Reviewer's report

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

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Reviewer: Timm Harder

Reviewer's report:

The revised version of MS 1172942562270580 by Szewczuk et al. has considered, mostly satisfactorily, the majority of remarks made in my previous review. Only major remark #5 regarding reasons why not a real-time RT PCR has been used as a standard diagnostic tool has been left out. The issue of rRT-PCR and the semiquantitative nature of the Ct values obtained by this method as a well-established standard method in clinical settings has also been raised by the co-reviewer.

The revised manuscript has improved and will be an interesting contribution to the field of diagnosis of influenza-like illness. However, my principal concern about lack of transparency in primer targets remains. I emphasize “principal” as I think that in a scientific correspondence the reader should be given all opportunities to evaluate the conclusions made by the authors. Here we have to rely, e.g., on the authors statement that no mismatches occurred in alignments of primers and sequences from reference strains as listed in the tables. But how many strains have been tested? How about sequences of the new H1N1v? For me this is a principal stumbling block. So it is to the editor’s judgement and the journal’s policy how to treat this issue.

Concerning the statistics maybe the authors should precisely state their intentions in the manuscript to conduct a “descriptive study and not to control for specimen variation in quality, quantity, operator performance or any large variety of confounding variables” as mentioned in their remarks labelled “4. …statistics…”

As far as the new H1N1v is concerned a sentence has been added to the discussion stating that assays for the NP and M genes are now available. Do the authors mean MT assays? Maybe I missed it, but it would be helpful if the authors could state that, based on their alignments, detection of H1N1v by the INF-A MT assay is also likely?

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