

# DETECTION OF VIRUSES CAUSING LOWER RESPIRATORY TRACT INFECTIONS USING MULTIPLEX RT-PCR AND MICROCHIP CAPILLARY ELECTROPHORESIS



J. M. Gras, B. Léger, R. Schröder, P. Goffinet, N. Hougardy  
Clinical and molecular Laboratory, Cliniques Sud Luxembourg, Arlon, Belgium



## 1. Background

- Lower respiratory tract infections are very common in children
- Many cases are caused by Respiratory Syncytial Virus (RSV), but other viruses such as human Metapneumovirus (hMPV) or Adenoviruses can be also responsible for this clinical condition
- Multiplex PCR-based techniques could be particularly interesting by detecting numerous viruses in a single test

## 2. Objectives

- In this work, we investigate the clinical utility of a multiplex RT-PCR that allows the detection of 14 viruses implicated in respiratory infections
- Separation of the PCR products was performed using microchip capillary electrophoresis, an innovative technology that allows the detection of PCR products with limited costs

## 3. Methods

- During January 2009, 36 children (M/F: 22/14; mean age (range): 1.15 years old (0.32 -2.89 years)) presenting lower respiratory infection symptoms benefited nasopharyngeal aspirations
- These samples were tested for RSV, Influenza A, Influenza B, Para-Influenza viruses and Adenoviruses using immunochromatographic or immunofluorescence assays
- These aspirations were frozen at -20°C and subsequently used for RT-PCR
- Extraction was performed using EasyMAG™ automated system (bioMérieux sa, Marcy l'Etoile, France)
- RT-PCR system used was RespiFinder DC TwoStep Kit (PathoFinder BV, Maastricht, The Netherlands)
- PCR products were detected using microchip capillary electrophoresis (Experion™, Bio-Rad, Hercules, CA, USA)
- Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA)

## References

- Reijans M, Dingemans G, Klaassen CH et al. RespiFinder: a new multiparameter test to differentially identify 15 respiratory viruses. JCM 2008; 46(4):1232-1240
- Li SFY, Kricka LJ. Clinical Analysis by Microchip Capillary Electrophoresis. Clin Chem 2006; 52:37-45.

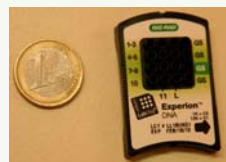
## 4. Technology used

### Viruses detected by RespiFinder DC kit

Rhinovirus – Influenza A – Influenza B – hMPV – Adenovirus – Parainfluenza 1, 2, 3, 4 – RSV-A, RSV-B, Coronavirus NL 63 – Coronavirus OC43 – Coronavirus 229E



The Experion™ system (Left to right): electrophoresis station, priming station and vortex



Close up on an Experion™ chip, as compared to an one Euro coin

## 6. Conclusion

- RespiFinder RT-PCR coupled with microchip capillary electrophoresis detection seems to be more sensitive than classical immunochromatographic or immunofluorescence tests
- It also allows the detection of co-infections that were particularly frequent in this work
- Our study also confirms that hMPV plays an important role in the pathogenesis of lower respiratory tract infections in children
- Further works will focus on the comparison of this conventional multiplex RT-PCR coupled with microchip capillary electrophoresis with several real-time PCR methods for respiratory virus detection

## 5. Results

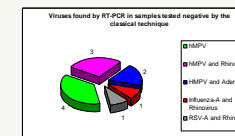
### Example of results



Various examples of results. Smaller peaks represent upper and lower markers as well as Internal Size Marker (ISM) used to interpret results

### Results discussion

- RSV testing, either by immunochromatographic or immunofluorescence technique, was positive in 23 cases
- The corresponding results by RT-PCR were RSV-A in 9 cases, RSV-B in 1 case, co-infection RSV-A and other viruses in 5 cases, co-infection RSV-B and other viruses in 5 cases, hMPV infection in 2 cases. One patient who was tested positive by the rapid RSV technique was negative by RT-PCR
- Two patients were positive for the respiratory screen "flu" immunofluorescence testing; it corresponded to two cases of hMPV infection
- Classical testings were negative in 11 patients; RT-PCR showed 4 cases of hMPV infection and various types of co-infection in 7 cases



## Support



Travel grant approved by Mdeon  
Visum N° 09/V/1/1560/020021

## Contact

jeremie.gras@gmail.com