Clinical Validation of SARS-Cov-2 RNA by Multiplex rRT-PCR Detection for Molecular Diagnosis of COVID-19 using CE-IVD Assay for Nasopharyngeal swab and Saliva Samples

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Introduction

The detection of SARS-CoV-2 RNA by real-time reverse transcription–polymerase chain reaction (rRT-PCR) is used to confirm the clinical diagnosis of COVID-19 by molecular diagnostic laboratories. Sample collection using nasopharyngeal (NP) swabs requires healthcare workers wearing personal protective equipment to collect samples, the swabs can be uncomfortable for the patients during collection. Saliva specimen can be an alternative to the NP. In this study we evaluated the multiplex UltraGene Assays for the detection of SARS-CoV-2 RNA for NP and saliva specimens.

Aim

The objective of this study was to evaluate the multiplex UltraGene Assays for the detection of SARS-CoV-2 RNA for NP and saliva specimens.

Methodology

Sample types → **RNA extraction** → **PCR amplification** → **Data interpretation**

1

Or

Nasopharyngeal and saliva specimens

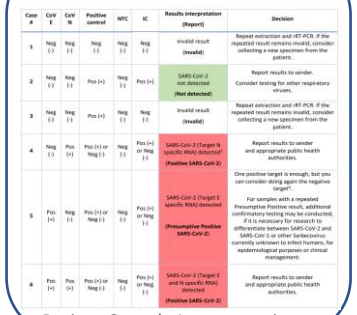
2

Roche MagNa Pure

3

UltraGene48,96 qPCR instrument

4



Patient Sample Interpretation

Results

- We obtained 100% clinical reproducibility for the international QCMD panel including the sample with the lowest viral load (2.30 Log10 Copies/ml) was detected.

SARS-CoV-2 $TCID_{50}$ / mL	Concentration (Level)	Mean Ct Gene N		Mean Ct Gene E		Mean Ct IC	
		NP	Saliva	NP	Saliva	NP	Saliva
316000	1:10 (L1)	17,28	15,01	17,41	15,26	26,00	25,99
31600	1:100 (L2)	21,00	18,33	21,45	18,42	22,71	21,57
3160	1:1000 (L3)	24,06	21,34	24,79	21,39	22,86	21,85
316	1:10000 (L4)	28,17	25,03	28,96	25,88	23,02	21,78
31	1:100000 (L5)	34,09	30,25	36,43	30,89	22,85	22,05
3	1:1000000 (L6)	No results	35,37	No results	37,02	No results	21,96
0,3	1:10000000 (L7)	No results	No results	No results	No results	No results	No results

1 Initial LoD determination for nasopharyngeal (NP) and saliva using USA-WA1/2020 strain

Microorganism	ID	Test Concentration	Replicates detected / total	SARS-CoV-2 Test Result
Human coronavirus OC43	EQA	10715 dPCR cp/ml	(0/1)	Not detected
Human coronavirus NL63	EQA	43651 dPCR cp/ml	(0/1)	Not detected
Adenovirus type 3	ZeptoMetrix #NATPPA-BIO	No titer available	(0/3)	Not detected
Human Metapneumovirus	ZeptoMetrix #0810161CF	3.80×10^{4} $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 1	ZeptoMetrix # 0810014CF	3.39×10^{3} $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 2	ZeptoMetrix # 0810015CF	4.17×10^{5} $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 3	ZeptoMetrix # 0810016CF	8.51×10^{7} $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 4	ZeptoMetrix # 0810017CF	1.51×10^{4} $TCID_{50}$/mL	(0/3)	Not detected
Influenza A	ZeptoMetrix # 0810036CF	$1 \times 10^{5.15}$ $TCID_{50}$/mL	(0/3)	Not detected
Influenza B	ZeptoMetrix # 0810255CF	$1 \times 10^{6.50}$ $TCID_{50}$/mL	(0/3)	Not detected
Respiratory syncytial virus	ZeptoMetrix # 0810040ACF	5.01×10^{5} $TCID_{50}$/mL	(0/3)	Not detected
Rhinovirus	ZeptoMetrix #NATPPA-BIO	No titer available	(0/3)	Not detected
Chlamydia pneumoniae	ZeptoMetrix #NATPPA-BIO	No titer available	(0/3)	Not detected
Haemophilus influenzae	ZeptoMetrix # 0801679	2.27×10^{9} CFU/mL	(0/3)	Not detected
Streptococcus pneumoniae	ZeptoMetrix	2.26×10^{9} CFU/mL	(0/3)	Not detected

2 Wet testing : No cross-reactivity observed

Conclusion

- Multiplex UltraGene Assays
- Detection of SARS-CoV-2 RNA
- NP and saliva specimens
- Highly sensitive detection

IVD
Qualitative in vitro diagnostics

For use with downstream sequencing instruments

CE

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Results:

The limit of detection was 0,000001 TCID50/mL or 1*10-6 TCID50/mL for SARS-CoV-2 for both NP swab and saliva specimens. No cross-reactivity was observed. The results interpretation agreement for saliva specimen between nasopharyngeal swab was perfect (Cohen's Kappa score=1). We obtained 100% clinical reproducibility for the international QCMD panel including the sample with the lowest viral load (2.30 Log10 Copies/ml) was detected.

Conclusions:

- The CE-IVD (FDA EuA submission) UltraGene Combo2screen SARS-CoV-2 (E/N) multiplex rRT-PCR Assay shall enable highly sensitive detection of SARS-CoV-2 RNA, reducing reagent use, cost and time required by clinical laboratory technicians.
- Large scale SARS-CoV-2 saliva testing shall be a powerful solution in preventing spread of this virus and helping to control the COVID-19 pandemic including in asymptomatic patients.