Reviewer's report

Title: Evaluation of real-time PCR for diagnosis of Bordetella pertussis infection

Version: 1 Date: 2 January 2006

Reviewer: Karen Carroll

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General
The manuscript by Knorr, et. al. represents a detailed description of several real-time PCR molecular assays for detection of B. pertussis and the differentiation between B. holmesii and B. pertussis. The manuscript presents some new information. See specific recommendations outlined below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

p. 2 Abstract
The authors should re-organize the abstract as follows:
Shorten the background section.
Objectives and methods – there is improper use of a semicolon here. The authors should number the objectives for easier reading, e.g. “The objectives of the study were 1) to assess…..2) to employ….. and so on.
It is important that the authors state in the abstract the number of clinical samples tested. The abstract contains no data of substance. The results should be expanded to provide a summary of the key points from the results.

p. 3 Processing of specimens—Did the Regan-Lowe media that was used to collect 12% of the samples contain cephalexin? This is important for data analysis because B. holmesii is susceptible to this antibiotic. This could give PCR positive, culture negative results.

Since different volumes were potentially used for the PCR and culture, how was this information factored into the data analysis? What proportion of samples had DFA slides submitted along with the swab for culture? If possible this point should be addressed in the Results section.

Table 1 IS481 HP a “G” is missing from the end of the reverse primer sequence.

Table 1 IS481MB, compared to the published reverse primer sequence a “G” is missing. The authors should verify the accuracy of all primers and probes as listed in Table 1.

The Results section should be re-organized, especially the first section. It is extremely difficult to follow. There authors may wish to begin with the discussion of the analytical validation, followed then by a discussion of the clinical portion of the study. Also for the clinical portion of the study, the authors do not indicate what is considered a “true positive” for the purposes of data analysis. All of this should be spelled out.

The Venn diagram in Figure 2 can be deleted in favor of expanding the text.

What was done to evaluate analytical specificity? Did the authors test non-Bordetella sp. such as
Haemophilus, Alcaligenes or other coccobacillary gram negative bacilli? What about the remaining Bordetella sp. such as B. hinzii and B. avium? Even though the authors tried to verify culture and DFA negative, PCR positives with other primers, the fact remains that there are 28 PCR positive, culture and DFA negative samples which could represent cross-reactivity with other species that may not have been evaluated. Also as indicated above if the transport media contained cephalixin this could have generated false negative culture results.

Results p. 7. The authors should explicitly state the results for the IS1001 MB assay. It is implied that there were only 2 positives using this assay, which were culture confirmed as B. parapertussis. Is this correct?

It is unclear how the authors will confirm the IS481 assays with a much less sensitive B. pertussis TPR assay in actual clinical practice. What will they tell clinicians when the IS481 is positive but the TPR assay is negative?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

p. 2 line 6 in the Background section. Remove the word “and” after “nucleic acid amplification”.

p. 3 Processing of specimens—“Thenafter”? Should it be “then, after”?

Figure legends: In figure legends 4, 5 and 6 the word “parapertussis” is misspelled.

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Discretionary Revisions (which the author can choose to ignore)

Background: On p. 3, the authors may wish to expand the discussion on the clinical relevance of B. holmesii as a respiratory pathogen. Few manuscripts have addressed this, but those that have reported on their recovery/detection by PCR or culture from NP swabs have found a very low prevalence. Most of the literature concerns this species’ role in bacteremia.

Using the strategy described in the conclusions the authors may miss B. parapertussis if culture is performed only on PCR positive samples since the IS481 assay does not detect B. parapertussis. The authors did see at least two cases in their survey. The authors may wish to comment upon this point.

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What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have not competing interests.