Reviewer's report

Title: Indeterminate tcdB using a Clostridium difficile PCR assay: a retrospective cohort study

Version: 2 Date: 2 April 2013

Reviewer: Maja Rupnik

Reviewer's report:

In this manuscript Leis et al., describe analysis of samples that are recognized as negative by commercial C. difficile molecular test (Cepheid), although by manual inspection there is some amplification of target tcdB gene. Authors did show that large proportion of these ‘negative’ tests are associated with clinically relevant CDI.

Reviewing a manuscript without proper line numbers is very difficult and I hope that my comments will be clear.

General:

In abstract 'IND' is defined and used, while in the manuscript only 'indeterminate assay' is used. Was this done because of the word limit in abstract? Would be better if authors would use both (or just one) term consistently in entire manuscript.

Literature: especially in the Background some citations are not entirely appropriate:

Literature 2 is a paper from 2001; since then many papers on molecular testing were published; suggestion to cite one of the recent reviews on this topic, for instance Carrol K., 2011, Anaerobe

Literature 3 is suitable, but many more studies have been published on Cepheid test sensitivity and specificity and authors should cite more of them here and also give the ranges of reported sensitivity and specificity levels.

Minor:

Abstract (Methods): '…was defined…', specify by whom (authors? Or by Cepheid?)

Abstract (last paragraph): 'IND results should be reported…' this sentence is not very clear. Much better is the explanation given in the Discussion- how should this result be reported. Please modify this sentence in Abstract (include explanation on how to report)

Pg. 6 – again, specify the definition (e.g. …was identified IN THIS STUDY as detection…)

Pg. 6, Clinical data collection: 'these patients' change to 'patients with indeterminate…'

Pg. 7 – is isolation and/or typing done in this lab routinely or only in specific
cases (studies?). Please specify (see also below the comment on ribotype prevalence)

Pg. 9 In first paragraph include also the information how many of IND samples were from relapses, or more precisely how many patients with IND result had confirmed CDI previously (and in what time interval)

Pg. 9. Was some other testing also required for IND samples (viruses, other pathogens). If no, add this information. If yes, specify which other tests were done and what was the result (if applicable also include this in Table)

Pg. 10 Is information on ribotypes isolated in general in this lab available. And if so, how prevalent are the ribotypes shown on Fig. 1 within this lab? (Probably at least some of them are among very prevalent – 014, 020 – this further supports author’s results that IND results are not associated with specific ribotype. Otherwise, there would be a theoretical possibility that IND results are associated with several extremely rare ribotypes and none of them is recognized by Cepheid test)

Table 2 What does ‘expert user’ mean? Add the explanation.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests