Reviewer’s report

Title: Rapid semi-automated quantitative multiplex tandem PCR (MT-PCR) assays for the differential diagnosis of influenza-like illness

Version: 3 Date: 14 December 2009

Reviewer: Eric C.J. CJ Claas

Reviewer’s report:

The authors have improved the manuscript according to the comments provided. The additional information (assay development) raises the question whether this is actual multiplex PCR or a combined set of separate reactions on the same target.

Referring to the detailed response (with the same numbers) to the reviewer’s comments:

My main concern was that the assay was performed as a nested PCR. The authors do not comment on this significant risk with the assay on respiratory samples that may contain low Ct values even without pre-amplification.

2) The comment was not that quantitative determination of respiratory viruses is not useful, but that Ct value in real-time PCR can be the marker for this. In the absence of standardized controls, any deduced value is a surrogate quantitative marker. The examples on the value of VL detection are chronic or latent reactivating infections, not acute infections.

4) These responses do not answer the question whether the discrepant results could be the results of contamination. A second, confirmatory PCR on another target would be the proof of true positivity.

5) See my question above. To me it is unclear which (and if) primer pairs are combined for multiplex application.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests