COMPARISON OF LUMINEX xTAG RESPIRATORY PANEL FAST ASSAY WITH NxTAG RESPIRATORY PATHOGEN PANEL FOR DETECTION OF RESPIRATORY PATHOGENS IN NASOPHARYNGEAL SECRETIONS

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INTRODUCTION AND PURPOSE

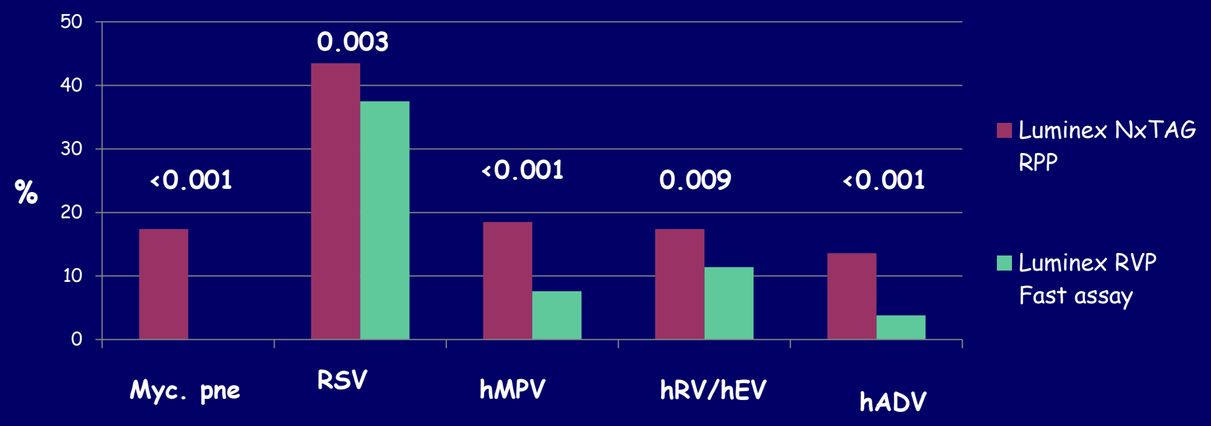
Etiologic diagnosis of respiratory infection is essential but, unfortunately, clinical symptoms of disease due to different infectious agents are very similar and only laboratory assays can identify the causative agents. The advent of nucleic acid amplification tests has significantly improved etiologic diagnosis of respiratory infections. However, testing for all respiratory viral or atypical bacterial targets using singleplex polymerase chain reaction (PCR) is expensive and laborious. On the other hand, multiplex real-time PCR can be technologically challenging and can result in a loss of sensitivity. Multiplex assays for amplification and detection of a panel of respiratory viruses using suspension microarrays might provide a practical solution. To improve viral detection and to simultaneously identify atypical bacteria, a new assay, the Luminex NxTAG Respiratory Pathogen Panel (RPP) was developed, optimizing primer design and PCR protocols. This study compares the sensitivity and specificity of this new assay to that of the Luminex RVP Fast assay v2 (the first assay able to identify simultaneously 19 different viral types and subtypes targets in respiratory secretions, criticized because of reduced sensitivity, mainly in samples with low viral load, in comparison with real-time PCR). Results obtained with both assays were compared with those due to singleplex real-time PCR assays specifically devoted to the identification of single infectious agents.

METHODS

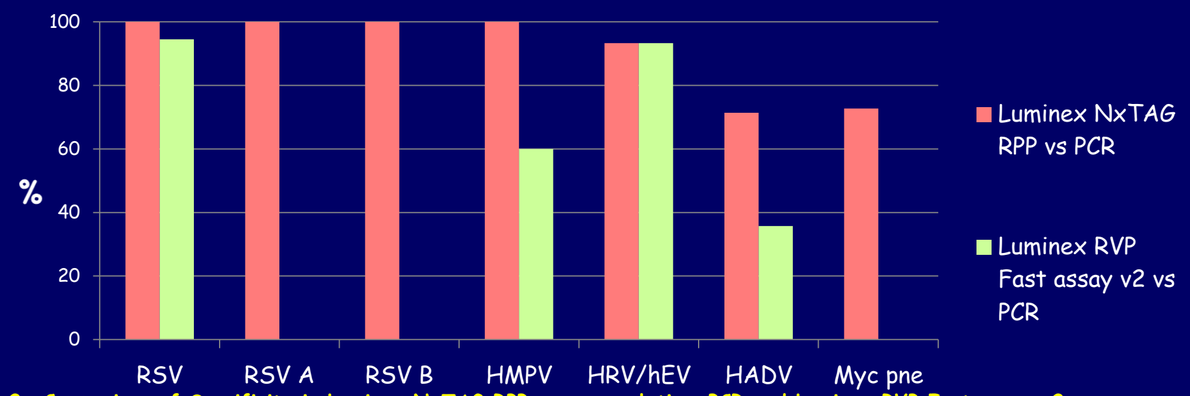
A total of 185 nasopharyngeal swabs collected from otherwise healthy children admitted to our Unit for respiratory tract infections in February 2015 were evaluated tested for infectious agents using the Luminex NxTAG RPP and the Luminex RVP Fast assay v2 (both produced by Luminex Molecular Diagnostics, Inc., Toronto, ON, Canada). Both these assays simultaneously detect influenza A viruses (non-specific influenza A, A/H1N1, A/H3N2, influenza A/H1N1 2009), influenza B virus, respiratory syncytial virus (RSV), parainfluenzaviruses (types 1-4), human adenovirus (hADV), human metapneumovirus (hMPV), human coronaviruses (229E, NL63, OC43 and HKU1), human enterovirus/rhinovirus (hEV/hRV) and human bocavirus. Moreover, the NxTAG RPP assay is able to differentiate RSV type A from RSV type B and to detect *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*. The remaining extracts were tested for RSV A and B, hRV, hMPV, hADV and *M. pneumoniae* using validated real-time PCRs

RESULTS

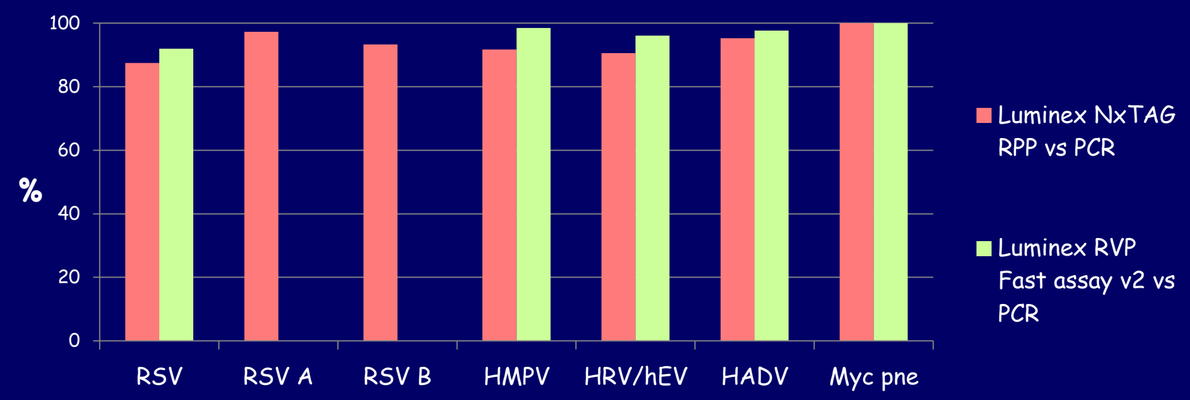
Tab. 1- Comparison of Luminex NxTAG RPP to Luminex Respiratory Virus Panel Fast assay v2: % of positive test for different pathogens



Tab. 2- Comparison of sensitivity in Luminex NxTAG RPP *versus* real-time PCR and Luminex RVP Fast assay v2 *versus* real-time PCR



Tab. 2- Comparison of Specificity in Luminex NxTAG RPP *versus* real-time PCR and Luminex RVP Fast assay v2 *versus* real-time PCR



CONCLUSIONS

To the best of our knowledge, this is the first study that has measured the efficiency of the Luminex NxTAG RPP assay for detection of respiratory viruses and atypical bacteria. Available data seem to indicate that the Luminex NxTAG RPP assay has exceeded some of the limitations of the previous Luminex assays. The most important advances seem related to the detection of hADV, the differentiation between RSV A and B and the identification of *M. pneumoniae*.
However, for a more complete evaluation of the Luminex NxTAG RPP assay further studies are needed. More samples have to be evaluated and all the viruses and atypical bacteria for which this assay have been prepared have to be tested. Moreover, accurate cost analyses have to be made. Until further data become available, the routine use of this assay cannot replace real-time PCR specific for single agents in clinical practice.