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Evaluation of Four Commercial Real-Time PCR Assays for Detection of *Bordetella* spp. in Nasopharyngeal Aspirates[▽]

Philippe Lanotte,[1,2] Chloé Plouzeau,[3] Christophe Burucoa,[3,4] Carole Grélaud,[5] Sophie Guillot,[6] Nicole Guiso,[6] and Fabien Garnier[5]*

CHRU de Tours, Service de Bactériologie-Virologie, Hôpital Bretonneau, Tours, France[1]; Université François Rabelais, Tours, France[2]; CHU de Poitiers, Laboratoire de Bactériologie, Hôpital La Milétrie, Poitiers, France[3]; Université de Poitiers, Poitiers, France[4]; CHU de Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, Limoges, France[5]; and Institut Pasteur, Centre National de Référence de la Coqueluche et Autres Bordetelloses, Unité de Prévention et Thérapie Moléculaires des Maladies Humaines, 25 rue du Docteur Roux, 75015 Paris, France[6]

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We evaluated the performances of 4 commercial real-time PCR kits for *Bordetella pertussis* IS*481* sequence detection in nasopharyngeal aspirates by comparison with an in-house real-time PCR assay. Among them, the Simplexa Bordetella pertussis/parapertussis assay (Focus Diagnostics), the SmartCycler *Bordetella pertussis*/*parapertussis* assay (Cepheid), and Bordetella R-gene (Argene) present sensitivities over 90%. One kit proved unsuitable for routine clinical use.

Nucleic acid amplification tests (NAAT), including PCR and, more recently, real-time PCR, overcome some of the limitations of culture and serological methods for the diagnosis of *Bordetella* infection (1, 6, 12). NAAT targets include IS*481* for *Bordetella pertussis* (this gene is also present in *B. holmesii* and sometimes in *B. bronchiseptica*) (12, 13), the pertussis toxin promoter or porin gene BP3385 for *B. pertussis* (10), and IS*1001* for *B. parapertussis* (5, 13). NAAT methods are highly sensitive and may be either genus or species specific, depending on the choice of primers and targets. Real-time PCR accelerates the diagnostic process by combining amplification and detection (12, 13). These methods have proved more sensitive than the equivalent gel-based system (9, 12), and many medical laboratories have developed in-house tests. A voluntary external molecular quality control procedure for these in-house methods was set up in France in 2009 by the National Reference Centre for Whooping Cough and Other Bordetelloses (Institut Pasteur, Paris) (3), following a study performed in eight hospital laboratories throughout France to assess the performance of in-house methods and adaptations of the techniques developed by Reischl et al. (11), Kösters et al. (8), Caro et al. (3), and Templeton et al. (13). Commercial molecular diagnostic kits are now available but have never been comparatively tested.

The aim of this study was to compare the performances of 4 commercial real-time PCR assays for the detection of *B. pertussis*, using as a reference an in-house method evaluated during the French external molecular quality control (3).

Eighty-one nonredundant nasopharyngeal aspirates (NPA) from patients with suspected pertussis were tested in the bacteriology laboratories of three French teaching hospitals (Tours, Poitiers, and Limoges). Sixty-six samples tested positive with our in-house PCR, while 15 had tested negative but were from patients with a suspicion of pertussis. The samples and DNA extracts were stored at −20°C until use. DNA was extracted with the Invisorb spin cell minikit (Invitek, Berlin, Germany) in Tours and with the QIAamp DNA minikit (Qiagen) in Poitiers and Limoges by following the manufacturers' instructions. The principles and procedures of the two extraction methods are very similar. Tenfold serial dilutions of DNA from *B. pertussis* strain Tohama, containing 238 copies of IS*481*, were used to determine the analytical sensitivity of each method. DNA extracted from each of the 81 samples was tested with the in-house real-time PCR assay using primers Bp481F (5′-CCGAACCGGATTTGAGAAAC-3′) and Bp481R (5′-TAGGAAGGTCAATCGGGCAT-3′), which target a 100-bp fragment of IS*481*. Detection was based on hybridization of the Bp481S probe (5′–6-carboxyfluorescein [FAM]–CCGGCCGGATGAACACCCATAA–6-carboxytetramethylrhodamine [TAMRA]–3′). Primers were purchased from Eurogentec (Seraing, Belgium) and were used at a concentration of 10 μmol/liter. Amplification was performed with a SmartCycler device (Cepheid, Maurens-Scopont, France), with 5 μl of DNA, 12.5 μl of premix Ex Taq (TaKaRa, Foster City, CA), 4.2 μl of water, 0.4 μl of each primer, and 2.5 μl of Bp481S probe (1 μM). The thermal cycling conditions were as follows: 1 cycle of 15 s at 95°C, followed by 45 cycles of 5 s at 95°C and 10 s at 60°C. Each laboratory tested the specimens collected in its host hospital and used the same protocols as the other laboratories. The SmartCycler II apparatus (Cepheid) was used for all assays.

The four commercial PCR kits, all based on TaqMan technology, were Bordetella R-gene (Argene, Verniolle, France), the Simplexa Bordetella pertussis/parapertussis assay (Focus Diagnostics; distributed in France by Eurobio, Courtabeuf, France), the *Bordetella pertussis* real-time PCR kit (catalog no. RD-0061-02 [Shanghai ZJ Bio-Tech; distributed in France by

* Corresponding author. Mailing address: Laboratoire de Bactériologie-Virologie-Hygiène, Centre Hospitalier Universitaire de Limoges, 2 avenue Martin Luther King, 87042 Limoges Cedex, France. Phone: 33 5 55 05 63 48. Fax: 33 5 55 05 67 22. E-mail: fabien.garnier@unilim.fr.

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TABLE 1. Comparison of main parameters of 4 real-time PCR kits tested

Manufacturer or source	Name of kit	Target pathogen(s)	Target gene(s)	Kit includes:				Reagent storage temp (°C)	Final reaction vol[b] (μl)	Vol (μl) of DNA from sample added	No. of cycles used for comparison (no. of cycles recommended by manufacturer)	No. of tests with kit	Apparatus for which kit is available
				Ready-to-use mix containing primers and probe	IC[a] detection	Extraction controls	Positive control						
In-house		*B. pertussis*	IS*481*	No	No	No		−20	20	5	45		
Argene	Bordetella R-gene	*B. pertussis*	IS*481*	Yes	Yes (10 μl IC to add in sample before extraction)	Yes	Yes	−20	25	10	45 (45)	60	LightCycler (LC480/LC2.0), SmartCycler, ABI Prism, Rotor-Gene
Shanghai ZJ Bio-Tech (distributed in France by BioAdvance)	*Bordetella pertussis* real-time PCR kit	*B. pertussis*	IS*481*	No	Yes (1 μl IC to add in mix before amplification)	No	Yes (10^7 copies/ml)	−20	25	2.5	45 (40)	25	LightCycler (LC480), iCycler (iQ4/iQ5), SmartCycler II, ABI 7000/7300/7500/7900, Rotor-Gene 6000, Mx3000P/3005P, MJ Option2/Chromo4
Cepheid	SmartCycler *Bordetella pertussis/parapertussis* assay	*B. pertussis* and *B. parapertussis*	IS*481* (*B. pertussis*), IS*1001* (*B. parapertussis*)	Yes	Yes (contained in mix [20 copies/μl])	No	No	+4 (beads)	25	5	45 (40)	40	SmartCycler II
Focus Diagnostics (distributed in France by Eurobio)	Simplexa Bordetella pertussis/parapertussis assay	*B. pertussis* and *B. parapertussis*	IS*481* (*B. pertussis*), IS*1001* (*B. parapertussis*)	No	Yes (5 μl IC to add in sample before extraction)	Yes	Yes	−20	25	5	45 (45)	48	LightCycler LC480, SmartCycler II, ABI 7500

[a] IC, internal control.
[b] As recommended by the manufacturer of the SmartCycler apparatus.

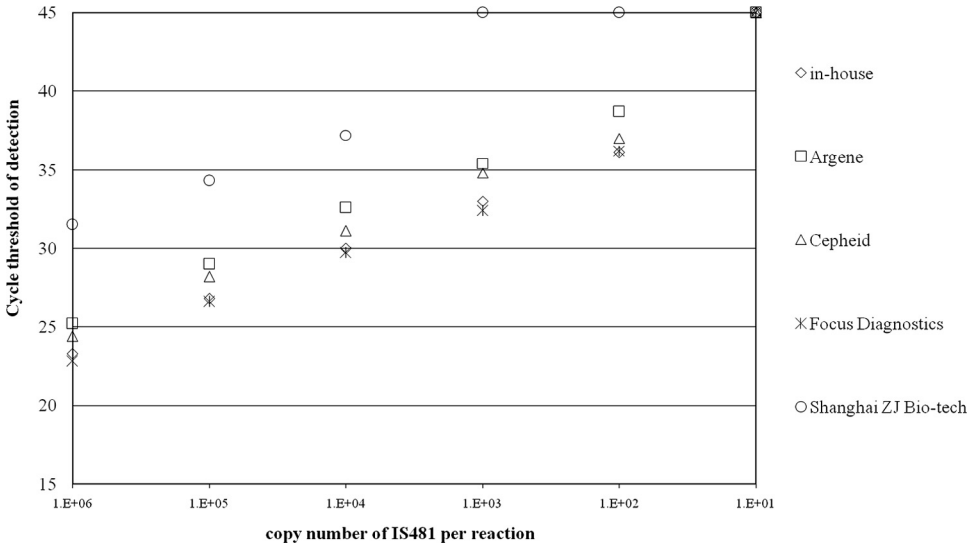

FIG. 1. Representation of the crossing threshold obtained for each commercial kit and the in-house real-time PCR for the detection of the *Bordetella* IS*481* sequence.

BioAdvance, Bussy-Saint-Martin, France]), and the SmartCycler *Bordetella pertussis*/*parapertussis* assay (Cepheid). The different kits were used according to the recommendations of the manufacturers, including cycle thresholds (C_T) of 7 for the Shanghai ZJ Bio-Tech kit, 15 and 30 for the Focus Diagnostics kit, and 30 for the other two methods. Differences between the methods are shown in Table 1.

The analytical sensitivity of each method, tested in duplicate, is shown in Fig. 1. The C_T values were similar for the different methods with up to 10^4 copies of IS*481* per reaction, except that the C_T of the Shanghai ZJ Bio-Tech test was about 7 cycles above that of the other methods whatever the number of copies. Only the in-house method and the Argene, Cepheid, and Focus Diagnostics kits were positive with the dilution containing 100 copies of IS*481* per reaction.

Each clinical DNA specimen was tested with the in-house method and with the 4 commercial kits. All the DNA specimens were thawed and tested on the same day (no refreezing occurred between the different assays). The results are shown in Table 2. No false positives were noted for any assay. With the in-house method as the gold standard, clinical sensitivity was 97% with the Focus Diagnostics kit, 93.9% with the Cepheid kit, 90.9% with the Argene kit, and 51.5% with the Shanghai ZJ Bio-Tech kit. The Focus Diagnostics kit had the lowest mean C_T (Table 2), which was not significantly different from that of the in-house assay. The Argene and Cepheid kits had slightly but not significantly higher mean C_T values than the in-house assay. In contrast, the Shangai ZJ Bio-Tech kit had significantly higher mean C_T values than the in-house assay. It is noteworthy that the manufacturer of the Shanghai ZJ Bio-Tech kit recommends using only half the amount of DNA used in the other kits. The performance of the Focus Diagnostics kit was unaffected when a C_T of 15 rather than 30 was used (data not shown).

Internal controls are included to detect PCR inhibition. Beta-globin was used for this purpose in the in-house method in a separate vial. The Argene and Focus Diagnostics kits also include extraction controls (Table 1). The list of apparatus on which kits can be used is shown in Table 1. Two kits (the Cepheid and Focus Diagnostics assays) are claimed to detect *B. pertussis* and *B. parapertussis* in the same tube, while the other two kits and the in-house method target only IS*481*. *B. holmesii*, which also possesses IS*481*, cannot be distinguished from *B. pertussis* with these kits. This species rarely induces

TABLE 2. Clinical sensitivities and mean C_T values for real-time PCR kits[a]

Kit	No. of positive specimens identified/total no. of positive specimens	Sensitivity (%)	Specificity (%)	Mean C_T ± SD	*P* value[d]
In-house kit	66/66	100	100	25.5 ± 8.6	
Argene kit	60/66	90.9	100	27.1 ± 9.5	NS
Cepheid kit	62/66	93.9	100	25.8 ± 9.2	NS
Focus Diagnostics kit	64/66	97	100	24.8 ± 8.8[b]	NS
Shanghai ZJ Bio-Tech kit	34/66	51.5	100	37.3 ± 8[c]	<0.01

[a] Data for the four real-time PCR kits tested are in comparison to results of the in-house real-time PCR assay for detection of the *Bordetella* IS*481* sequence in 66 *Bordetella*-positive specimens.
[b] As recommended by the manufacturer, the C_T retained for the comparison was 15.
[c] As recommended by the manufacturer, the C_T retained for the comparison was 7.
[d] Determined by paired *t* test (2). NS, not significant.

pertussis-like symptoms and can be identified by real-time PCR-based on the *recA* gene (7).

In conclusion, all four kits tested here were highly specific, whereas their sensitivities were highly variable. The Argene, Focus Diagnostics, and Cepheid kits performed as well as the in-house method in terms of both analytical and clinical sensitivities. These three kits include an internal control to detect inhibitors, which is important in routine practice (4), but only two of them, the Argene and Focus Diagnostics tests, validate the extraction step. The Shanghai ZJ Bio-Tech kit needs to be improved if it is to be used in routine clinical settings.

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