Author's response to reviews

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

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Version: 4 Date: 3 April 2012

Author's response to reviews: see over
Submission for publication in *BMC Infectious Diseases*

**Our manuscript MS 1084908583595467; your letter from 26th March 2012**

Dear Dr. Diana Marshall,

Thank you very much for the favorable review of our manuscript “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”.

Enclosed please find the revised manuscript which was modified according to your and the reviewers` suggestions and a point-to-point response to all remarks. We thank the reviewers for their constructive comments and corrections. We gratefully accepted all the comments and corrections, corrected the remarks and added the missing information. All changes made are highlighted in the manuscript (underlined).

We hope that the manuscript is now suitable for publication in *BMC Infectious Diseases*.

Yours sincerely,

J. Dreier

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POINT-TO-POINT Response

Editorial Comments
In addition to the points raised by the reviewers, please also address the following editorial points:

1) Please include a statement in the methods section of your revised manuscript stating that ethical approval was not required and including the reason why this was the case for your study.
   The statement was included in the methods section.

2) Please move the Methods section of your manuscript so that it is between the Background and the Results sections.
   Methods were moved between the Background and the Results sections.

3) Competing interests - Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.
   The questions that are asked of authors are:
   Financial competing interests
   - In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.
   - Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.
   - Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.
   - Do you have any other financial competing interests? If so, please specify.
   Non-financial competing interests
   - Are there any non-financial competing interests (political, personal, religious, academic, ideological, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.
   The 'Competing interests' section was included and the authors declared that they have no competing interests.

Reviewer 1
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: 9 February 2012
Reviewer: Guus Simons

Reviewer's report:
Overall, the authors have addressed most of the comments. I am pleased with that. I still have some minor remarks:

Minor essential revisions:

Page 5: "Another study performed a comparison with clinical samples of the RespiFinder-19 with the precursor assay of the RVP". Please also indicate the % of sensitivity and specificity as shown a few lines before about the specificity and sensitivity of the RVP assay.
   The modification was performed according to the reviewers’ suggestion.
Page 12, line 5: "This study indicated a higher analytical sensitivity of the RespiFinder 19 in the detection of virus infections in clinical samples....." If you are analyzing clinical samples, I would prefer using the name clinical sensitivity rather than analytical sensitivity.

_The modification was performed according to the reviewers’ suggestion._

Page 28, Table 4: RespiFinder 19 and RespiFinder Smart 22 are CE-IVD marked products. Please change.

_The modification was performed according to the reviewers’ suggestion._

Page 28, Table 4: The authors state that "personal training/qualification" takes 3 days / high. It is our experience that technicians who are familiar with sequencing on a ABI system can deal with RespiFinder-19 very easily without almost any training.

_The modification was performed according to the reviewers’ suggestion._

**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.

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**Reviewer 2**  
**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.

Initially we were also skeptical of this high rate of adenovirus/parainfluenza-positive samples. Therefore, we already re-extracted samples and verified positive results with in house monoplex real-time PCR methods in our original study. However, all samples provide the same results. In this context, sequencing of amplicons for the determination of genotypes is not possible, because PCR amplicons are not available and the rest of the original material was depleted for re-extraction, verification by PCR. Consequently, growth of viruses is likewise not possible, furthermore we do not have the possibility at all to grow viruses in our lab. We also thought about a contamination, however, all samples were extracted and re-extracted with different lots of reagents, within different extraction series on different days and samples extracted in the same series show also negative results. Furthermore, samples were collected during a time period about two month and therefore contamination seemed improbable. An outbreak in the ICU is also unlikely, because as already mentioned, samples were collected in a two month period and patients with positive virus detection were not room neighbours. One explanation might be the detection of incidental but non-causal viruses or the constitution of our special patient cohort. All samples were taken from mechanically ventilated non-immunocompromised patients.

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.

Methods were moved between the Background and the Results sections.

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.

List of changes made:
- Our statement concerning the ethical approval was included in the Methods sections.
- Methods were moved between the Background and the Results sections.
A ‘Competing interests’ section was included between the Conclusions and Authors’ contributions.