

The new RIDA®GENE Parainfluenza real-time PCR is a sensitive and specific assay for the detection and differentiation of HPIV1, HPIV3 and HPIV2/4

A. Hiergeist[†], S. Stender[‡], L. Kastl[‡], A. Simons[‡]
[†] microBIOMix GmbH, Regensburg, Germany
[‡] R-Biopharm AG, Darmstadt, Germany

Objectives

Human Parainfluenza viruses (HPIV) cause both upper respiratory infections and lower respiratory infections. Mainly, HPIV1, 2 and 3, and sporadically HPIV4 account for the major community-acquired respiratory pathogens worldwide. There is a clear relationship between the different HPIV serogroups and presentation of respiratory disease, as well as the seasonal appearance of each of the four HPIV types (Figure 1).

Epidemics of HPIV1 and 2 mostly occur biannually in fall whereas HPIV3 epidemics in North America and Europe occur yearly in spring and summer.¹ Clinical symptoms are dependent on the different serogroup, croup, bronchiolitis, pneumonia and tracheobronchitis.² Similar symptoms occur in other respiratory tract infections, therefore a specific differential diagnosis by real-time PCR is an essential tool for detection of HPIV.

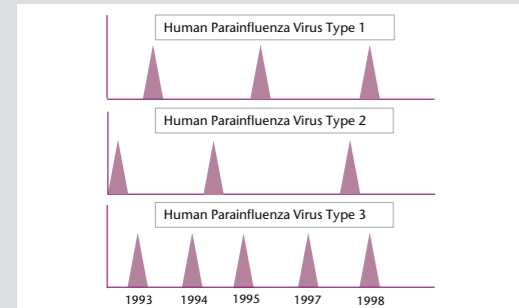


Figure 1: Seasonal appearance of the four human Parainfluenza types; adopted from Hall³

Picture 1: RIDA®GENE Parainfluenza Art. No. PG5805



Methods

The RIDA®GENE Parainfluenza real-time PCR assay simultaneously detects and differentiates HPIV1, HPIV3 and HPIV2/4 using fluorogenic target-specific hydrolysis probes. In a retrospective study, a total of 118 positive and negative respiratory samples (nose/throat swabs; pharyngeal lavage) were extracted

using the MagNAPure 96 (Roche). Extracted nucleic acids were analysed with the RIDA®GENE Parainfluenza real-time PCR assay on the LightCycler® 480II (Roche). Results were compared to a standard reference in-house real-time PCR assay (Figure 2).

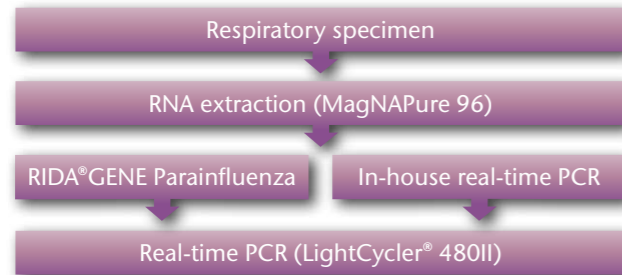


Figure 2: Study design

Results

Overall, 118 clinical samples were tested, of which 64 were nose and throat swabs and 54 was pharyngeal lavage material. 8 samples (6.8 %) were positive for HPIV1 and 9 samples (7.6 %) were HPIV3-positive using the RIDA®GENE Parainfluenza real-time PCR assay (Figure 3, Table 1).

101 samples negative for either HPIV serogroup were found with the RIDA®GENE Parainfluenza real-time PCR assay (Table 1). These results concur with the results obtained with the reference in-house real-time PCR assay, indicating a positive, negative and

overall agreement of 100 %, respectively (Table 2).

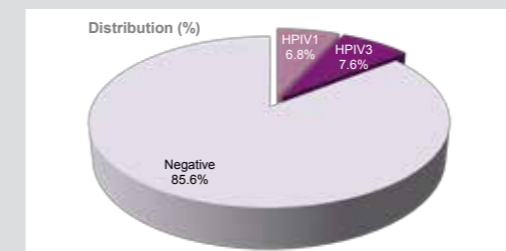


Figure 3: Distribution of Parainfluenza subtypes in % found by RIDA®GENE Parainfluenza

Table 1: In-house real-time PCR vs. RIDA®GENE Parainfluenza real-time PCR

		In-house real-time PCR		Total
		+	-	
RIDA®GENE Parainfluenza	+	17	0	17
	-	0	101	101
Total		17	101	118

Table 2: Clinical performance of the RIDA®GENE Parainfluenza real-time PCR assay

Positive agreement: 100 %

Negative agreement: 100 %

Overall agreement: 100 %

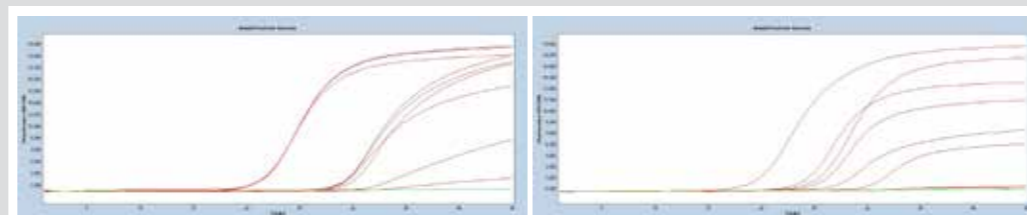


Figure 4: Example of Parainfluenza (HPIV1, HPIV3) run on the LightCycler® 480II

¹ Berman S. Epidemiology of acute respiratory infections in children of developing countries. Rev. Infect. Dis. 1991, 13:454-462.
² Henrickson KJ. Parainfluenza viruses. Clin. Microbiol. Reviews. 2003, 16(2): 242-263.
³ Hall CB. Respiratory Syncytial Virus and parainfluenza virus. N Engl J Med. 2001, 344(25): 1917-1928.

Conclusion

The RIDA®GENE Parainfluenza real-time PCR assay shows good correlation with an established in-house real-time PCR method. It is a highly sensitive and specific assay for the simultaneous detection and differentiation of HPIV1, HPIV3 and HPIV2/4. Results are available in less than 2 hours. An included Internal Control RNA (ICR) detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient and hence ensures reliable results.