Reviewer's report

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

Version: 1 Date: 16 December 2005

Reviewer: Saibal K Poddar

Reviewer's report:

General
In this manuscript authors report an evaluation study of a real time PCR assay for B.pertussis infection the protocol of which is published before [Reischl et al; J.Clin. Micro 2001, 39:1963-1966]. This PCR method targets IS481 region in the genome of the bacteria, but since this target is common in other closely related species particularly B.holmesii found in pertussis like illness, both B.pertussis and B.holmesii produce same PCR amplified signal, and thus the test lacks specificity. The study objective was to determine how this lack in specificity of assay impacts diagnostics by finding the number of B.holmesii infection sample present in suspected pertussis patients in Alberta population. They tested the method in 808 samples collected from patients during 2003 and 2004. They compared the results with that obtained by standard culture and DFA and another previously published PCR method targeting insertion sequence IS1001 [Tempelton et al; J.Clin.Micro 2003, 41:4121-4126] and also a PCR protocol standardized in their laboratory targeting pertussis toxin promoter region (TPR). The PCR targeting toxin promoter although is100 fold less sensitive than that targeting IS481, majority of IS481 positive B.pertussis samples tested were found positive, thus they concluded the test can be useful for confirming positive B.pertussis found by IS481 PCR test. Authors did not find any B.holmesii positive sample and came to a general conclusion that it is unnecessary to rule out B.holmesii for specimen that are positive for IS481 in Alberta population. This reviewer finds it difficult to accept this conclusion in general(even for future years) for a population, --incidence of B.holmesii infection might not be there during 2003-2004, but no body can tell whether or not it will occur in future years, as for example in Massachusetts almost 4% of the pertussis samples were found to be B.holmesii reported in a study during 1999 [Yih et al; Emerg. Infect. Dis 1999,5:441-443], and since then there has been no report of occurrence of significant B.holmesii positive in pertussis samples. Thus the concluding statement in page 2 "that this limitation of IS481 PCR .....has minimal diagnostic relevance in the Alberta population" is not appropriate.

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

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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)

Also Table 1 was unnecessary since primers and probes used in assays except that in TPR assay were identical to those in published papers; and primers and probe sequence information for TPR assay can be included in page 7 where the assay protocol is described. The TPR assay has been claimed as novel, although it appears to be an optimized with respect to reagents (primers, probe and other PCR components) an established probe hydrolysis based PCR method [well known as TaqMan probe based assay] using vendor (Roche Diagnostics) supported master mix. Although the experimental part is well described, for evaluation and use in other laboratories, TPR assay part should be with appropriate published references; so unfamiliar readers can understand how the
assay system works. In the discussion section page 9, the statement “The IS481 sequence of B.holmesii and B.pertussis differs by only 2 bases (unpublished data from our lab)” provides published information [Reischl et al; J.Clin. Micro 2001, 39:1963-1966], authors should use a published reference; so interested readers can find which two base sequences they are.

Discretionary Revisions (which the author can choose to ignore)

**What next?:** Accept after minor essential revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

'I declare that I have no competing interests'