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:** 30 November 2011

**Reviewer:** Guus Simons

**Reviewer's report:**

This is a well written and sound manuscript comparing three multiplex PCR assays for detection of respiratory pathogens. Multiplex PCR assays are gaining more ground in analyzing acute respiratory tract infections.

In the article quantified standarized control material in either a mixture of 13 or 4 viruses is used. In addition, two multiplex assays are compared on clinical specimens and hands of time and time of result of the three tests is given.

Minor Essential revisions:

1. The performance of the three multiplex assays was compared to monoplex real time PCR. However, no QPCR data such as threshold cycles values are shown. Strikingly, PCR conditions of up to 50 cycles are used. It is known that ct values > 40 are not relevant at all. It is stated that none of the three multiplex assays was capable of detecting 13 or even 4 viruses at the same time. Ct values of the undiluted, 1:10, 1:100 and 1:1000 are needed for a full comparison.

2. To assess the clinical performance only RVP and RespiFinder 19 are compared. No comparison was made with RespiFinder Smart 22 because the analysis of 100 sample was already started before RespiFinder Smart 22 was commercially available. But on page 3 it is stated that the detection rate of RVP and RespiFinder Smart 22 was only 22%? Fewer detections were found........RespiFinder Smart-22: 4%. Please clarify!!!

3. Page 3: Conclusions: Multiplex PCR tests have a broad spectrum of pathogens to test at a time, but a lack of sensitivity in comparison to monoplex assays. This conclusion is not correct. In table 2, data obtained with RespiFinder 19 and real time PCR show a very high level of concordance. This indicates that in real life (clinical specimens) RespiFinder 19 is as sensitive as monoplex real time PCR. Moreover, on page 9 it is stated that the viruses that were only detected with RespiFinder 19 showed high cycle threshold points (how high?) in monoplex PCR methods due to low virus concentrations indicating a high level of sensitivity of the multiplex assay.

4. Please explain why CMV is tested. In general CMV is not regarded as a respiratory pathogen.

5. Page 2: Background: Therefore, the identification of the causative viruses and bacteria is only feasible using.........
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:

Yes, I am the CEO of PathoFinder the manufacturer of RespiFinder 19 and RespiFinder Smart 22.

However, this research was performed independently and without any correspondence or influence by me or any PathoFinder employee.