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Comparative Evaluation of the Diagenode Multiplex PCR Assay on the BD Max System versus a Routine In-House Assay for Detection of *Bordetella pertussis*

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This study looked at 128 nasopharyngeal aspirates (NPA) and 162 throat swabs (TS) tested with the Diagenode multiplex assay on the BD Max system versus our in-house *Bordetella pertussis* PCR. Sensitivity and specificity were 97.3% and 100% for NPA and 88.3% and 98% for TS, respectively. Of positive NPA, 42.1% were coinfecting with respiratory viruses.

Pertussis remains one of the 10 leading causes of global childhood mortality in unvaccinated populations (1, 2). Although the number of laboratory-confirmed cases of pertussis decreased in November 2012 and decreased further in June 2013, activity in England and Wales has continued at raised levels compared to recent years (3).

Fast and accurate molecular diagnosis helps clinicians expedite appropriate treatment options and improve patient care. Real-time PCR remains the gold standard method used to diagnose pertussis in the laboratory (4, 5, 6, 7). The aim of this study was to evaluate performance of a CE marked, internally controlled multiplex real-time PCR from Diagenode (Diagenode Diagnostics, Liège, Belgium) for detection of *Bordetella pertussis* and *Bordetella parapertussis* on the BD Max system (Becton, Dickinson, Franklin Lakes, NJ, USA), a combined extraction and PCR platform.

Samples were collected over a 9-month study period from the beginning of May until the end of November 2012 and were submitted for *B. pertussis* testing by in-house real-time PCR to the local microbiology department. Samples were anonymized and tested retrospectively using the Diagenode internally controlled *B. pertussis* and *B. parapertussis* multiplex assay on the BD Max system.

There were two populations of samples. The first population was 128 nasopharyngeal aspirates (NPA) from pediatric patients. This group was 47.7% male (61/128) and 52.3% female (67/128), aged from 0 to 14 years (mean age, 1 year 1 month). The second population was throat swabs (TS) from mixed-age patients: 35.8% male (58/162) and 64.2% female (104/162), aged 0 to 85 years (mean, 24 years 5 months). NPA and TS were collected from patients and placed in 3 ml of universal transport medium (UTM) (Copan, Brescia, Italy). Transportation was via the normal laboratory van service. NPA and per-nasal swabs are shown from studies with culture identification to be superior for *B. pertussis* culture and are the recommended sample types. However, throat swabs have been used in studies using PCR and here were a pragmatic

choice to aid diagnosis in patients over 2 years (4, 5, 6, 8, 9). Samples from a Quality Control for Molecular Diagnostics (QCMD) panel containing 5 samples positive for *B. pertussis* and 1 sample positive for *Bordetella holmesii* were also tested (10). Handling and testing of specimens for the study were carried out in accordance with the approval of a local ethics board (South East Scotland SAHSC Human Annotated BioResource reference no. 10/S1402/33).

The test assay was compared to the validated in-house assay using extractions on the NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France) and PCR on the ABI7500 system (Applied Biosystems, Foster City, California). Assay performance was assessed by calculating sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively). Agreement between the Diagenode assay on the BD Max system and the in-house assay was assessed using Cohen's kappa. Two hundred microliters NPA or TS in UTM was extracted on the easyMAG system, eluted in 100 μ l extract, and run on the ABI7500 system. The in-house assay with phocine herpesvirus as the internal control used primers targeting IS481 (product size, 154 bp) adapted from an existing protocol (6, 10) using a VIC-labeled TaqMan probe instead of a Molecular Beacon. The protocol used 0.5 μ M (each) primer and 0.4 μ M probe (Bpfp, 5' TCA ATA GGT TGT ATG CAT GG 3'; Bprp, 5' GAT CAA TTG CTG GAC CAT T 3'; BpP, 5' YY CGG CCG GAT GAA CAC CCA TAA BHQ1 3').

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TABLE 1 Assay performance for detection of *Bordetella pertussis* using Diagenode kit

Sample type (n)	% (95% CI)					Cohen's kappa (95% CI)	% inhibition	IC mean C_T (range)
	Prevalence	Sensitivity	Specificity	PPV	NPV			
NPA (128)	29.7 (22.1–38.5)	97.3 (84.6–99.9)	100 (94.9–100)	100 (88.3–100)	98.9 (93.2–99.9)	0.981 (0.994–1.0)	0.6	27.1 (22.5–29.1)
TS (162)	37.0 (29.7–45.0)	88.3 (76.8–94.8)	98.0 (92.4–99.7)	96.4 (86.4–99.4)	93.5 (86.5–97.1)	0.879 (0.80–0.96)	0	28.0

TABLE 2 NPA and TS results (2 × 2 table)^a

Sample type and result (BD Max)	No. of samples with result by easyMAG/ABI7500:	
	Pos	Neg
NPA		
Pos	37	0
Neg	1	90
Throat swabs		
Pos	53	2
Neg	7	100

^a Pos, positive; Neg, negative.

Cycling conditions for the ABI7500 system were as follows: step 1, 1 cycle of 95°C for 3 min; step 2, 45 cycles of 95°C for 3 s, 55°C for 25 s, and 72°C for 10 s (plus detection).

Samples extracted and amplified on the BD Max system were prepared by mixing 375 µl of sample with an equal volume of proteinase K pretreatment (1 mg/ml proteinase K in 20 mM Tris, pH 8.3, 0.5% SDS buffer). Samples were then added to the sample buffer tube and extracted using the universal reagent strip, according to the manufacturer's protocol. The protocol used fluorogenic target-specific hybridization probes for IS481 and IS1001 for *B. pertussis* and *B. parapertussis*, respectively (6, 9). Coamplification of the two targets could indicate either coinfection with *B. pertussis* and *B. parapertussis*, cross-reactivity between IS1001 and IS481, or infection with *Bordetella holmesii*, which, like *B. parapertussis*, can cause pertussis-like symptoms (6). The IS481 (11) target is present in multiple copies in the genome of *B. pertussis* (12); however, it is also present in 8 to 10 copies per genome in *B. holmesii* (13) and in some human and animal isolates of *Bordetella bronchiseptica* (14, 15). This means that as a stand-alone PCR target, IS481 has limitations for the detection of *B. pertussis*; notwithstanding this, the IS481 is most commonly used as a screening test. PCR was performed on the BD Max PCR cartridge with the following thermo-profile: 10 min at 98°C (1 cycle) and a cycle program (45 cycles) of step 1, 15 s at 98°C, and step 2, 55.9 s at 64°C (plus detection).

Using the in-house real-time PCR, there was 29.7% and 37.0% prevalence of *B. pertussis* in NPA from pediatric patients and TS from mixed-age patients, respectively (Table 1). The Diagenode assay using NPA on the BD Max system showed very good diagnostic sensitivity (97.3%) and excellent specificity (100%), and PPV and NPV were 100% and 98.9%, respectively. Sensitivity, specificity, PPV, and NPV were all slightly lower for the TS, at 88.3%, 98%, 96.4%, and 93.4%, respectively. The two assays showed excellent agreement in the NPA group (Cohen's kappa = 0.981) and very good agreement in the TS group (Cohen's kappa = 0.879). Two throat swabs were found to be positive on the Diagenode assay on the BD Max system and negative on the in-house assay (Table 2). Both of these samples were positive for both targets on the Diagenode assay, so it is possible that there was some cross-reactivity from the IS1001 target. One of the two samples had a cycle threshold (C_T) value of over 37.3, and so it was only a low-level positive. The second throat swab that was positive with the Diagenode assay on the BD Max system and negative on the in-house assay had a C_T value of 17. This sample was found to be negative on repeat with the in-house assay. There was not enough of the sample to test it again on the BD Max system. The internal control (IC) in the Diagenode assay showed that there were 2 samples inhibited (0.6% inhibition) in the NPA group (mean C_T

TABLE 3 Workflow, test, and labor cost comparison of Diagenode assay on BD Max system and in-house assay on easyMAG and ABI system

Parameter	BD Max/Diagenode	easyMag/ABI in-house assay
Sample preparation time/test	1 min	4 min
PCR setup time/test	0 min	2 min
Total run time/24 samples, sample to result	1 h 45 min	3 h
Labor cost ^a	£5.20	£32
Labor time, including setup, quality checks, and analysis per run	30 min	1 h 15 min
Assay cost/test	£15	£5

^a Labor costs relate to the grade of staff able to run the assay.

IC, 27.1) and that there was no inhibition in the TS group (mean C_T IC, 27.9) (Table 1). There were no inhibited samples by the in-house assay. The sensitivity was 1 to 10 CFU/ml for both *B. pertussis* assays.

The NPA samples were tested for the routine respiratory panel: influenza A and B viruses, rhinovirus (RhV), parainfluenza viruses 1 to 3 (PF1 to -3), adenovirus (AdV), *Mycoplasma pneumoniae*, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) (16, 17, 18). Sixty-four of 128 NPA were positive for a respiratory virus, and 16 (42.1%) were coinfecting with pertussis bacteria and a respiratory virus (4/16 with PF3, 1/16 with RSV, and 11/16 with RhV). One patient was positive for both the IS481 and IS1001 targets using the Diagenode assay, suggesting possible *B. holmesii* infection or coinfection with *B. pertussis* and *B. parapertussis*. This highlights the value of a combined approach to testing for respiratory pathogens.

Six samples taken from the QCMD panel were tested using the Diagenode assay and achieved detection of all *B. pertussis*-positive cases and one of *B. holmesii* which was positive for IS481 and IS1001. The Diagenode assay showed 100% agreement with the in-house assay.

Those performing the Diagenode assay on the BD Max system found it straightforward and easy to use. A full run of 24 samples took around 1 h 45 min to prepare and perform (Table 3), which is comparable to running the assay using the in-house method; however, the walk-away functionality of the BD Max system means that hands-on time is kept to a minimum, reducing labor costs (Table 3).

In conclusion, the Diagenode assay performed very well for the detection of *B. pertussis* in pediatric NPA and also performed well when testing TS from a mixed pediatric and adult population. In NPA, there was very little inhibition detected, and there was no inhibition seen in TS. The assay was straightforward to perform and required very little hands-on time. The simplicity of the assay setup would lend itself to less specialist laboratories to enable more local rapid diagnostics.

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