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Testing implications of varying targets for *Bordetella pertussis*: comparison of the FilmArray Respiratory Panel and the Focus *B. pertussis* PCR assay

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ABSTRACT

Background The FilmArray Respiratory Panel (RP) detects multiple pathogens, including *Bordetella pertussis.* The multiplex PCR system is appropriate for a core laboratory or point of care due to ease of use. The purpose of this study is to compare the analytical sensitivity of the FilmArray RP, which targets the promoter region of the *B. pertussis* toxin gene, with the Focus real-time PCR assay, which targets the insertion sequence IS*481*.

Methods Seventy-one specimens from patients aged 1 month to 18 years, which had tested positive for *B. pertussis* using the Focus assay, were analysed using the FilmArray RP.

Results Forty-six specimens were positive for *B. pertussis* by both the Focus and the FilmArray RP assays. Twenty-five specimens were negative for *B. pertussis* using the FilmArray RP assay, but positive using the Focus assay.

Conclusions The FilmArray RP assays will detect approximately 1/3 less cases of *B. pertussis* than the Focus assay.

INTRODUCTION

The target with the greatest analytical sensitivity to detect *Bordetella pertussis* is the insertion sequence, IS*481*.[1–3] IS*481* is internally repeated between 50 and >200 times in each *B. pertussis* genome, resulting in a PCR assay with high analytical sensitivity. However, studies have identified the presence of IS*481* in *Bordetella holmesii*, an organism that also has been associated with cough-like illness.[1 4–6] When compared with *B. pertussis*, IS*481* is internally repeated 8–27 times within the *B. holmesii* genome.[5 7] The conundrum related to PCR testing for *B. pertussis* revolves around whether the assay implemented in a clinical laboratory should be highly sensitive (IS*481* target) or highly specific (pertussis toxin gene target).

The FilmArray Respiratory Panel (RP) (BioFire Diagnostics, Salt Lake City, Utah, USA) uses PCR to detect 20 respiratory pathogens, including *B. pertussis*.[8] The closed system makes testing easy, resulting in the system being amenable to less complex laboratories or point of care.[9] The genetic target for identification of *B. pertussis* is the promoter region of the pertussis toxin gene,[8] which is present in only one copy per *B. pertussis* genome. While this target is specific, with lack of cross-reactivity with *B. holmesii*, it should have lower analytical sensitivity compared with detection of the IS*481* locus. Previous analyses of the FilmArray RP reported high agreement between the results of FilmArray RP and other PCR assays for *B. pertussis*, suggesting that the analytical sensitivity of the FilmArray RP for *B. pertussis* may be equivalent to assays that target the insertion sequence IS*481*.[9 10]

The purpose of this study is to use clinical samples to compare the sensitivity of the FilmArray RP test, a closed system with on-board extraction coupled with the toxin gene target, to the Focus PCR assay (Focus Diagnostics, Cypress, California, USA), which amplifies insertion sequence IS*481*, following offline extraction.

METHODS

Specimens

Reserve samples, held in −80°C in universal transport medium in the microbiology laboratory at Children's Healthcare of Atlanta (Atlanta, Georgia, USA), were used for testing. All samples were initially collected using flocked swabs (Copan Diagnostics, Murrieta, California, USA) between September 2012 and June 2013. Flocked swabs are approved for collection of both viruses and bacteria, and have been verified for collection and performance of PCR by our laboratory. The samples were originally tested between September 2012 and June 2013 using the Focus assay that targets the insertion sequence IS*481*. This is a laboratory-developed test using analyte-specific reagents, with a limit of detection of five cells at a C_T of 40. Each sample was positive for *B. pertussis*. Residual specimen in universal transport medium, which was not used for the initial testing, was frozen within 48–72 h at −80°C.

For the comparison between the FilmArray RP assay and the Focus assay, samples were thawed and retested using the Focus IS*481*-based assay and FilmArray RP assay. Testing was performed the same day the specimen was thawed for the repeat Focus assay and within three days of thawing for the FilmArray RP assay.

FilmArray RP

Specimens were tested using the FilmArray RP per the manufacturer's instructions. At the time of testing, the FilmArray RP, including the test for *B. pertussis*, had been cleared by the US Food and Drug Administration. The lower limit of detection for *B. pertussis* was stated to be 4000 copies/reaction by the manufacturer. Briefly, for each sample, 1.0 mL of hydration solution (molecular reagent grade water) was added to the pouch to rehydrate the reagents. In total, 300 µL of the reserve sample was

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Table 1 Comparison of sample results tested by the Focus assay and FilmArray

Sample ID #	Original Focus C_T result	Retested Focus C_T result	FilmArray result
1	12.6	12.4	Pos
2	14.8	15.5	Pos
3	14.9	15.3	Pos
4	15	15.4	Pos
5	15	20.6	Pos
6	15.3	15.2	Pos
7	15.6	15.7	Pos
8	16.8	17.4	Pos
9	18.4	18.3	Pos
10	18.6	19.6	Pos
11	18.8	18.1	Pos
12	19.2	19	Pos
13	19.4	20	Pos
14	20.1	20.2	Pos
15	20.1	21.1	Pos
16	20.2	21.3	Pos
17	20.4	22.2	Pos
18	21	21	Pos
19	21.2	22.5	Pos
20	21.2	20.2	Pos
21	22.4	23.2	Pos
22	22.6	21.4	Pos
23	22.8	23.7	Pos
24	23.2	23.5	Pos
25	23.4	23.5	Pos
26	23.7	22.4	Pos
27	23.7	23	Pos
28	23.8	23.9	Pos
29	23.8	29.5	Pos
30	23.9	27.3	Pos
31	23.9	28	Pos
32	24.8	32.9	Pos
33	25.2	30.6	Pos
34	25.3	31.2	Pos
35	25.4	26.2	Pos
36	25.7	25.4	Pos
37	26.2	25.4	Pos
38	26.6	27.8	Pos
39	26.7	26.9	Pos
40	26.7	26.9	Neg
41	27	26.2	Pos
42	27.2	27	Pos
43	27.4	27.6	Neg
44	27.4	30.6	Pos
45	27.7	27.1	Pos
46	28.3	28.3	Neg
47	29.3	29.6	Pos
48	29	31.4	Neg
49	29.3	30.1	Neg
50	29.9	30.3	Neg
51	30.2	29.7	Neg
52	30.7	32.9	Neg
53	30.8	31.1	Neg
54	31.4	32.8	Neg
55	31.7	32.3	Pos
56	31.8	32.5	Neg
57	32	38.9	Neg
58	32.4	29.1	Neg
59	32.9	32	Neg
60	33	31.4	Pos
61	33.2	32.5	Neg
62	33.3	30	Neg
63	33.4	34.8	Neg
64	33.6	37.2	Neg
65	33.8	34.9	Neg
66	33.8	35.4	Neg
67	33.9	31.1	Neg
68	36.1	35.9	Neg
69	36.1	37.8	Neg
70	36.5	39.9	Neg
71	37.2	37.9	Neg
72	37.3	0	Neg
73	37.9	0	Neg
74	38	0	Neg

Neg, negative; Pos, positive.

added to 500 µL of sample buffer mix and thoroughly combined. Also, 300 µL of sample/sample buffer mix was added to the pouch, which was then loaded onto the instrument. Each run contained internal process controls for extraction, dilution and PCR.

Focus PCR-based IS*481* assay

DNA from 200 µL of the reserve sample was extracted using the NucliSENS easyMAG system according to the recommendations of the manufacturer (bioMérieux Diagnostics, Marcy l'Etoile, France). Also, 5 µL of extracted nucleic acid was combined with the Focus Diagnostics *B. pertussis* and *Bordetella parapertussis* analyte-specific reagent reaction matrix. Forty cycles of PCR were accomplished using a 3M Integrated Cycler (3M Health Care, St. Paul, Minnesota, USA).

RESULTS

Seventy-four samples originally positive for *B. pertussis* between September 2012 and June 2013 were retested using the Focus assay. Seventy-one were positive on repeat testing. The specimens that did not repeat as positive were associated with original C_T values between 37.3 and 38. These 71 samples were used for the analysis with the FilmArray RP.

Forty-six samples were positive for *B. pertussis* by both the Focus assay and the FilmArray RP assay. Twenty-five samples were negative for *B. pertussis* using the FilmArray RP assay, but positive using the Focus assay. The range of cycle thresholds at which all samples were positive using both the FilmArray RP assay and the Focus assay was 12.4–26.2. At C_T values between 26.9 and 32.9, there was variability in the correlation with 14 samples positive by both FilmArray RP and Focus assays, and 16 negative using the FilmArray RP assay and positive using the Focus assay. At C_T values from 34.8 to 39.9, the FilmArray RP assay was negative and the Focus assay was positive.

The 25 discrepant samples were submitted to the *Bordetella* laboratory at the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia USA) and tested for *B. pertussis* and *B. holmesii* according to their published assay.[2] Two samples were negative for *B. pertussis*. Twenty-three samples were positive for the IS*481* gene target. Of these, 10 samples were also positive for

the toxin gene and deemed positive for *B. pertussis*. Thirteen were negative for the toxin gene and deemed intermediate for *B. pertussis*. *B. holmesii* was not detected in any of the 25 samples.

DISCUSSION

The rationale for this study is straightforward. We wanted to evaluate the analytical sensitivity of the FilmArray RP compared with a real-time PCR assay for the detection of *B. pertussis* using a large number of clinical samples. We hypothesised that the FilmArray RP, which targets the pertussis toxin gene, would have less analytical sensitivity than the Focus assay, which targets the insertion sequence IS*481*. Two studies did not find a loss of analytical sensitivity for *B. pertussis* when using the FilmArray RP.[9 10] However, the studies were designed to evaluate the entire panel, and so *B. pertussis* was not a primary focus of the studies. One study evaluated nine clinical samples, comparing the FilmArray RP with a laboratory-developed test targeting the insertion sequence IS*481*, and there was 100% correlation.[10] From evaluation of the C_T values, all C_T values were 30 or lower, suggesting that the burden of organisms was high in all the samples.

We did identify a loss of analytical sensitivity for *B. pertussis* using the FilmArray RP, which detected *B. pertussis* in 65% (46/71) of samples that tested positive using the Focus assay. FilmArray-negative samples occurred only at Focus C_T values ≥26.9. The reason for the difference in analytical sensitivity could be

1. detection of *B. holmesii* by the Focus assay instead of *B. pertussis* (ie, false-positive tests);
2. differences in the extraction methods;
3. differences in the gene target.

We did not identify *B. holmesii* using the reference method performed at the CDC, nor would it be logical that there would be such a high prevalence of *B. holmesii* in our population. While we did not conduct a detailed evaluation of the extraction methodologies, the FilmArray RP uses a greater sample volume than the easyMag, which was the nucleic acid purification method used prior to amplification with the Focus assay. It is likely, therefore, that the difference lies in the PCR target, as IS*481* is known to be present in multiple copies, resulting in increased analytical sensitivity.[2 11] Our data differ from those from Qin *et al*,[12] who demonstrated little difference between their limit of detection for the toxin promoter region and IS*481*. The performance specifications of their assay were reported to detect a similar limit of detection for each gene, indicating that their assay was not optimised for maximal sensitivity for the IS*481* target. We acknowledge the need for multitarget analysis to sort out the optimal diagnostic regimen for diagnosis of pertussis, but focus this study on two commercially available systems (table 1).

Key messages

- FilmArray RP is less sensitive than the Focus assay targeting the insertion sequence IS481 for *B. pertussis* detection.
- Caution should be executed when examining kits for diagnosis of *B. pertussis* as they may differ.

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