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: 2  Date: 9 December 2011

Reviewer: Marek Smieja

Reviewer's report:

Summary: Dabisch-Ruthe and colleagues examine the analytic sensitivity of three commercial multiplex assays for detection of respiratory viruses. When 13 viruses are all analyzed together, all three assays miss a number of the included viruses, although performance is considerably better using a mix of only four viruses together. The RVP assay detected lower concentrations of influenza, RSV, and rhinovirus, whereas the two RespiFinder assays detected lower concentrations of coronaviruses and adenoviruses. For clinical evaluation, 100 tracheal aspirate specimens from ventilated patients in the Intensive Care Unit are assayed by two or three kits. A large number were found to have parainfluenza or adenovirus by the RespiFinder19 kit, but not by the other kits.

Major Compulsory Revisions

1. The authors’ objectives for conducting this study are not adequately described. Was the objective to compare the analytic sensitivity for each individual virus with each of the three commercial multiplex assays, and therefore to have some comparison for which viruses are best detected with which assay? Or was the purpose simply to determine the relative sensitivity for mixtures of 4-13 viruses in the same sample? Those are two very different questions. I see limited utility in attempting to detect 13 infections in the same sample (we have seen up to 6 infections simultaneously in a child), although having done this, it is appropriate to briefly report it without over-interpretting the information. Thus, a brief paragraph summarizing that all multiplex assays performed terribly when tested with this highly artificial mocked experiment is adequate, and may lead to some new thinking on how interference between targets may affect detection. Generally we are most interested in whether we can reliably detect influenza and RSV (which are the only two viruses for which we currently have effective treatment and prevention), and it is interesting that some of the kits detected these viruses even in the presence of 13 viruses in total.

2. If the primary purpose was to compare assays for analytical sensitivity for each virus, these should be done separately for each virus and not initially combined. Statistical analysis should then be used to appropriately determine whether increased analytical sensitivity has been demonstrated or not. I would suggest repeating the experiments with individual viruses, in replicates of at least 3 for each dilution, and using probit regression or other appropriate statistical models.
to prove a difference in detected concentrations. If the primary purpose is NOT to compare the individual viruses, but only to examine their interaction within multiply infected mocked specimens, then the authors need to be much clearer in describing this as their purpose, and more open in the limited clinical relevance of such experiments. Even here, unless an adequate number of replicates are done, no statistically significantly difference will be observed. Hence, all of the reported differences could simply be due to chance.

3. The authors provide virtually no description of their patients. Usually nasopharyngeal swabs or aspirates, and not tracheal aspirates (TA) or broncho-alveolar lavage specimens, are the preferred specimen for detection of respiratory tract viruses. Were NPS or NPA samples collected? Were the TA samples collected for clinical diagnostic purposes? TA samples may be used as a supplement, but there is much less published on their clinical utility. We also need to know who these patients were—were these all children, were they severely immunocompromised? Was there an outbreak of adenovirus or parainfluenza within the hospital or ICU? I have seen only a small number of patients end up on a ventilator as a result of these infections, usually these are co-infections with important bacterial pathogens. A more rigorous clinical evaluation would require consecutive or randomly sampled patients.

4. The authors conclude that the RespiFinder 19 was more sensitive. This may be true for parainfluenza and adenovirus, but would be unlikely to be the case for influenza or RSV. Alternate explanations include non-specificity of the RespiFinder, contamination, or detection of incidental but non-causal virus. These should be discussed.

5. The methods should be described before the results. I believe that some of the BMC instructions to the author specify that methods should be at the end, but this is a bizarre placement and certainly most readers expect to see methods before results.

6. The tables should be altered. The top of table 1 is of marginal interest, and might be better summarized in text. The bottom of Table 1, showing the four multiple infections per sample, is really the main result and needs to be expanded and reformatted in a clear manner.

- Minor Essential Revisions

1. All instances of the word “sensitivity” refer to analytical sensitivity, not clinical sensitivity, and therefore the more correct term is analytical sensitivity.

2. Words such as “impossible”, “not possible”, “systematic comparison” and “clinical usability” are used incorrectly and should be replaced. Without examining every virus in replicates and at various dilutions, and without including every commercial respiratory virus multiplex available (Luminex, Seegene, Resplex etc), this cannot be described as a systematic comparison.

3. The virus combinations described under results, page 6-7 (which should be described under methods instead) appear incorrect, with 4, 3 [panel 2] and 5 [panel 3] virus mixes described. Elsewhere, 4 viruses per mix are described. This should be corrected.
4. Reference 23 appears to be an abstract, but there is a 2011 citation of the same title. This reference should be updated from the 2009 citation to the 2011 citation.

5. Legionella is included on page 8 for the viral results; clearly this is not a virus.

- Discretionary Revisions

Table 2 and Figure 2 are of marginal benefit and could be eliminated.

**Level of interest:** An article of limited interest

**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.

**Declaration of competing interests:**

I declare that I have no competing interests.