FITNESS GENES

FitnessGenes®
SARS-CoV-2
Multiplex RT-PCR Test:
Information Guide

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Test description

Application	Qualitative PCR test for the detection of the SARS-CoV-2 N/E genes
Type of detection	Ribonucleic acid (RNA) of SARS-CoV-2
Sample type	Saliva specimen
RT-PCR limit of detection	750 copies/ml

The FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test has been designed specifically for the detection of SARS-CoV-2 genomic RNA extracted from human saliva. The test uses primer/probe sets which have been designed to target and detect the presence of the SARS-CoV-2 nucleocapsid (N) and envelope (E) genes. The primer/probe sets used are a selection of the best performing Centers for Disease Control and Prevention (CDC) and the World Health Organisation (WHO) approved sequences. It also targets the human RPP30 gene and this is used as an internal RNA extraction control.

The multiplex design of the test means that the presence of multiple genes can be detected simultaneously in the same test sample. This lowers material and reagents costs, and reduces the time required for each test. Accordingly, this kit is well suited to higher throughput and large-scale testing.

Since the start of the SARS-CoV-2 outbreak, deep nasopharyngeal (i.e. nasal and/or throat) swabs have been the most commonly used sample collection method. However, this method requires collection from deep inside either the throat or nasal cavity, which is often extremely uncomfortable for the individual providing the sample. Nasopharyngeal swabs are also difficult to self-administer and often require a trained medical professional to collect a valid sample proficiently. This method is also likely to induce coughing or sneezing, which increases the risk of viral transmission and necessitates the use of personal protective equipment (PPE) by those collecting the samples.

By contrast, saliva collection is a non-invasive method which can be self-administered by the sample provider. The only requirement is that they are capable of producing an adequate amount of saliva (~2ml). Saliva collection also greatly reduces the risk of viral transmission and obviates the need for PPE. It has also been shown to have comparable detection sensitivity for SARS-CoV-2 when compared with nasal/throat samples[1-5]. Saliva samples are also easy to use in an automated lab process and are therefore better suited to higher throughput and large-scale testing.

NOTE: This test has also been shown to work with lower respiratory tract (e.g. - bronchoalveolar lavage, sputum, tracheal aspirate) and upper respiratory tract (e.g. - nasopharyngeal fluids, nasal swab) samples.

1. Kojima, N. et al., Self-Collected Oral Fluid and Nasal Swabs Demonstrate Comparable Sensitivity to Clinician Collected Nasopharyngeal Swabs for Covid-19 Detection, medRxiv, 2020.04.11.20062372, (2020), doi:10.1101/2020.04.11.20062372
2. Wyllie, A. L. et al., Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs, medRxiv, 2020.04.16.20067835, (2020), doi:10.1101/2020.04.16.20067835
3. To, K. K. W. et al., Consistent detection of 2019 novel coronavirus in saliva, Clin. Infect. Dis., (2020), doi:10.1093/cid/ciaa149
4. Azzi, L. et al., Saliva is a reliable tool to detect SARS-CoV-2, J. Infect., (2020), doi:10.1016/j.jinf.2020.04.005
5. Zheng, S. et al., Saliva as a Diagnostic Specimen for SARS-CoV-2 by a PCR-Based Assay: A Diagnostic Validity Study, SSRN Electron. J., (2020), doi:10.2139/ssrn.3543605

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Possible test results

Sample	RPP30 Result	N Result	E Result
Positive user sample	+	+	+
	-	+	+
Negative user sample	+	-	-
Inconclusive user sample	+	+	-
	+	-	+
	-	+	-
	-	-	+
Failed user sample	-	-	-

Positive

The presence of both the SARS-CoV-2 N and E genes was detected. The user was therefore positive for the SARS-CoV-2 virus at the time the sample was collected.

They should self-isolate and follow the NHS[1] and Public Health England[2] (PHE) guidelines.

Negative

The presence of both the SARS-CoV-2 N and E genes was **not** detected. The user was therefore negative for the SARS-CoV-2 virus at the time the sample was collected, and no further action is required.

However, a negative test result does not preclude infection post sample collection. If COVID-19-like symptoms develop (e.g. high temperature, a new continuous cough, a loss or change of smell and/or taste), then the user should follow NHS[1] and PHE[2] guidelines and self-isolate while also requesting another test. Symptomatic individuals can get a free test via the NHS[3].

Inconclusive

The presence of only one of the SARS-CoV-2 N or E genes was detected.

While the result of the test did not reach the criteria for a confirmed positive result, on the balance of probabilities, there is a high chance that the user is infected with the SARS-CoV-2 virus. The user should self-isolate, request another test (this is provided free of charge by FitnessGenes®), await the results, and then proceed accordingly.

Failed

The presence of both the SARS-CoV-2 N and E genes, as well the human RPP30 gene, was not detected; or, the user sample did not contain adequate amounts of viable RNA to perform the test. Another test should be requested (this is provided free of charge by FitnessGenes®).

If the user is not currently displaying COVID-19-like symptoms, then they do not need to self-isolate.

If the user is displaying symptoms (e.g. high temperature, a new continuous cough, a loss or change of smell and/or taste), then they should follow NHS[1] and PHE[2] guidelines and self-isolate. Symptomatic individuals can get a free test via the NHS[3].

NOTE: The chances of a failed test can be greatly reduced by reading and carefully following the instructions provided with the saliva collection tube.

1. https://www.nhs.uk/conditions/coronavirus-covid-19/testing-and-tracing/what-your-test-result-means/
2. https://www.gov.uk/government/publications/covid-19-stay-at-home-guidance
3. https://www.nhs.uk/conditions/coronavirus-covid-19/testing-and-tracing/get-an-antigen-test-to-check-if-you-have-coronavirus/

Test validation

To validate and understand the limit of detection of the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test, the following experiments were carried out.

Synthetic copies of SARS-CoV-2 RNA (ATCC® VR-3276SD™) were serially diluted (1000, 100, 10, 5 and 1 copies) in molecular biology grade water. These dilutions were then tested using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

All dilutions containing synthetic RNA produced Ct values of less then 40, which are considered positive signals as per CDC guidelines[1], confirming the presence of both the E and N genes (**Figure 1** and **Table 1**).

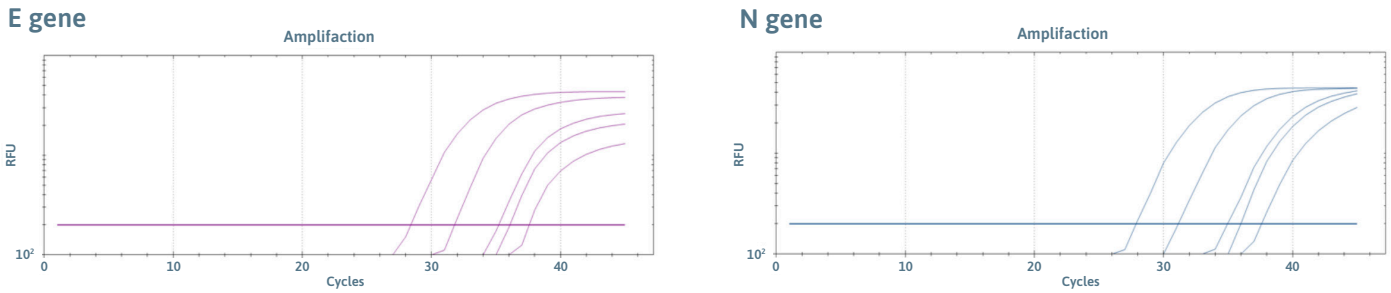

Figure 1. RT-PCR data of samples containing 1000, 100, 10, 5, 1 and 0 copies of synthetic SARS-CoV-2 RNA, tested using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

Table 1. RT-PCR triplicate data of samples containing 1000, 100, 10, 5, 1 and 0 copies of synthetic SARS-CoV-2 RNA, tested using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

Total copies	E gene			N gene		
	Replicates	Average Ct	St dev	Replicates	Average Ct	St dev
1000	3/3	28.25	0.10	3/3	27.78	0.14
100	3/3	31.49	0.22	3/3	30.95	0.22
10	3/3	35.12	0.11	3/3	34.48	0.43
5	3/3	36.09	0.42	3/3	35.94	0.77
1	3/3	37.05	0.60	3/3	37.27	0.52
0	0/3	N/A	N/A	0/3	N/A	N/A

Synthetic SARS-CoV-2 RNA was then spiked into negative saliva samples at viral loads equivalent to 2500, 1000, 500 and 200 copies/ml. RNA was then extracted from these samples using an RNA extraction kit and eluted in 50 µl of the elution buffer. 5 µl of the eluate was then tested using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

A positive signal (Ct < 40) was detected for the N gene in all samples containing synthetic RNA (**Table 2**). However, at an equivalent viral load of 250 copies/ml, no positive signal was detected for the E gene.

Test validation cont.

Table 2. RT-PCR triplicate data of samples containing 2500, 1000, 500, 200 and 0 copies/ml of synthetic SARS-CoV-2 RNA, extracted using an RNA extraction kit, and then testing using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

Copies/ml	E gene			N gene		
	Replicates	Average Ct	St dev	Replicates	Average Ct	St dev
2500	3/3	35.04	1.01	3/3	35.41	0.82
1000	3/3	36.50	1.83	3/3	36.00	0.64
500	3/3	37.23	1.20	3/3	37.49	2.09
200	0/3	N/A	N/A	3/3	38.19	0.88
0	0/3	N/A	N/A	0/3	N/A	N/A

A final experiment was then carried out to refine the limit of detection of the test. A further 20 negative saliva samples were spiked with synthetic SARS-CoV-2 RNA at viral loads equivalent to 750 and 500 copies/ml. RNA was then extracted from these samples using an RNA extraction kit and eluted in 50 µl of the elution buffer. 5 µl of the eluate was then tested using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

At an equivalent viral load of 750 copies/ml, all 20 replicates returned a positve signal (Ct < 40) for both the E and N genes (**Table 3**). At 500 copies/ml only 17/20 replicates returned a positive signal for the E gene. Therefore 750 copies/ml was considered as the reliable limit of detection for the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test.

Typical viral loads in SARS-CoV-2 infected individuals are in the range of 10,000 to 100,000,000 copies per/ml[2-4].

Table 3. RT-PCR data of 20 negative saliva samples containing 750 and 500 copies/ml of synthetic SARS-CoV-2 RNA, extracted using an RNA extraction kit, and then testing using the FitnessGenes® SARS-CoV-2 Multiplex RT-PCR test and a BioRad CFX96 qPCR instrument.

Copies/ml	E gene			N gene		
	Replicates	Average Ct	St dev	Replicates	Average Ct	St dev
750	20/20	36.22	0.68	20/20	36.38	1.00
500	17/20	36.75	1.52	20/20	37.44	1.17

1. Centers for Disease Control and Prevention, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, 1–59, (2020), https://www.fda.gov/media/134922/download
2. Wyllie, A. L. et al., Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs, medRxiv, 2020.04.16.20067835, (2020), doi:10.1101/2020.04.16.20067835
3. To, K. K. W. et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, Lancet Infect. Dis., (2020), doi:10.1016/S1473-3099(20)30196-1
4. Yoon, J. G. et al., Clinical significance of a high SARS-CoV-2 viral load in the Saliva, J. Korean Med. Sci., (2020), doi:10.3346/JKMS.2020.35.E195

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www.fitnessgenes.com

FitnessGenes Ltd
Bicester Innovation Centre, Commerce House,
Telford Road, Bicester, Oxfordshire, OX26 4LD