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BRIEF REPORT

# Low Detection Rate of *Bordetella pertussis* Using the BioFire FilmArray Respiratory Panel 2*plus*

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Syndromic respiratory panels are increasingly used worldwide. Their performance for detection of *Bordetella pertussis* needs to be evaluated. We found that the FilmArray Respiratory Panel 2*plus* (RP2+) assay, which uses the pertussis toxin promoter target for *B. pertussis*, can only detect highly charged samples. Negative RP2+ results should not be interpreted as an absence of *B. pertussis* in clinical samples.

**Keywords.** *Bordetella pertussis*; FilmArray respiratory panel; molecular diagnosis; whooping cough.

*Bordetella pertussis*, the main agent of whooping cough (also called pertussis), continues to cause infections despite high levels of vaccination and has even re-emerged in several countries [1]. Clinical diagnosis of whooping cough is difficult given the nonspecificity of symptoms in adults and vaccinated individuals [2]. Co-infections with viruses (eg, respiratory syncytial virus) may also confound a diagnosis of pertussis in infants, especially if these are the only agents tested for [3]. The laboratory confirmation of whooping cough is therefore essential for accurate diagnosis and treatment of the index case, as well as for surveillance purposes and prevention of disease transmission. Laboratory confirmation mostly relies on direct detection methods on a nasopharyngeal (NP) specimen, such as culture and quantitative polymerase chain reaction (PCR). PCR is the most sensitive approach when performed within the 3 first weeks after disease onset, but molecular *B. pertussis* detection methods suffer from a trade-off between sensitivity and specificity [4]. The target with the highest analytical sensitivity is the insertion sequence IS*481*, which is repeated >240 times in the *B. pertussis* genome [5]. However, IS*481* is also present in *Bordetella holmesii* (range, 28–36 copies) [6], which can also be detected in respiratory samples from patients with whooping cough–like illness [7], as well as in some *Bordetella bronchiseptica* isolates (≤1 copy) [6]. *B. pertussis*–specific PCRs targeting the pertussis toxin promoter region *ptxP* have been described, but these have reduced sensitivity compared with IS*481*, as only a single copy is present in the genome [4].

The FilmArray Respiratory Panel 2 plus (RP2+) is a multiplex in vitro diagnostic test for the simultaneous and rapid detection of 22 respiratory pathogens (18 viruses and 4 bacteria species including *B. pertussis*) directly from NP samples. It consists of automated nucleic acid extraction, nested multiplex PCR, and analysis of generated end point melting curve data. This multiplex panel presents the advantages of speed and could lead to pertussis diagnosis when the symptoms are not typical. The RP2+ as the previous RP genetic target for *B. pertussis* is *ptxP* [8]. In a recent multicenter evaluation of the FilmArray RP2 in nasopharyngeal swab samples, 2 out of 3 positive *B. pertussis* samples (according to the comparator RP) were detected [9].

The present study aimed to evaluate the performance of the FilmArray RP2+ for detection of *B. pertussis* on clinical samples when compared with a simplex quantitative PCR (qPCR) using the same target, with the IS*481* qPCR considered the reference standard.

## METHODS

### Sample Selection Based on *B. pertussis* Detection With an In-house *ptxP* qPCR

An in-house control *ptxP* real-time PCR targeting the promoter region of the pertussis toxin gene (named *ptx*A–Pr) was used as the comparator. This assay (*ptxP* qPCR) was previously shown to have a lower limit of detection (LLOD) of 30 CFU per PCR reaction [7]. The selected samples were clinical nasopharyngeal aspirates/swabs from patients with pertussis symptoms (n = 13) and were completed with quality control clinical samples (Quality Control for Molecular Diagnostics: www.qcmd.org; n = 5), all stored at −80°C at the French National Reference Center of Whooping Cough. All the selected samples were previously analyzed by qPCR and found positive for IS*481* and negative for hIS*1001* (specific for *B. holmesii* detection). The samples were collected continuously between 2018 and 2019 and tested using the in-house *ptxP* qPCR. Among these samples, we selected 16 to represent a range of positive *ptxP* threshold cycle (Ct) values and 2 samples for being below the detection limit of *ptxP* qPCR (no Ct value with *ptxP*) while

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being positive on IS*481* qPCR, with Ct values of 34 and 36 for this higher-copy target. The selected samples were coded with a letter designation to be tested blindly with FilmArray RP2+.

All French bacteriological samples were collected, coded, shipped, managed, and analyzed according to the National Reference Center protocols and received approval from the French Supervisory Ethics Authority (CNIL, No. 1474593).

#### FilmArray RP2+ Analyses

Samples were tested using the FilmArray RP2+ according to the manufacturer's instructions [8]. Briefly, for each sample, 1.0 mL of hydration solution (water) was added to the pouch to rehydrate the reagents. In total, 300 µL of the reserve sample was added to 500 µL of sample buffer mix and thoroughly combined. Then, 300 µL of sample/sample buffer mix was added to the pouch, which was then loaded onto the instrument (system 2.0). Each run contained internal process controls for extraction, dilution, and PCR. The FilmArray test consists of automated nucleic acid extraction, nested multiplex PCR, and analysis of generated end point melting curve data. This process took approximately 1 hour per run. Valid run results were reported as "detected" or "not detected" for each target.

#### Performance Analysis

To compare the performance of RP2+ and in-house *ptxP* qPCR, all samples were analyzed in parallel by IS*481* qPCR (Argene *Bordetella* R-gene, Biomerieux). The detection rate was estimated based on negative or positive test results of RP2+ and in-house *ptxP* qPCR compared with IS*481*, which is considered the reference standard.

## RESULTS

The results are presented in Figure 1. Twelve samples were positive for *B. pertussis* using both the FilmArray RP2+ and the in-house *ptxP* qPCR. The range of *ptxP* Ct values for these samples was 18–35. In contrast, 4 samples were found to be negative using the FilmArray RP2+ assay; all had a Ct value >35 using the in-house *ptxP* qPCR. These 4 discrepant samples were retested by the in-house *ptxP* qPCR, starting from the clinical material, thus including extraction and amplification, and were confirmed positive with Ct values >35 (37–40). Finally, the 2 samples that were negative with the in-house *ptxP* assay but positive using the qPCR IS*481* with a Ct value of 34 and 36, respectively, were also negative with RP2+ (Figure 1). Therefore, while the detection rate for *B. pertussis* of in-house *ptxP* qPCR was fixed at 89% in our study, the detection rate of RP2+ was 67%, revealing a lower detection rate for RP2+, when considering qPCR IS*481* the reference standard.

## DISCUSSION

The FilmArray respiratory multiplex panel is being increasingly used for diagnosis of respiratory infections. It presents the advantages of multipathogen testing, allowing for detection of nonanticipated pathogens using broad diagnosis coverage. However, multiplexing often comes at the expense of analytical

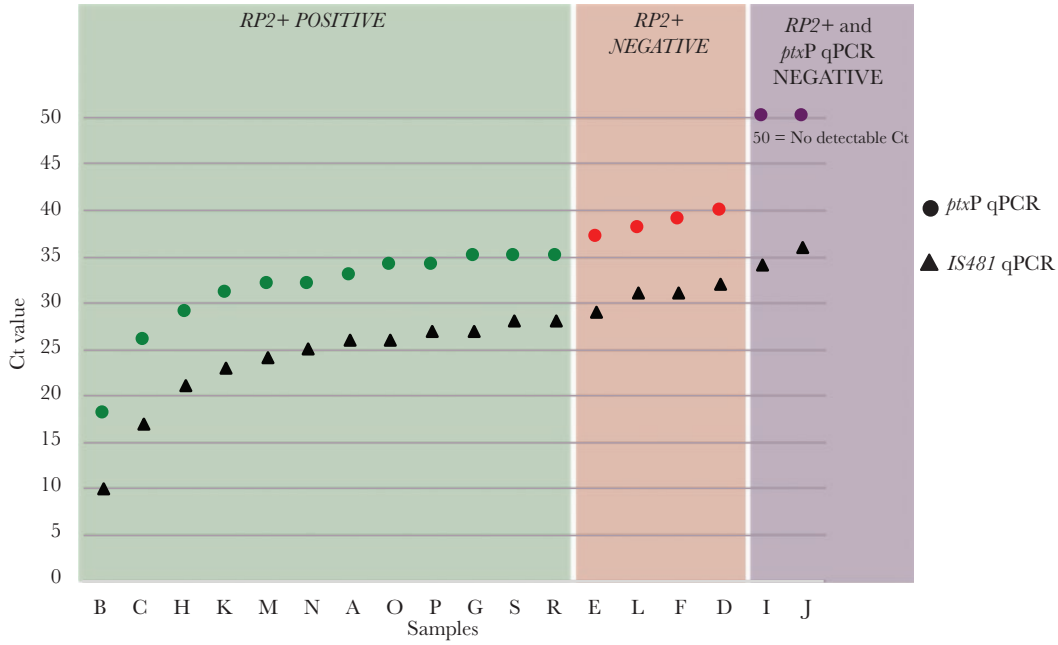


**Figure 1.** Comparison of Ct values obtained with the FilmArray RP2+ and *ptxP* qPCR. Samples (n = 18, A to R) were ordered by increasing Ct value of the *ptxP* qPCR. The Ct values of the reference standard IS*481* qPCR are indicated by black triangles. Green, red, and purple circles denote samples that were positive for both the *ptxP* qPCR and RP2+ methods (green), *ptxP* qPCR only (red), or none (purple). Abbreviation: qPCR, quantitative polymerase chain reaction.

sensitivity [10]. This issue is critical for detection of *B. pertussis*, which can be present at low levels in clinical samples, and the abundance of which quickly decreases over time despite symptoms being present [10, 11]. Previous studies have compared the performance of the first version of the FilmArray RP to qPCR targeting the insertion sequence IS*481*, which is the most frequently used target for the molecular diagnosis of pertussis. Two of these studies did not find a loss of analytical sensitivity for *B. pertussis* when using the FilmArray RP, but they were designed to assess the entire panel and *B. pertussis* was therefore not their primary focus [12, 13]. Furthermore, in the Pierce et al. study, all samples detected positive by FilmArray RP had low Ct values (<30) with real-time PCR targeting IS*481* [13]. Another study compared the performance of the FilmArray RP with the Focus qPCR assay (Focus Diagnostic, Murrieta, CA, USA), which targets IS*481*. Seventy-one specimens from patients, which had been tested positive for *B. pertussis,* were analyzed and compared. The authors concluded that the FilmArray RP assay detects ~30% less cases of *B. pertussis* than the Focus assay [14]. In a recent multicenter evaluation of the FilmArray RP2 in nasopharyngeal swab samples, only 3 positive *B. pertussis* samples were tested and compared with the previous FilmArray RP [9].

Here, we evaluated the performance of the FilmArray RP2+ to detect *B. pertussis* using a comparative design based on an in-house simplex *ptxP* target assay. The use of archived frozen clinical samples allowed us to select clinical samples representing a wide range of abundances of *B. pertussis*. We selected a range of positive samples based on *ptxP*, a single copy target that is known to be very specific, with the advantage of discriminating between *B. pertussis* and other *Bordetella* spp., *B. holmesii* in particular [7], but less sensitive than the multicopy golden standard IS*481* target for the molecular diagnosis of whooping cough.

As observed in our report, the samples with a *ptxP* qPCR Ct value >35 (corresponding to *IS481* >28) were not detected with the FilmArray RP2+. Hence, although the FilmArray RP2+ shares the advantage of higher specificity than the standard qPCR targeting IS*481*, it appears less sensitive than the simplex *ptxP* qPCR assay and a fortiori than the reference standard. Depending on the age of the patient, vaccination status, duration of the cough before biological sampling, and sample quality, a value of Ct IS*481* >28 is not infrequent [15, 16]. In 2019, we received at the French National Reference Center of whooping cough 50 clinical samples, one-third of which had a *ptxP* Ct value >35, implying that one-third of samples would have been missed using RP2+ if it would not have been complemented by qPCR IS*481*. Most of these samples were from previously vaccinated children or adults, who are more likely to present with atypical symptoms inducing a delay between symptom onset and sampling >3 weeks. This could explain a low bacterial load. Overall, although the number of tested samples is limited, this work calls for caution when interpreting Bp-negative results when the FilmArray RP2+ is used. Depending on the clinical and epidemiological contexts, it may be important to control the negativity of samples using more sensitive, dedicated *B. pertussis* diagnostic methods. Besides, other additional qPCR with good sensitivity targeting sequences such as hIS*1001* can be used to discriminate between *B. pertussis* and *B. holmesii*, which is also involved in pertussis-like symptoms.


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***Author contributions.*** S.G., S.B., and J.T. contributed to the conception and design of the study. S.G., A.M., N.A., and L.C. contributed to the acquisition of data. J.T., S.G., and A.L. analyzed and interpreted the data. S.G. and J.T. wrote the first draft of the article. J.T. and S.B. supervised the study. All authors revised the manuscript for important intellectual content and approved the final version submitted for publication.


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