Reviewer's report

Title: Comparison of three multiplex PCR assays for the detection of respiratory viral infections: evaluation of xTAG Respiratory Virus Panel Fast assay, RespiFinder 19 assay and RespiFinder SMART 22 assay

Version: 3 Date: 13 February 2012

Reviewer: Marek Smieja

Reviewer’s report:

The authors have adequately addressed most of the major compulsory revisions, but have not adequately addressed revisions #4 and #5. I have further clarified these two points, below:

1. In revision #4, I remarked that the high rates of adenovirus/parainfluenza 2 in the “clinical samples” might be due to higher test sensitivity, and named alternative explanations such as non-specificity, contamination, or detection of incidental but non-causal virus. I think these comments need to be addressed more carefully, since the current study is simply not adequate for a clinical laboratory to consider using the assay in a clinical context. I have reviewed our regional laboratory records in Hamilton, Canada, for 24,218 nasopharyngeal specimens submitted over the past 5 years to our laboratory. These represent results from DFA, culture, or, in the past 2 years, in-house PCR. I identified only 29 adenovirus positives in adults (roughly 6 per year, or roughly 0.3% of the 8000 adult specimens; by contrast, we had over 200 in children). In the current study, 21% (21 of 100) adult post-operative patients were ADV positive—this represents as many as we have seen in 8000 adult patients in 5 years, and, frankly, sounds unlikely! Similarly, for parainfluenza-2, I found only 25 in adults (5 per year, or 0.3%, versus over 100 in children). Again, the rate of 11% positives in the current study represents about half of all the positive tests we have seen in 8000 adults in 5 years. So, we need to ask: are these extremely high reported rates real? Non-specificity has been (partially) addressed, but specimen contamination (at source), amplicon contamination (in the lab) and detection of non-clinically relevant amounts of virus have not been excluded. It’s also possible that tracheal aspirates (which are not a common specimen for virology) are vastly superior to nasopharyngeal swabs, but that would need parallel specimen collection to answer. Ideally, I would have liked to see: re-extraction of the original material, and verification by a PCR with a different target than the multiplex uses. I would also have gone back to the original material and attempted to grow adenovirus and parainfluenza from the discordant samples; finally, I would have sequenced the amplicons to determine whether they were all the same genotype (indicating either an outbreak in the ICU, or contamination) or different genotypes (making the above unlikely). Are any of these possible? My conclusion to this part of the discussion is that the assay “may” be more sensitive with clinical samples, but that adequate, parallel, blinded validations need to be carried out with prospectively collected samples in future.
2. In revision #5, I mentioned that Methods should be described before the results. I checked the placement of the methods section in the last 10 manuscripts accepted at BMC ID. Nine of the ten had their methods section after background, and before the methods section; and one had no methods section at all. As I stated previously, I would place the methods section before the results; that is where readers expect to see it.

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

Quality of written English: Needs some language corrections before being published

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