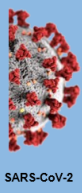
Validation of GENOMICA's *qCOVID-19 Respiratory Combo* for the detection of SARS-CoV-2 on swab and saliva samples



Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological agent responsible for the biggest pandemic the world has experienced in a century. Products based on Real Time RT-PCR technology have been widely used for the diagnosis of the infection allowing infected individuals to be quarantined appropriately.

qCOVID-19 Respiratory COMBO is a novel real-time multiplex RT PCR kit for the qualitative detection of the Influenza A M gene, the Influenza B NP gene, and the RSV A/B M gene on the MIX FLU-RSV; and the SARS-CoV-2 N and ORF ab genes on the MIX COVID-19 providing results with high sensitivity and specificity from nasopharyngeal swab and saliva samples.

The versatility of the kit allows to use just one Mix for the detection of the corresponding target depending on requirement (i.e., SARS-CoV-2 before and after Flu season).

Introduction

After SARS-CoV-2 was first reported in Wuhan, China, in December, it spread rapidly across the world and presents a very real and current threat globally. Real Time RT PCR detection kits for this new microorganism were developed quickly, and are of vital importance, to diagnose patients in order to treat and quarantine the infected and break the contagion chain (1).

With the arrival of winter and the regular flu season to the Northern Hemisphere (affecting over 80% of the world's population) an increase in the incidence of respiratory infections occurred (2).

Considering that infections by SARS-CoV-2, Influenza and respiratory syncytial virus share similar symptoms, it is necessary for diagnostic kits to differentiate between them. This differentiation allows for correct management of patients infected with SARS-CoV-2 to avoid further community transmission allowing better control of the infection and reduced hospitalization and fatalities (3, 4).

GENOMICA has developed the qCOVID-19 Respiratory COMBO which is used for the qualitative detection of SARS-CoV-2, Influenza A, Influenza B and Respiratory Syncytial Virus A/B (RSV A/B) by Real-Time multiplex RT PCR in respiratory samples. The data collected during the clinical validation is detailed below and the results show its outstanding performance and suitability for the detection of SARS-CoV-2, FluA, FluB and RSV.

Materials and Methods

Equipment

qCOVID-19 Respiratory COMBO have been validated using the following thermal cyclers: Applied Biosystems 7500, Thermofisher's QuantStudio 5, QuantStudio 12K Flex Real Time and CFX96 Bio-Rad.

The mixtures of oligonucleotides and probes pr vided are based on the so-called TaqMan® principle.

- MIX FLU-RSV of the kit (Inf-A, Inf-B and RSV A/B viruses) is detected through the HEX/VIC, FAM, and CY5 channels respectively and the internal control probe is detected in the TAMRA channel (for Applied Biosystem and Thermofisher equipment) or ROX (for CFX96 from Bio-Rad).
- MIX COVID-19 of the kit (SARS-CoV-2) is detected through the FAM and HEX/VIC detection channels and the internal control probe of the MIX COVID-19 that can be detected in the CY5 channel.

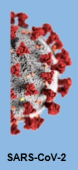
Validation procedure

528 - nasopharyngeal swab samples were included in the clinical validation for the detection of SARS-CoV-2 and human respiratory samples for the detection of Influenza A, Influenza B and (RSV A/B).

Samples were collected from the routine microbiology service at the Hospital La Fe, Hospital Clínic Universitari de Valéncia and Hospital Universitario La Paz. All of them were analyzed using routine hospital techniques and qCOVID-19 Respiratory Combo.

In addition, 359 swab samples for SARS-CoV2 and 355

Validation of GENOMICA's *qCOVID-19 Respiratory Combo* for the detection of SARS-CoV-2 on swab and saliva samples



human respiratory samples for the rest of respiratory viruses were used to determine the diagnostic utility parameters.

The clinical validation of saliva samples for the detection of SARS-CoV-2 was performed with 72 individual samples (directly and diluted) and 46 combined samples in batches of 10 in collaboration with the Complutense University of Madrid, Spain, as part of the COVID-LOT study.

Samples were collected from the following hospitals: The Hospital Universitario Puerta de Hierro (HUPH: Majadahonda, Madrid, Spain) and the Hospital Universitario Infanta Sofía (HUIS: San Sebastián de los Reyes, Madrid) (5, personal communication).

The samples obtained for the sensitivity and specificity tests were analyzed following the protocol detailed in the product kit manual.

Results

Diagnostic Utility Parameters

The sensitivity obtained for qCOVID-19 Respiratory Combo (Table 1) was very high, above 97%, for all the microorganisms detected: 97,97% for SARS-CoV-2, 99,93% for Influenza A, 97,44% for Influenza B and 98,37% for RSV. In all the cases specificity was 99-100%.

	n=	tp	fn	fp	tn	Sensitivity	Specificity	PPV	NPV
SARS-CoV-2	246	241	5	0	641	97,97	100,00	100,00	99,23
Influenza A	298	296	2	0	585	99,33	100,00	100,00	99,66
Influenza B	117	114	3	0	766	97,44	100,00	100,00	99,61
RSV	184	181	3	1	698	98,37	99,86	99,45	99,57

Sensitivity (TP/(TP+FN); 95% CI. Positive predictive value (TP/FP+TP)

Specificity (TN/TN+FP); 95% CI Negative predictive value (TN/TN+FN)

Table 1. Results of diagnostic sensitivity and specificity with samples.

Clinical Utility Parameters

The Clinical Utility Parameters obtained in different hospitals confirm the high sensitivity of the kit: 98,23% for the detection of SARS-CoV-2, 98,90% for Influenza A, 95,16% for Influenza B and 96,77 for RSV. The specificity is 100% for the first three microorganisms and 99,77% for RSV.

Sensitivity from saliva samples

The study of sensitivity in saliva samples was carried out in collaboration with the Complutense University of Madrid as part of the COVID-LOT study.

Of the 72 samples analyzed, the concordance between the nasopharyngeal swab sample and saliva is 90.3%. The Positive Predicted Value (PPV) was 100%, the Negative Predicted Value (NPV) was 82.9%".

N=528	N=	TP	FN	FP	TN	Sensitivity	Specificity	PPV	NPV
SARS-CoV-2	113	111	2	0	415	98,23	100,00	100,00	99,52
Influenza A	181	179	2	0	347	98,90	100,00	100,00	99,43
Influenza B	62	59	3	0	466	95,16	100,00	100,00	99,36
RSV	93	90	3	1	434	96,77	99,77	98,90	99,31

Sensitivity (TP/(TP+FN); 95% CI. Positive predictive value (TP/FP+TP)

Specificity (TN/TN+FP); 95% CI Negative predictive value (TN/TN+FN)

Table 2. Clinical parameters.

Conclusion

The data collected demonstrates the suitability of qCOVID-19 Combo for the detection and differentiation of SARS-CoV-2, Influenza A, Influenza B and RSV. The outstanding performance of this kit makes it a well validated tool and a good candidate for respiratory diagnostic in the clinical and hospital setting. The validation of saliva samples to detect SARS-CoV-2 target it is a big advantage that can potentially reduce the time and cost of the assay.

References

1. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25 (3). PMID: 31992387.
2. Belongia EA, Osterholm MT. COVID-19 and flu, a perfect storm. Science. 2020 Jun 12;368 (6496):1163. PMID: 32527804.
3. Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? J Microbiol Immunol Infect. 2020 Aug;53(4):505-512. PMID: 32482366.
4. Guan WJ,Ni ZY,Hu Y, et al.Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med.2020 Apr 30;382(18):1708–1720. PMID: 32109013.
5. C. B. F. Vogels et al., SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. medRxiv 10.1101/2020.08.03.20167791, 2020.2008.2003.20167791 (2020).