Human Coronavirus (HCoV) and Human Parainfluenzavirus (HPIV) are frequently involved in respiratory infections in young children, the elderly, and the immunocompromised. The bioMérieux HCoV/HPIV r-gene® kit allows a rapid detection of Human Coronavirus and Human Parainfluenzavirus using the 9 nucleic PCR.

The HCoV/HPIV r-gene® kit helps to identify the search for respiratory infection pathogens. This easy-to-use kit can detect the 4 HCoV species (NL63 / OC43 / HKU1 and 229E) and the 4 HPIV types (HPIV-1 / HPIV-2 / HPIV-3 and HPIV-4) with a high level of sensitivity.

**MATERIAL AND METHODS**

- **Extraction step:**
  Nuclear acids were extracted from 200μl nasopharyngeal samples, after a proteinase K pre-treatment, on NucleicEasy® easyMAG system with the Specific B protocol and eluted in 50μL.
  A negative extraction and amplification control (W0) was added from the lysis step to check contamination during the whole process of extraction and amplification.

- **Amplification step:**
  0.15μL of diluted to 1/100 reverse transcriptase (RT) was added to 15μL of HCoV/HPIV r-gene® (ref. 71-045, bioMérieux) amplification premix. Then 10μL of diluted eluted samples, W0 and Positive Control (PC45) were added.
  HCoV were detected at 530nm; HPIV were detected at 560nm on Bio-Rad Dx Real Time System (Dx RTS).

Analytical performance of the HCoV/HPIV r-gene® assay was established:
- **Performance on External Quality Assessment Panel (EQA Panel)**
- **Analytical sensitivity**
- **Precision determination**
- **Analytical specificity**

**EQA Panels:**
- These panels were obtained from Quality Control for Molecular Diagnostics (QCMD), an Independent International External Quality Assessment (EQA) / Proficiency Testing (PT) organisation.
- The QCMD 2012 Coronavirus RNA EQA panel included 10 samples with various concentrations of Human Coronavirus OC43, NL63 and 229E in 1 negative sample.
- The QCMD 2012 Parainfluenzavirus RNA EQA panel included 10 samples containing various concentrations of Human Parainfluenzavirus types 1, 2, 3 and 4 in negative sample.

Extractions and amplifications steps have been performed as described above.

Analytical sensitivity:
The limits of detection (LoD) of the HCoV/HPIV r-gene® assay on the 4 species of Human Coronavirus and the 4 types of Human Parainfluenzavirus have been determined.

Titrated viral cultures or quantified plasmid (HKU1) were spiked in a previously negative-characterized nasopharyngeal sample for the 8 pathogens tested. Dilutions were performed in a negative nasopharyngeal sample (NS).

A wide range of dilutions of each parameter has been tested with one replicate of each dilution to determine the concentrations corresponding to the LoD at 100% and 0%.

At least 4 new dilutions were adjusted between the 2 LoD previously obtained: at least 1 dilution at 0% and 1 dilution at 100% of detection and at least 2 dilutions between 5% and 95% were tested 15 times, from extraction to amplification step, for the determination of the LoD at 95%. Extractions and amplifications steps have been performed as described above. Obtained results were processed using MiniLab16 statistical analysis software.

**Precision determination:**
The studies for the precision determination of the HCoV/HPIV r-gene® kit were carried out on spiked samples containing the 4 species of Human Coronavirus and the 4 types of Human Parainfluenzavirus. Titrated viral cultures were diluted in negative nasopharyngeal sample. Dilutions tested corresponded to concentrations determined at 1,000, 100, 10 & 0.01 times the respective LoD at 5% value (determined as described) of each parameter. Extractions and amplifications steps have been performed as described above.

In the intra-experiment variability determination: 10 RT-PCR assays in the same run from one eluted sample.

In the inter-experiment variability determination: 10 eluted samples in 10 successive RT-PCR assays.

The mean of Ct’s, the standard deviation and the coefficient of variation (C.V) were determined for each dilution of each parameter.

Analytical specificity:
Sixty-four potentially cross-reacting pathogens including 37 viruses and 27 bacteria, were evaluated with HCoV/HPIV r-gene® kit. These pathogens can cause respiratory infections or can be present in the respiratory tract.

Each potential cross-reactant was individually spiked at a high load into a negative respiratory sample. Each viral load was determined by molecular diagnostic CE-marked kits using PCR or RT-PCR technology or according to ATCC data.

Each bacterial sample was checked by qualitative PCR CE-marked or a 16S RNA quantitative RT-PCR or according to the ATCC data. Extractions and amplifications steps have been performed as described above.

**RESULTS**

- **Coronavirus and Parainfluenzavirus EQA RNA 2012 QCMD Panels:**
  - Panel members are designated ‘Core’ proficiency samples on the base of scientific information, clinical relevance and clinical experience. Laboratories are expected to carry out the analysis and submit the results, in order to show acceptable proficiency; >QCMD-2013 general announcement.
  - Core samples are considered as challenging (‘Education’) due to very low concentrations, they are clearly detection limits.

  - The 5 ‘Core’ positive Coronavirus samples of the QCMD 2012 Coronavirus RNA EQA panel and the 4 ‘Core’ positive Parainfluenzavirus samples of the QCMD 2012 Parainfluenza RNA EQA panel and have been detected with HCoV/HPIV r-gene® kit.
  - The Core negative samples of both panels are undetected with the HCoV/HPIV r-gene® kit as expected.
  - 4/4 ‘Education’ HCoV samples (challenging samples) and 4/5 ‘Education’ HPIV samples (challenging samples) are detected with HCoV/HPIV r-gene®.

Analytical sensitivity:
The concentrations values of LoD at 95% obtained by the HCoV/HPIV r-gene® kit and determined by Minitab® software for Human Coronavirus 229E, OC43, NL63 and HKU1 and Human Parainfluenzavirus types 1, 2, 3 and 4 are:

- Precision determination:
The mean Ct’s obtained for each dilution tested, the standard deviations and coefficients of variation were determined and reported in the following tables.

Inter experimental assay

The coefficients of variation ranged between 0.44 and 2.03 % for the HCoV and between 0.47 and 1.57% for the HPIV.

**CONCLUSIONS**

Analytical performance studies of the HCoV/HPIV r-gene® RT-PCR assay demonstrated robustness and reliable detection of all 4 species of Human Coronavirus and the 4 types of Human Parainfluenzavirus in respiratory samples. The high quality level associated with its compatibility with the major extraction and real time PCR platforms allow an immediate integration of HCoV/HPIV r-gene® (ref: 71-045) in most diagnostic labs.