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Development of a new diagnostic tool for the detection of Human Coronavirus & Human Parainfluenzavirus in a duplex RT-PCR

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INTRODUCTION

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 5' nuclease PCR.

The HCoV/HPIV r-gene® kit helps to simplify 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:

Nucleic acids were extracted from 200µL nasopharyngeal samples, after a proteinase K pre-treatment, on NucliSENS® 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 1/10th reverse transcriptase (RT) was added to 15µL of HCoV/HPIV r-gene® (ref. 71-045, bioMérieux) amplification premix. Then 10µL of eluted samples, W0 and Positive Control (PC45) were added.
HCoV are detected at 530nm and HPIV are detected at 560nm on Bio-Rad Dx Real Time System (Dx RTS).

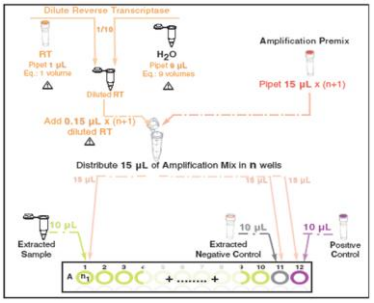


Figure 1: Protocol for HCoV and HPIV Detection

Analytical performance of the HCoV/HPIV r-gene® assay were 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 and 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 and 1 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% of detection, 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 Minitab16 statistical analysis software.

✓ Precision determination:

The studies for the precision determination of the HCoV/HPIV r-gene® kit were carried out on simulated 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 95% value (determined as described above) of each parameter. Extractions and amplifications steps have been performed as described above.

- In the intra-experiment variability determination: 10 RT-PCRs assays in the same run from one eluted sample.
- In the inter-experiment variability determination: 10 eluted samples in 10 successive RT-PCRs assays.

The mean of CTs, 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 a 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 basis of scientific information, clinical relevance and clinical experience (...). Laboratories are expected to correctly analyze and report the core proficiency samples in order to show acceptable proficiency.» QCMD-2012-general-announcement.
Consequently the other samples are considered as challenging ("Educational") due to very low concentrations, they are clearly detection limits.

QCMD 2012 Coronavirus RNA EQA Programme — HCoV/HPIV r-gene® REF# 71-045 Results: Dx RTS Bio-Rad

Panel Code	Sample Content	Sample Type	Sample Status	Dilution factor	Expected Result	HCoV CT (530 nm)	HPIV CT (560 nm)
CV12.01	Coronavirus – OC43	Core	Frequently detected	1.0E-4	Positive	30.06	Negative
CV12.02	Coronavirus – NL63	Core	Detected	1.0E-3	Positive	25.06	Negative
CV12.03	Coronavirus – 229E	Core	Frequently detected	1.0E-4	Positive	27.81	Negative
CV12.04	Coronavirus – OC43	Educational	Detected	1.0E-6	Positive	36.40	Negative
CV12.05	Coronavirus – NL63	Educational	Detected	1.0E-5	Positive	31.54	Negative
CV12.06	Negative	Core			Negative	Negative	Negative
CV12.07	Coronavirus – 229E	Educational	Detected	1.0E-5	Positive	31.33	Negative
CV12.08	Coronavirus – OC43	Core	Detected	1.0E-5	Positive	33.05	Negative
CV12.09	Coronavirus – NL63	Core	Detected	1.0E-4	Positive	28.05	Negative
CV12.10	Coronavirus – 229E	Educational	Detected	1.0E-6	Positive	33.66	Negative

QCMD 2012 Parainfluenzavirus RNA EQA Programme — HCoV/HPIV r-gene® REF# 71-045 Results: Dx RTS Bio-Rad

Panel Code	Sample Content	Sample Type	Sample Status	Dilution factor	Expected Result	HCoV CT (530 nm)	HPIV CT (560 nm)
PINF 12.01	Parainfluenza virus Type 1	Educational	Detected	1.0E-4	Positive	Negative	34.18
PINF 12.02	Parainfluenza virus Type 2	Educational	Detected	1.0E-5	Positive	Negative	34.16
PINF 12.03	Parainfluenza virus Type 2	Core	Frequently detected	1.0E-3	Positive	Negative	27.40
PINF 12.04	Parainfluenza virus Type 3	Core	Frequently detected	1.0E-3	Positive	Negative	26.83
PINF 12.05	Parainfluenza virus Type 1	Educational	Detected	1.0E-4	Positive	Negative	38.28
PINF 12.06	Negative	Core			Negative	Negative	Negative
PINF 12.07	Parainfluenza virus Type 1	Educational	Infrequently detected	1.0E-5	Positive	Negative	Negative
PINF 12.08	Parainfluenza virus Type 4	Educational	Infrequently detected	1.0E-3	Positive	Negative	34.09
PINF 12.09	Parainfluenza virus Type 2	Core	Frequently detected	1.0E-4	Positive	Negative	30.14
PINF 12.10	Parainfluenza virus Type 1	Core	Detected	1.0E-3	Positive	Negative	33.40

- 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 "Educational" HCoV samples (challenging samples) and 4/5 "Educational" 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 Minitab16 software for Human Coronavirus 229E, OC43, NL63 and HKU1 and Human Parainfluenzavirus types 1, 2, 3 and 4 are:

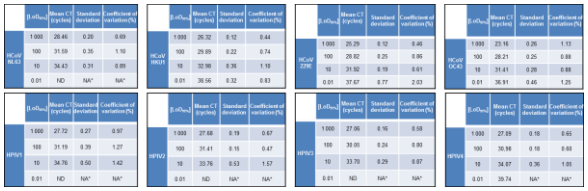
Targeted viruses	LOD 95% (TCID50/mL)
Coronavirus 229E	0.197
Coronavirus OC43	86.854
Coronavirus NL63	0.002
Coronavirus HKU1	18.066 cp/mL

Targeted viruses	LOD 95% (TCID50/mL)
Parainfluenza virus type 1	379.788
Parainfluenza virus type 2	529.930
Parainfluenza virus type 3	245.261
Parainfluenza virus type 4	0.213

✓ Precision determination:

The mean CTs obtained for each dilution tested, the standard deviations and coefficients of variation were determined and reported in the following tables.

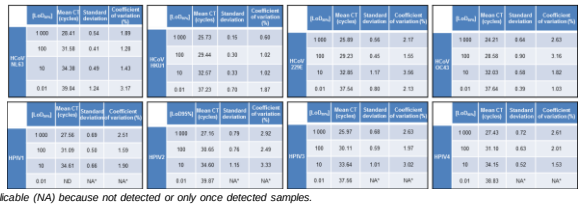
Intra experimental assay



*Note : * not applicable (NA) because not detected or only once detected samples.*

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

Inter experimental assay



*Note : * not applicable (NA) because not detected or only once detected samples.*

The coefficients of variation ranged between 0,6 and 3,56% for the HCoV, and between 1.53 and 3,33% for the HPIV.

✓ Analytical specificity:

The specificity of recognizing of primers and probes selected to detect Human Coronavirus and Human Parainfluenzavirus with the HCoV/HPIV r-gene® assay was determined by sequence analysis (viral, bacterial and Human) using Internet databases. Analytical specificity of HCoV/HPIV r-gene® kit was experimentally checked on the following pathogens:

- **Viruses:** HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8 / BKV / Adenovirus 3 / Bocavirus 1 / Coxsackievirus B2, A9, Echovirus 9, 25, 30, Poliovirus S3 / Parechovirus 1, 2 / Rhinovirus 14, 87, 1B / Influenza A, Influenza B / VRS A, VRS B / hMPV A, hMPV B / Mumps / HPIV1, 2, 3, 4 / HCoV NL63, OC43, 229E and HKU1.
- **Bacteria :** *Acinobacter baumannii, Bordetella bronchiseptica, Bordetella parapertussis, Bordetella pertussis, Branhamella (Moraxella) catarrhalis, Chlamydophila pneumoniae, Citrobacter freundii Citrobacter koseri, Enterobacter cloacae, Enterobacter kobei, Escherichia coli, Haemophilus influenzae, Haemophilus parainfluenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Legionella pneumophila, Morganella morganii, Mycoplasma pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Raoultella ornithinolytica, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Stenotrophomonas maltophilia, Streptococcus agalactiae, Streptococcus constellatus.*

⇒ No cross-reaction of the HCoV/HPIV r-gene® kit was observed with all pathogens tested.

Note : Tests were also performed on Human DNA extracts negative for Coronavirus and Parainfluenzavirus. These test demonstrated that there is no amplification of sequences of Human origin with HCoV/HPIV r-gene® kit.

CONCLUSIONS

Analytical performance studies of the HCoV/HPIV r-gene® RT-PCR assay demonstrated robustness and reliable detection of the 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.