Reviewer's report

Title: The Development of a GeXP-based Multiplex reverse transcription-PCR Assay for Simultaneous Detection of Sixteen Human Respiratory Virus Types/subtypes

Version: 1 Date: 26 May 2012

Reviewer: Alexander Lai

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Summary:

This manuscript describes a multiplex detection method for 16 human respiratory viruses. The method is based on a slight modification of Beckman Coulter's GenomeLab GeXP Genetic Analysis System in which the authors attached viral specific primers to the GeXP's tag primers, i.e., the viral specific primers for viral RT-PCR, and the GeXP primers for capillary detection. The authors tried this new technique by using a total of 126 nasopharyngeal aspirate specimens from hospitalized children diagnosed with pneumonitis or bronchopneumonia and fever. To determine the lower limit of detection, cloned plasmid DNAs (from the PCR products) for all 16 viruses were diluted to 10 to 10,000 copies, and subjected to this new test alone, or as a premix.

Comments:

The scientific value of this manuscript is very low and unexciting, as this work is purely a method development. In addition, there are two significant flaws that need to be addressed if this manuscript is to be published:

1. The statement that the detection limit is 20-200 copies for single virus and 1000 copies for premix (mixture of all 16) is meaningless, as the copy number is referring to plasmid DNA, while the described technique is a RT-PCR (and the tested viruses are RNA viruses). The sensitivity should be referring to copies of RNA rather than DNA;

2. Table 2 is conceptually wrong, and it should be deleted! For example, the 100% sensitivity and 100% specificity for FluB was based on ONE positive only! Sensitivity and specificity are calculated based on a comparison of the new test to established gold standard (May it be by virus culture, or by other well established laboratory test), not one new test to another! The number of positive and negative specimens should be sufficient large for proper statistical analysis.

While having all 16 pairs of primers in a single RT-PCR reaction without interference is quite impressive, however, the conclusion regarding savings in cost and in time is premature without detailed analysis of the cost of materials and the cost of equipment.
Level of interest: An article of limited interest

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests