

Diagnosis of multiple respiratory pathogens with the Multiplex Ligation-dependent Probe Amplification (MLPA).

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ABSTRACT

The MLPA of the RespiFinder Plus ® kit detects multiple viral and bacterial respiratory pathogens. During the winter of 2008-09 we introduced the MLPA in the routine diagnostics, replacing the viral culture. The MLPA improved the diagnosis of respiratory pathogens considerably. With the MLPA more pathogens were detected, the sensitivity increased, and the turn-around time and hands-on time were greatly reduced.

Introduction

The MLPA of the RespiFinder Plus ® kit is able to detect seven viral and four bacterial pathogenic species simultaneously. The objective of this report is to evaluate the MLPA as a diagnostic tool. We tested its performance on clinical respiratory samples and on QCMD panels.

Material and methods

Clinical samples (774) were obtained from the hospital (mainly pediatric patients) and general practitioners during the winter season of 2008-09.

Five QCMD viral proficiency panels were tested. The panels included all viral species indicated in figure 1. To mimic clinical samples with multiple infections, the panels were tested as mixed samples, each containing one sample of the five panels.

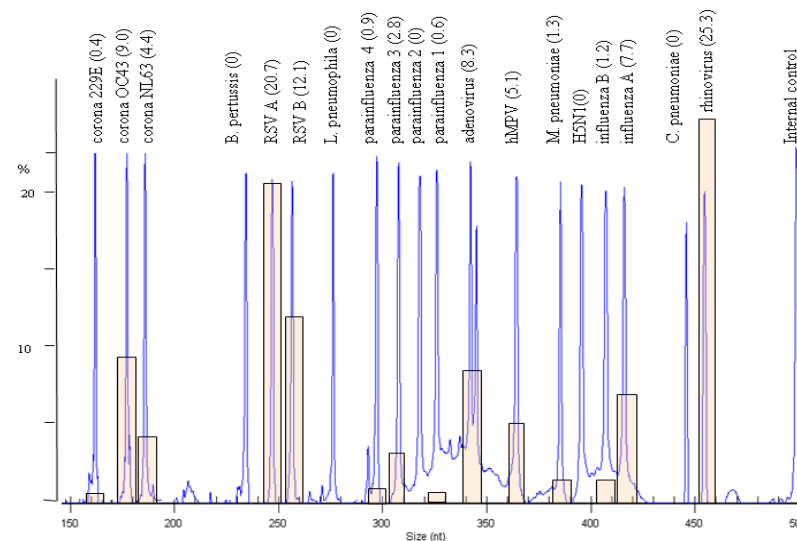


Figure 1. Electropherogram (blue) of the MLPA fragments of each of the pathogens and the internal control. On the horizontal axis the size (bp) of the fragments is indicated. Above each peak the virus or bacterial pathogen is indicated. Between parenthesis is indicated the percentage of the pathogens that were found by MLPA in the 774 clinical samples of the 2008-09 season. The bars visualize the percentages of each of the pathogens.

Results

The MLPA produces for each target pathogen a DNA fragment of characteristic length. The fragments were detected and sized by capillary electrophoresis on a Beckman sequencer. The minimal turn-around time to final result is nine hours. In figure 1 the percentages of viral pathogens in the clinical samples are presented. 84% of the samples were positive for one or more pathogens. In 26% of these samples multiple pathogens were detected.

Of the QCMD panels all viral species and subtypes were correctly analyzed. We missed ten of the viruses with a low titer, probably due to competition in the MLPA by other viruses. On average these ten samples were missed by 49% of the participants of the five QCMD proficiency panels.

Conclusions

The MLPA of the RespiFinder Plus ® kit is a useful asset for a diagnostic laboratory due to:

- the simultaneous analysis of a large panel of respiratory viruses and bacteria.
- a good specificity and sensitivity, but competition might obscure double infections
- a good turn-around time, hands-on time and cost effectiveness