneumoniae* (ATCC 29342), *Chlamydophila pneumoniae* (ATCC VR-1310) and a clinical strain of *Neisseria mucosa* isolated at BC Children's Hospital. *Bordetella* species were streaked on charcoal agar medium (Oxoid) and grown at 37°C for 72 h in a humidified environment. Bacterial suspension was prepared in phosphate-buffered saline to a turbidity equivalent to a 0.5 McFarland standard, titred by colony counting and cryopreserved at −80°C. The titred bacterial stocks were diluted in TE8 buffer (10 mM Tris, 1 mM EDTA; pH 8.0) and used directly in the PCR reactions. Other bacteria were grown on blood or chocolate agar plates (Oxoid) overnight at 37°C in a 5% CO_2 atmosphere, and a bacterial suspension was prepared in TE8 buffer to a turbidity equivalent to a 0.5 McFarland standard. The suspension was further diluted 1:10 in TE8 before being used for PCR.

Specimens

A total of 107 nasopharyngeal wash specimens were used in the present study, including selected PCR-positive (by a SYBR Green PCR [15]) specimens since August 2009, and specimens submitted between September 2011 to October 2012 to the Microbiology and Virology laboratory of BC Children's Hospital for *B pertussis* PCR. Testing was performed exclusively on retrospective, residual samples that were stored at −80°C. To maintain patient anonymity, each sample was coded and all patient identifiers were removed to ensure that personnel involved in the present study were unaware of any patient information. Ethics approval was not considered to be necessary because studies that involve the secondary use of anonymous human biological materials are exempted from review by the local Research Ethics Board of the University of British Columbia. DNA from 0.2 mL nasopharyngeal wash specimens was extracted using the QIAsymphony virus/bacteria kit in an automated DNA extraction platform QIAsymphony SP (Qiagen, USA).

PCR

The titred bacterial suspensions and DNA extracts from 107 nasopharyngeal wash specimens were analyzed using Taqman real-time PCR or by SYBR Green PCR with the primers and probes shown in Table 1. For Taqman real-time PCR assays, 5 µL of sample

Organism	Accession No.	Sequence	Base position
B. Pertussis	CP002695.1	TGAACCATGCATACAACCTATTGAATCTTCACAGTTAGCCCGCGCGCGATTCCGGATTAAGGGGACAGG	892567-892635
B. bronchiseptica	BX640448.1	GGGACTTGTTGCATTGCGATGCGCCGCCCTAGG.....	243022-242954
B. parapertussis	BX640433.1	GGAGCTTGTTGCATTGCGATGCGCCGCCCTAGG.....	180753-180685
B. holmeseii	DQ420073.1	.....C...GTCGGCGTTCTCGTCGTAGAAGCTGGCCCGC	150-82

Figure 1) *DNA sequence alignment of a segment of a* Bordetella pertussis *gene for a porin (POR) protein with similar sequences from different* Bordetella *species. Underlined sequences indicate the forward primer, probe and reverse primer, respectively (from left) for the new POR polymerase chain reaction assay. Dots indicate homology with the sequence in the top row*

TABLE 2
Specificity of different *Bordetella pertussis* polymerase chain reaction assays

		C_T						
		Published assays					In-house assays	
Sample	CFU/reaction	OMP	IS481	BPTP	BP283	PRN	PT-P	POR
Bordetella species								
B pertussis (ATCC BAA-589)	4.7×10^3	22	15.9	22.8	24.3	24.5	33.4	24.9
B pertussis (ATCC 9340)	3.75×10^3	22.5	16.7	26.3	0	25.2	0	25.2
B pertussis (ATCC 9797)	3.8×10^3	21.9	15.7	25.4	0	24.5	0	24.5
B pertussis (patient isolate)	0.1×10^3	27.2	19.8	29.6	27.6	31.1	37.9	31.8
B parapertussis (ATCC 15237)	0.7×10^3	22.1	34.7	0	0	0	0	0
B parapertussis (patient isolate)	0.1×10^3	29.9	0	0	0	0	0	0
B holmesii (ATCC 51541)	3.2×10^3	28.9	18.3	0	0	0	0	0
B bronchiseptica (ATCC BAA-588)	2.7×10^3	23.2	33.5	33.1	0	39.6	0	0
Non-*Bordetella* species								
Haemophilus influenzae (ATCC 10211)	*0.05 M	0	0	0	0	0	0	0
Pseudomonas aeruginosa (ATCC 27853)	*0.05 M	0	0	0	0	0	0	0
Klebsiella pneumoniae (ATCC 13882)	*0.05 M	0	0	0	0	0	0	0
Staphylococcus aureus (ATCC 43300)	*0.05 M	0	0	0	0	0	0	0
Staphylococcus epidermidis (ATCC 12228)	*0.05 M	0	0	0	0	0	0	0
Streptococcus agalactiae (ATCC 13813)	*0.05 M	0	0	0	0	0	0	0
Streptococcus mitis (ATCC 6249)	*0.05 M	35.4	0	0	0	0	0	0
Streptococcus pneumoniae (ATCC 49619)	*0.05 M	0	0	0	0	0	0	0
Streptococcus pyogenes (ATCC 19615)	*0.05 M	37.8	0	0	0	0	0	0
Corynebacterium pseudodiphtheriticum (ATCC 10700)	*0.05 M	0	0	0	0	0	0	0
Neisseria mucosa (patient isolate)	*0.05 M	0	0	0	0	0	0	0
Mycoplasma pneumoniae (ATCC 29342)	†C_T=30	0	0	0	0	0	0	0
Chlamydophila pneumoniae (ATCC VR-1310)	†C_T=31	0	0	0	0	0	0	0

**Sample concentration equivalent to 10-fold diluted, 0.5 McFarland standard; †Sample concentration equivalent to cycle threshold (C_T) values according to respective real-time polymerase chain reaction assays. ATCC American Type Culture Collection; BPTP* Bordetella parapertussis *toxin promoter; CFU Colony forming units; M McFarland; OMP Outer membrane porin protein; POR Porin; PRN Pertactin; PT-P Pertussis toxin promoter*

extract or diluted bacterial suspensions was mixed with 20 µL of a master mix containing 12.5 µL of Taqman Universal PCR Master Mix (Applied Biosystems, USA) and primers and probes to final concentrations shown in Table 1. Thermal cycling was performed in a ABI7500 Fast instrument (Applied Biosystems, USA) with one cycle of 95°C for 10 min, followed by 45 cycles consisting of 95°C for 15 s and 60°C for 60 s. The SYBR Green PCR was performed with the same parameters except that the reaction mix contained 12.5 µL 2XiTaq SYBR Green PCR Master mix (Bio-Rad, USA) and the thermal cycling was performed in a SmartCycler (Cepheid, USA) with one cycle of 95°C for 2 min, followed by 45 cycles consisting of 95°C for 10 s and 64°C for 60 s. The SYBR Green PCR reactions were subjected to meltcurve analysis after the final amplification cycle. Samples with discrepant results from different assays were analyzed by sequencing PCR products using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in an ABI 3130 genetic analyzer according to the manufacturer's instructions. For Probit regression analysis, *B pertussis* strain ATCC BAA-589 stock suspension was serially diluted to 10, 50, 100, 200, 500 and 1000 colony forming units (CFU)/mL, and eight replicates of each dilution were tested by real-time PCR; 95% detection limit was determined as described previously (16).

RESULTS

Real-time PCR assays targeting the IS*481* element (IS*481*), *B pertussis*/*B parapertussis* gene for an outer membrane porin protein (OMP), pertussis toxin (*ptx*) promoter (BPTP), pertactin gene (PRN), and a putative thialase gene (BP283) (Table 1) (11-15) were selected from the literature. In addition, two new sets of primers and Taqman probes were designed based on *ptx* promoter (PT-P) and *B pertussis* gene for a porin protein (BPTD_0837) (POR). The new *ptx* promoter assay was designed through modification of the primer and probe sequences described by Grogan et al (12) in an attempt to further improve the specificity of the assay. By aligning the genomes of several different *Bordetella* species, it was noted that the published probe sequence has only a single nucleotide mismatch with the corresponding sequences of *B bronchiseptica* and *B parapertussis* genome. In contrast, the newly designed probe has >3 nucleotide differences with the corresponding sequences of *B bronchiseptica* and *B parapertussis* genome. The new POR assay was designed by exploiting the nucleotide sequence variation between the *B pertussis* gene for a porin protein and the corresponding sequences in the genomes of *B parapertussis*, *B bronchiseptica* and *B holmesii* (Figure 1).

All PCR assays were first tested using a panel comprised of ATCC strains and patient isolates of various *Bordetella* species and other bacteria commonly found in the respiratory tract (Table 2). The OMP

TABLE 3
Detection of *Bordetella pertussis* in nasopharyngeal samples from patients

	IS481 (Tatti et al [11])	BPTP (Grogan et al [12])	BP283 (Probert et al [13])	PRN (Vincart et al [14])	POR (in-house assay)
True-positive samples, n	20	19	19	19	20
False-positive samples, n	1	1	0	0	0
True-negative samples, n	85	86	87	87	87
False-negative samples, n	0	1	1	1	0
Sensitivity, %	100	95	95	95	100
Specificity, %	98.8	98.8	100	100	100

**Samples were considered to be positive if the cycle threshold values were ≤40 and typical amplification curves were observed. BPTP* Bordetella parapertussis *toxin promoter; POR Porin; PRN Pertactin*

A

Sample	C_T values from different assays						Sequencing
	POR	PRN	BP283	BPTP	OMP	IS481	
1	38.4	38.3	0	36.0	*36.0	30.2	*Pertussis*
2	*37.5	0	38.1	0	34.5	31.6	*Pertussis*
3	0	0	0	*35.5	0	32.2	*Bronchiseptica*

B

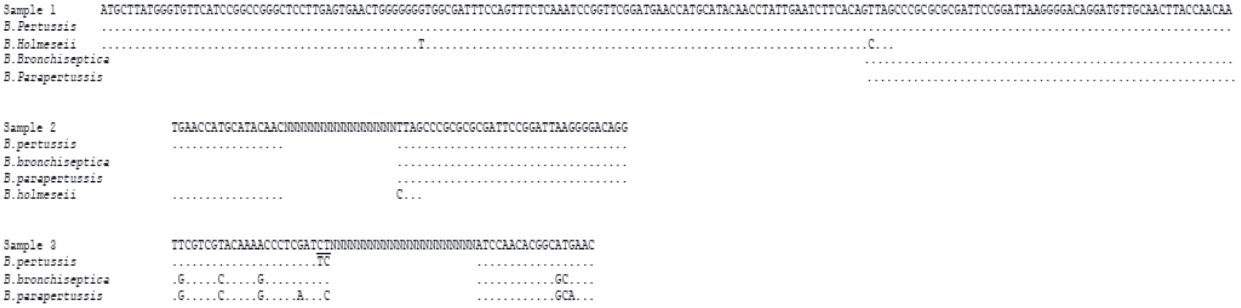

Figure 2) *Further analysis of discrepant samples.* **A** *Table showing parallel results of various PCR assays and sequencing for three discrepant samples (*Polymerase chain reaction [PCR] products that were sequenced).* **B** *Alignment of sequences obtained from different PCR products with genome sequences from different* Bordetella *species (GeneBank accession nos. HE965805 [*Bordetella pertussis*], BX640433 [*Bordetella parapertussis*], HE965806 [*Bordetella bronchiseptica*] and DQ420073 [*Bordetella holmesii*]). Dots indicate homology with the derived sequences. For samples 2 and 3, only partial sequences were obtained because these PCR products are very small in size. For sample 3, two bases right after the forward primer (underlined) indicate that the sequence is obtained from* B bronchiseptica *genome. C_T Cycle threshold; N Low-quality basecalls; OMP Outer membrane porin protein; POR Porin; PRN Pertactin*

assay (15) is a SYBR Green-based, shared primer assay designed to amplify both *B pertussis* and *B parapertussis*-specific DNA, but with different size PCR products that can subsequently be differentiated by their dissociation curves. When this PCR assay was applied to the panel of different *Bordetella* species and other respiratory bacteria, false-positive amplification was observed with *B holmesii, B bronchiseptica*, *S mitis* and *S pyogenes*, but the dissociation curves were distinct from that of *B pertussis* and *B parapertussis* strains (Table 2). Apart from this assay, none of the other assays showed any cross-reactivity with other bacteria tested in the present study that do not belong to the genus *Bordetella*. The analytical sensitivity of IS*481* assay for *B pertussis* was much higher (by cycle threshold >7) than other assays. The assay detected all *B pertussis* strains in the test panel, but also resulted in the false-positive detection of *B parapertussis, B holmesii* and *B bronchiseptica* strains, confirming the nonspecific nature of this target.

Among the single-target Taqman assays, the BPTP assay nonspecifically detected *B bronchiseptica* along with *B pertussis* strains, while the *ptx*-promoter assay, PT-P, designed in the present study lacked sensitivity and failed to detect all *B pertussis* strains. The BP283 assay was specific to *B pertussis* but failed to detect two of the four *B pertussis* strains tested. The PRN assay detected all *B pertussis* strains but it weakly detected *B bronchiseptica* at a relatively high concentration (≥5.4×10^5 CFU/mL). In contrast, the newly designed POR assay did not exhibit any cross-reactivity with DNA from any of the non-*B pertussis* strains.

Based on these results, the IS*481*, BPTP, BP283, PRN and POR assays were selected for further analysis of clinical samples. Among the 107 nasopharyngeal wash specimens, 21 (19.6%) samples were positive according to the IS*481* assay, 20 (18.7%) samples were positive according to the BPTP and POR assays, and 19 (17.7%) samples were positive according to the BP283 and PRN assays (Table 3). Samples that produced discrepant results were further analyzed by sequencing the PCR products. There were three discrepant samples: a sample that was positive according to all assays except the BP283 assay; a sample that was missed by the PRN and BPTP assays; and a sample that was only positive using the BPTP and IS*481* assays. Sequencing of PCR products confirmed that the first two samples were, in fact, *B pertussis*, while the third sample was *B bronchiseptica* (Figure 2). Taking into account the definitive sequence identification, the performances of all five *B pertussis*-specific assays were comparable, but the newly designed POR assay correctly detected all samples with a clinical sensitivity equal to that of IS*481* assay. Based on a Probit regression analysis, the 95% detection limit of the POR assay was 4 CFU/reaction.

DISCUSSION

The diagnosis of whooping cough caused by *B pertussis* could be difficult, particularly at the early stages because the signs and symptoms of the disease closely resemble those of other common respiratory illnesses, such as those caused by *Mycoplasma pneumoniae, Corynebacterium*

pneumoniae and adenoviruses. Moreover, other related *Bordetella* species, such as *B parapertussis*, *B bronchiseptica* and *B holmesii*, are also known to cause respiratory illness in humans, although generally with a milder clinical presentation (3,8). Specificity of diagnostic and surveillance methodologies for *B pertussis* is, therefore, critical for the effective management and control of pertussis disease. However, PCR testing for pertussis, which is now in widespread use, has variable specificity and needs further standardization and optimization (1,9). In the present study, we aimed to identify or develop a highly specific and sensitive, real-time PCR assay for *B pertussis* that can replace the PCR assays based on IS*481*, which is a repeat element present in the genomes of different *Bordetella* species at variable copy numbers (5). We tested and compared the performance characteristics of both previously published and in-house developed PCR assays that target different genes and genomic regions specific to *B pertussis*, in addition to that of IS*481*-based PCR assays. We observed remarkable variation in the specificity of different PCR assays while assessing a sample panel, comprised of reference strains of various *Bordetella* species. However, the sensitivity and specificity of these assays remained approximately 95% in the clinical samples.

The discrepant clinical sample results obtained by the different *B pertussis* specific real-time PCR assays are consistent with those obtained using the ATCC panel of different *Bordetella* species. The BP283 assay, which failed to detect two known ATCC strains of *B pertussis*, also failed to detect one of the *B pertussis*-positive clinical samples. The IS*481* and BPTP assay, which detected a known ATCC strain of *B bronchiseptica*, also produced one false-positive result among the clinical samples, which was eventually confirmed to be positive for *B bronchiseptica* by sequencing. The results of using the PRN assay on the ATCC panel suggests that this assay could also falsely detect *B bronchiseptica*, particularly at bacterial concentrations $>10^5$ CFU/mL in clinical samples. The novel real-time PCR assay that targets the porin gene of *B pertussis* demonstrated superior performance over all other *B pertussis* PCR assays tested in the present study.

The higher analytical sensitivity, but poor specificity of the IS*481* PCR, is well described in the literature and is a generally accepted phenomenon (5-7,11). However, an independent comparison of the most common single-target PCR assays for *B pertussis* has not been reported to date. Therefore, the results of the present study could be useful for guiding clinical microbiologists in the selection of an appropriate real-time PCR assay for *B pertussis*. The new POR gene-based PCR assay described may serve as a valuable new tool for diagnostic laboratories seeking to improve the specificity of *B pertussis* detection, with sensitivity comparable with that of IS*481* PCR assays.

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