False-Positive Diagnostics of Bordetella Pertussis using IS481 PCR is Limited in Danish Patients

Høgh, Silje Vermedal; Agergaard, Charlotte Nielsen; Skov, Marianne Nielsine; Kemp, Michael

Published in:
The Open Microbiology Journal

DOI:
10.2174/1874285801913010051

Publication date:
2019

Document version
Final published version

Document license
CC BY

Citation for published version (APA):

Terms of use
This work is brought to you by the University of Southern Denmark through the SDU Research Portal. Unless otherwise specified it has been shared according to the terms for self-archiving.
If no other license is stated, these terms apply:

• You may download this work for personal use only.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk
False-Positive Diagnostics of *Bordetella Pertussis* using IS481 PCR is Limited in Danish Patients

Silje V. Hoegh, Charlotte N. Agergaard* , Marianne N. Skov and Michael Kemp

Department of Clinical Microbiology, Odense University Hospital, Winsløwparken 21, 2, DK-5000 Odense, Denmark.

Abstract:

Background:
*Bordetella pertussis* is routinely detected using real-time PCR based on the multicopy insertion sequence IS481, which is not specific for *Bordetella pertussis*.

Objective:
The aim of this retrospective study was to evaluate the proportion of other *Bordetella* species misidentified as *Bordetella pertussis* using IS481-targeted real-time PCR.

Methods:
Clinical specimens from 228 Danish patients (median age 15 years, 0 to 90 years old) formerly identified as positive for *Bordetella pertussis* (IS481+) by routine PCR in 2011-2015, were subjected to real-time PCR targeting the insertion sequences IS1002 and IS1001.

Results:
The results showed that 2.3% of the samples were false-positive for *Bordetella pertussis*.

Conclusion:
In conclusion, we found that misidentification of *Bordetella pertussis* using IS481 PCR is limited in Danish patients.

Keywords: *Bordetella pertussis*, Real-time PCR, IS481, IS1002, IS1001, False-positive.

1. INTRODUCTION

Whooping cough is a highly contagious respiratory disease. It causes both local outbreaks and epidemics despite being vaccine-preventable. Whooping cough continues to be a significant cause of morbidity and infant mortality worldwide. Ten different species compose the genus *Bordetella*, four of which are known to infect humans: *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella holmesii* and *Bordetella bronchiseptica*. Humans are the sole reservoir for *B. pertussis*, which is the primary cause of whooping cough or pertussis. *B. parapertussis* may be responsible for up to 20% of the cases of pertussis-like disease, but the illness is often less severe than the one caused by *B. pertussis* [1]. *B. holmesii* is associated with bacteremia, infective endocarditis, and respiratory illness, particularly in functionally or anatomically asplenic patients. However, *B. holmesii* has also been isolated from nasopharyngeal specimens of immunocompetent individuals with pertussis-like illness. *B. bronchiseptica* is primarily an animal pathogen, but will occasionally infect humans and, like *B. holmesii*, *B. bronchiseptica* may cause invasive disease. Known cases describe bacteremia, peritonitis and respiratory tract infection in immunocompromised patients [2, 3].

PCR is the prevailing method used for detection of *Bordetella pertussis* in clinical samples, being fast, sensitive and less laborious compared to culturing. Molecular detection of *B. pertussis* is commonly based on insertion sequence IS481, which is present in multiple copies (>50 copies) in the *B. pertussis* genome, increasing the sensitivity of the PCR [4]. A high sensitivity of the assay is necessary in order to

DOI: 10.2174/1874285801913010051, 2019, JJ, 51-54
document the high incidence of pertussis, continuously found in the local population [5]. However, the target sequence IS481 is also present in the genomes of B. holmesii (about 8-10 copies), and of some B. bronchiseptica [1]. In this study, a method described by Roorda et al. [6] involving a PCR assay targeting IS1001 and IS1002 as well as IS481 is used for specific detection of B. pertussis. IS1002 is present in the genomes of B. pertussis and B. parapertussis, while IS1001 is found in all B. parapertussis and in a few B. bronchiseptica. Thus, a specimen containing B. pertussis will be positive for IS481 and IS1002 and negative for IS1001 Table 1 [1].

### Table 1. Bordetella species and presence of insertion sequences.

<table>
<thead>
<tr>
<th>Presence/No. of Copies per Genome</th>
<th>Species</th>
<th>IS481</th>
<th>IS1001</th>
<th>IS1002</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. pertussis</td>
<td>+/50</td>
<td>-</td>
<td>+/4-10</td>
<td></td>
</tr>
<tr>
<td>B. parapertussis</td>
<td>-</td>
<td>+/-20</td>
<td>+/9</td>
<td></td>
</tr>
<tr>
<td>B. holmesii</td>
<td>+/-10</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B. bronchiseptica</td>
<td>-</td>
<td>+/-7</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* Isolates of B. bronchiseptica can sometimes contain few copies of IS481 and/or IS1001.

The objective of this study was to retrospectively evaluate the proportion of other Bordetella species potentially misidentified as B. pertussis in patient samples collected between 2011 and 2015 in our region using IS481 based real-time PCR (rt-PCR).

### 2. MATERIALS AND METHODS

Clinical specimens were sputum and throat swabs collected from 228 Danish patients identified as positive for B. pertussis by routine PCR in 2011-2015. Stored extracted DNA (NucliSENS® easyMAG®, bioMérieux) previously positive for IS481 was reanalyzed by rt-PCR targeting the insertion sequences IS1002 and IS1001. Samples had been kept for quality control and were anonymized, therefore patients did not have to provide informed written consent.

Target sequences of the PCR were IS481, IS1001, and IS1002 Table 2. Phocine Herpes Virus (PhHV) was added to the samples as internal control in order to monitor the DNA extraction as well as the PCR amplification efficiency. Furthermore, negative control, as well as positive target controls were included in each run.

PCR assays for detection of IS481 were performed in 25 µl reaction volume containing 1× Lightcycler® 480 Probes master (Roche), 0.5 µM of IS481 forward and reverse primers, 0.2 µM of the IS481 probe and 5 µl extracted DNA. The amplification of IS1001, IS1002 and PhHV were run in separate reaction mixes containing 1× Lightcycler® 480 Probes master (Roche), 1.0 µM of each primer, 0.2 µM of the probe and 5 µl of extracted DNA. Amplification was carried out on a Lightcycler® 480 (Roche). The temperature profile included initial denaturation of 10 min at 95°C followed by 45 cycles of 95°C for 10 sec, 60°C for 30 sec, and 72°C for 1 sec. The Crossing Point (Cp) values were determined by the 2nd Derivative Max method using the Lightcycler® 480 software. A Cp value <40 combined with an acceptable curve was considered positive.

### 3. RESULTS

The Cp values obtained from the IS481 PCR-positive samples were on average 5.3 Cp values lower than the Cp values obtained from the IS1002 PCR, presumably due to differences in copy number of the target sequence (approximately >50 and 10 copies, respectively). Of the 228 clinical samples positive for B. pertussis (IS481+) by routine PCR, we therefore only included samples with a Cp value of <32 in the IS481 PCR in order to rule out that any negative result in the IS1002 PCR was due to lower sensitivity. 131 samples obtained a Cp value <32 in the IS481 PCR. 128 of these were also positive in the IS1002 PCR confirming the species identity as B. pertussis (IS481+ and IS1002+). Hence the three IS1002 negative samples were subjected to PCR targeting IS1001; all three were found negative (IS481+, IS1002- and IS1001-) and therefore did not contain B. parapertussis or IS1001 positive B. bronchiseptica DNA. Thus, three samples (2.3%) had been misidentified as B. pertussis. Presumably, these samples contained either B. holmesii or, less likely, B. bronchiseptica.

Patients had a median age of 15 years (0-90 years-old) with 59% of females.

Table 2. Real-time PCR assays targeting the three IS elements and the internal control Phocine Herpes virus (PhHV).

<table>
<thead>
<tr>
<th>PCR Assay</th>
<th>Label</th>
<th>Sequence (5′-3′)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481</td>
<td>Forward primer</td>
<td>CGG ATG AAC ACC CAT AAG CAT</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>CGA TCA ATT GCT GGA CCA TTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>FAM-CCC GAT TGA CCT TCC TAC GTG GAC TBHQ1</td>
<td></td>
</tr>
<tr>
<td>IS1001</td>
<td>Forward primer</td>
<td>AAT TGC TGC AAG CCA ACC A</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>CCA GAG CCG TTT GAG TTC GT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>FAM-ACA TAG ACC GTG AGC AG-MGB</td>
<td></td>
</tr>
<tr>
<td>IS1002</td>
<td>Forward primer</td>
<td>CTA GGT CGA GCC CTT GTC A</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>GCG GCC AAG CCA CTT GTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>FAM-CAT CGT CCA GTG CTG CAT CAC CC-BHQ1</td>
<td></td>
</tr>
<tr>
<td>PhHV</td>
<td>Forward primer</td>
<td>GCG GTA ATC ACA GTG TGA ATC</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>GCG GTT CCA AAC GTA CCA A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>FAM-ATT TTA TGT GTG CGC CAT CTG GTG C-BHQ1</td>
<td></td>
</tr>
</tbody>
</table>
4. DISCUSSION

In this retrospective study, we found that in 131 samples identified with routine non-discriminating B. pertussis IS481 rt-PCR, the bacterium had been misidentified in three samples. The three samples all originated from male adolescents (ages 14, 15 and 21 years-old) and presumably contained B. holmesii. This is consistent with observations from France and the Netherlands of B. holmesii in nasopharyngeal-samples from adolescents and young adults [7, 8]. We found a false-positive rate of 2.3%. Other studies have reported a much higher incidence of false-positive B. pertussis. Njamkepo et al. [7] found that the proportion of B. holmesii falsely identified as B. pertussis with IS481 PCR was 20.3% of samples collected from French adolescents and adults (n=59). From Chilean patients with suspected pertussis Miranda et al. [9] detected B. holmesii DNA in 11.1% of IS481-positive samples (n=99). In Norway, Reinton et al. [10] detected B. holmesii DNA in 5.8% of B. pertussis positive samples (n=87) and no B. bronchiseptica DNA in 375 other B. pertussis positive samples. In other European countries, no B. holmesii DNA was detected in nasopharyngeal samples from Finnish (n=2804), Dutch (n=6903) and Belgian (n=1493) patients with pertussis-like illness [3, 11]. Moreover, in Swiss patients, no B. holmesii DNA was detected in 194 B. pertussis positive samples [2].

The implications of misidentification of B. pertussis are currently unclear. Still, correct identification of B. pertussis is considered important for several reasons. Firstly, in vitro studies suggest that macrolides used to treat B. pertussis infections are less effective against B. holmesii and probably also against B. bronchiseptica. Secondly, a false-positive B. pertussis may cause prescription of unnecessary post-exposure prophylaxis, and thirdly, B. holmesii or B. bronchiseptica misdiagnosed as B. pertussis may suggest vaccine failure and lead to excessive public health interventions [1 - 3, 12].

It has been suggested to use multiple target PCR assays for detection of B. holmesii and B. bronchiseptica in concordance with IS481 to rule out false-positive B. pertussis or to use confirmatory PCR assays for specific detection of B. pertussis [13 - 15]. However, this will significantly increase workload, cost and turnaround time. As the confirmatory assays are less sensitive than the IS481 PCR, they may come out negative in samples with low amounts of B. pertussis, resulting in false-negative reports. In a clinical setting, sensitivity, as well as rapid diagnostics must be prioritized to ensure appropriate treatment and prevention of further transmission.

CONCLUSION

We have shown that false-positive diagnostics of B. pertussis using IS481 PCR is limited in Danish patients. This is consistent with the results from other studies [2, 3, 11, 12]. Our priority remains to diagnose B. pertussis infection. Thus, the rt-PCR assay targeting IS481 combines high sensitivity with an acceptable false-positive rate of 2.3% if the Cp is set to <32. Supplementary assays should not be necessary on a routine basis but may be applied in situations with clinical suspicion of false-positive results.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>Insertion Sequence</td>
</tr>
<tr>
<td>PhHV</td>
<td>Phocine Herpes Virus</td>
</tr>
<tr>
<td>Cp</td>
<td>Crossing Point</td>
</tr>
</tbody>
</table>

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors have no conflict of interests.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES


[5] Statens serum institut, Denmark, EPI-news No 16, April 2016 [http://dx.doi.org/10.1371/journal.pone.0088936] [Accessed 2018 November 2];


