Covid-19 FluoGene Q

Intended Use:

TaqMan based Real-Time Detection of SARS-CoV-2 specific genes

The assay provides rapid screening of individuals suspected of SARS-CoV-2 infection including the discrimination of Omicron BA.1 and BA.2 variants and aids the diagnosis of suspected COVID-19 disease in patients.

IVD

Article No.	Article	Reactions/Test	Tests/Kit
003 COV 096	Covid-19 FluoGene Q	1	96
003 COV 960	Covid-19 FluoGene Q	1	960
003 COV 500	Covid-19 FluoGene Q	1	500

CHANGES TO VERSION 1.4:

- Section 2.1: Adapted available kits.

TABLE OF CONTENTS

1 INTRODUCTION

Coronavirus are enveloped non-segmented positive-sense RNA viruses and belong to *Coronaviridae* family. There are six coronavirus species known to cause human diseases. Four viruses (229E, OC43, NL63 and HKU1) cause common cold symptoms and the other two (severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)) are zoonotic and producing more severe complications. SARS-CoV and MERS-CoV have caused more than 10,000 cumulative cases in the past two decades, with mortality rates of 34% MERS-CoV and 10% SARS-CoV.

In December 2019, some people that worked at or lived around the Huanan seafood market in Wuhan, Hubei Province, China, have presented pneumonia of unknown cause. Deep sequencing analysis of the respiratory samples indicated a novel coronavirus, which was named firstly 2019 novel coronavirus (2019-nCoV) and lately SARS-CoV-2.

Human-to-human transmission of the SARS-CoV-2 has been confirmed, even in the incubation period without symptoms, and the virus causes severe respiratory illness like those SARS-CoV produced. Although the pneumonia is the principal illness associated, a few patients have developed severe pneumonia, pulmonary edema, acute respiratory distress syndrome, or multiple organ failure and death. Centers of Disease Control and Prevention (CDC) believes that symptoms of SARS-CoV-2 may appear in as few as 2 days or as long as 14 days after exposure, being the most common fever, cough, myalgia and dyspnea. Less common symptoms are sore throat, headache, diarrhea and vomiting. It seems that older males with comorbidities have been more affected.

Diagnosis of SARS-CoV-2 is performed detecting conventional causes of pneumonia early by next-generation sequencing or real-time RT-PCR methods. Several assays that detect the SARS-CoV-2 are currently available, such as China CDC (gene targets, ORF1ab and N), Charité – Germany (gene targets, RdRP, E, N) or US CDC (gene targets, three N primers, RdRP).

WHO recommends lower respiratory specimens (sputum, endotracheal aspirate, or bronchoalveolar lavage) for the identification of SARS-CoV-2. However, if the collection is not possible, upper respiratory tract specimens such as a nasopharyngeal aspirate or combined nasopharyngeal and oropharyngeal swabs should be collected. In addition, other clinical specimens as blood, urine and stool may be collected to monitor the presence of the virus.

Viruses tend to rapidly mutate and evolve but so far, the Covid-19 FluoGene Q is able to detect all known variants, as of today 10th March 2022. The kit features a high sensitivity due to the additional detection of the *N1* and *E* genes of SARS-CoV-2 on the FAM channel. No known mutation affects the N1 and E primer / probe binding sites.

The delta variant B.1.617.2 of SARS-CoV-2 superseded other variants but now was itself superseded by the Omicron variant B.1.1.529 (Weekly status report RKI, 2022-03-03). The Omicron variant has two subvariants, the former main subvariant BA.1 and the newer subvariant BA.2, which itself seems to replace BA.1 (Weekly status report RKI, 2022-03-03). As different variants comprise the risk of easier infection, altered or more or less severe symptoms, as well as the risk of vaccination escape the knowledge of the exact infection of a patient can be highly valuable. The current version of the Covid-19 FluoGene Q – VoXcreen is able to detect a general SARS-CoV-2 infection and discriminate between an Omicron BA.1 or BA.2 variant infection.

1.1 Principle of Method

Covid-19 FluoGene Q is a reagent system based on real-time PCR technology for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA in respiratory samples.

The assay is designed for specific detection of **two SARS-CoV-2 genes** proposed by the Institute of Medical Virology, University Hospital Frankfurt am Main, Goethe University, Frankfurt am Main:

Toptan *et al.* (2020): Optimized qRT-PCR approach for the detection of intra- and extra-cellular SARS-CoV-2 RNAs. Int. J. Mol. Sci. 2020, 21(12), 4396; https://doi.org/10.3390/ijms21124396

An additional primer/probe set to detect the **human RNase P gene** (internal control) in control samples and clinical specimens is also included in the multiplex assay.

The test consists of three processes in a single vial assay:

- Reverse transcription of target RNA to cDNA
- PCR amplification of target cDNA
- Detection of PCR amplicons by fluorescent dye labelled probes

The isolated RNA target is transcribed generating complementary DNA (cDNA) by reverse transcriptase (RT) which is followed by polymerase chain reaction (PCR) for the amplification of a conserved region of *M* and *N1 genes* for SARS-CoV-2 using specific primers and a fluorescent-labeled probe.

Covid-19 FluoGene Q is based on the standard hydrolysis probe system known as TaqMan® probe detection and utilizes the 5´ exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bounded to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence can be measured on real-time PCR platforms.

Covid-19 FluoGene Q consists of:

- Buffer Mix (includes dNTPs and buffer)
- Enzyme Mix (DNA polymerase and reverse transcriptase)
- Primer / Probe Mix for two SARS-CoV-2 genes, Omicron BA.1 and BA.2 variants, and the human RNase P gene
- Positive Control (SARS-CoV-2 genes) with Internal Control (human RNase P gene) and BA.1 variant
- Positive Control (SARS-CoV-2 genes) with Internal Control (human RNase P gene) and BA.2 variant
- Water (Nuclease-free)

N1 & *E* genes are amplified and detected in FAM channel, *RNaseP* gene is amplified and detected in JOE channel, while HEX or VIC channels work as well (depending on the equipment used select the proper detection channel), Omicron BA.1 specific deletion is detected in ROX channel, and Omicron BA.2 specific deletion is detected in Cy5 channel.

2 MATERIAL

2.1 Components of Covid-19 FluoGene Q Kit (96 tests & 500 tests)

			003 COV 096		003 COV 960	
Component	**Vial Label**	**Color**	**Vial**	**Volume**	**Vial**	**Volume**
Buffer Mix	BM	Green	1x (1.5 mL) vial	1050 µL	10x (5 mL) vial	50000 µL
Enzyme Mix	EM	Yellow	1x (0.5 mL) vial	20 µL	10x (0.5 mL) vial	1000 µL
Primer / Probe Mix	PPM	Blue	1x (1.5 mL) vial	210 µL	10x (1.5 mL) vial	10000 µL
Positive Control BA.1	CTR BA.1	Red	1x (0.5 mL) vial	100 µL	10x (0.5 mL) vial	2500 µL
Positive Control BA.2	CTR BA.2	Red	1x (0.5 mL) vial	100 µL	10x (0.5 mL) vial	2500 µL
Nuclease-free water	Water	White	1x (1.5 mL) vial	850 µL	10x (1.5 mL) vial	10000 µL

			003 COV 500	
Component	**Vial Label**	**Color**	**Vial**	**Volume**
Buffer Mix	BM	Green	1x (5 mL) vial	5000 µL
Enzyme Mix	EM	Yellow	1x (0.5 mL) vial	100 µL
Primer / Probe Mix	PPM	Blue	1x (1.5 mL) vial	1000 µL
Positive Control BA.1	CTR BA.1	Red	1x (0.5 mL) vial	250 µL
Positive Control BA.2	CTR BA.2	Red	1x (0.5 mL) vial	250 µL
Nuclease-free water	Water	White	1x (1.5 mL) vial	1000 µL

2.2 Storage and Shelf-Life

Covid-19 FluoGene Q is shipped on dry ice. The components of the kit should arrive frozen. After receipt the kits should be stored between –20°C and -35°C. The shelf-life of the individual components and the entire system are stated on the respective label. Storage between +2°C and +8°C should not exceed a period of two hours. Protect Primer/Probe Mixes (PPM) from light.

We recommend dividing the PPM (Primer/Probe) mix into aliquots to avoid repeated freeze and thaw cycles. PPM as well as Positive Control have been validated to be stable after 6 freeze-thaw cycles.

2.3 Positive Control

SARS-CoV-2 Positive Controls (CTR BA.1 and CTR BA.2) are ready to use. They contain a standard number of SARS CoV-2 RNA specific sequences, human RNase P sequence, and SARS-CoV-2 BA.1 or BA.2 variant sequences respectively. To ensure PCR run validity, the CTR BA.1 of should be positive (Cq ≤38) for the three channels, FAM, JOE, ROX, while Cy5 should stay negative. For CTR BA.2 the three channels, FAM, JOE, Cy5, should be positive (Cq ≤38), while ROX should stay negative.

Recommendations:

- Since it contains high copies of the template, the recommendation is to open and handle it carefully in order to avoid any contamination. Thaw the PCR tube, vortex and spin down before pipetting a 5µL volume for the positive control reaction.
- Keep the positive control during PCR set-up on ice.
- Freeze it quickly again after use.

2.4 Additionally Required Materials and Devices

- Real-time PCR Cycler with the following filters: FAM, VIC or JOE or HEX, ROX, Cy5.
- Real-time PCR plates (e.g. ABI MICROAMP 96-WELL RXN PLATE Art. No.: N8010560)
- Optical foil for sealing the PCR plates (**inno-train**, Art. No.: 00207KF10)
- 8-well PCR strips (e.g. ABI MICROAMP FAST 8-Tube Strip, 0,1mL Art. No. 4358293)
- 8-well PCR cap strips (e.g. ABI MICROAMP OPTICAL 8-Cap Strips Art. No. 4323032)
- Tray for working with PCR strips (e.g. ABI MICROAMP FAST 96-WELL TRAY Art. No. 4358305)
- RNA extraction kit / sample RNA (e.g. BEXS12 Ready Viral RNA/DNA Bead kit, Art. No.: GX01054)
- 1.5 ml tubes (low binding, nuclease free)
- Pipettes (1 – 1000 µL)
- Pipette tips (with filters, nuclease free)
- Benchtop microcentrifuge for 1.5 ml tubes
- Plate centrifuge
- Vortex
- 80% molecular grade ethanol
- For use with FluoQube Real-time cycler, FluoPad, compression pad for PCR (**inno-train,** Art. No.: 002 07C P01)
- FluoApp, applicator for application of the optical foil (**inno-train**, Art. No.: 002 07F A01)

Covid-19 FluoGene Q has been validated on the following equipments: Applied Biosystems 7500 Fast Real-Time PCR System, Applied Biosystems Quantstudio 6 pro, inno-train FluoQube, Analytik Jena Q-Tower.

2.5 Facilities/Training Requirements

Samples should be handled in a Biosafety Level 2 facility; World Health Organization Interim guidance on laboratory biosafety from 12 Feb 2020 should be followed. Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures.

3 PROCEDURE

3.1 Sample preparation

Extracted RNA is the starting material for Covid-19 FluoGene Q.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. The time point(s) of sampling is important for the detection of SARS-CoV-2 RNA: the best time for qRT-PCR testing of swab samples is 4 – 12 days after infection.

It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are tested for nucleic acid extraction:

- GXP Mag Viral RNA Kit (GenXPro)
- MagMAX Viral RNA Isolation Kit (Thermo Fisher)
- BEXS12 Ready Viral DNA/RNA Bead Kit (**inno-train**, Art. No.: GX01054)

Alternative nucleic acid extraction systems and kits might also be appropriate.

If using a spin-column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 5-10 min at maximum speed, using a new collection tube, prior to the elution of the nucleic acid.

3.2 Precautions

Due to the closed Covid-19 FluoGene Q system there is no risk of contamination as no PCR product can leak out after the PCR preparation. According to existing regulations we recommend the following safety measures:
To avoid contamination within the PCR method:

- Spatial separation of the pre-PCR area (RNA isolation, storage, PCR sample) from the post-PCR area (Real-time cycler)
- Components of the post-PCR area must not get into the pre-PCR area.
- Use pipette tips with aerosol protection in the pre-PCR area.
- Keep the positive control on ice while doing the PCR setup.

The purpose of the kit is RNA detection, but RNA is chemical instable and RNases are ubiquitous present. This kit should always be used in a RNase-free environment and with special cautiousness. Always wear gloves, work on a sterile bench which was cleaned of RNases and use RNase-free materials, like reaction vials and pipette tips. Inappropriate handling can lead to false negative results.

3.3 Setup of PCR

⇨ Thaw RNA sample and allow them to get room temperature.
⇨ Keep enzyme mix on ice
⇨ Mix components (by pipetting or gentle vortexing) and centrifuge briefly before use.
⇨ Pipette the following volumes into each well. One well is used for one test. A positive and a negative control (Nuclease-free Water) must be included for each run and for each assay.

Reagents	**Volume per Reaction**
Buffer Mix (BM)	10 µl
Enzyme Mix (EM)	0.2 µL
Primer / Probe Mix (PPM)	2 µL
Sample, Negative Control, or Positive Control (CTR BA.1/CTR BA2)	7.5 µL
Nuclease-free Water	0.3 µL
Total Reaction Volume	**20 µL**

⇨ If several Covid-19 tests are planned, it is helpful to create a master mix that contains everything except the RNA. Dispense 12.5 µL aliquots into the desired number of wells of the PCR plate.
⇨ Add 7.5 µL of sample RNA resp. the positive or the negative control to the pre-dropped master mix.

- ⇨ Cover the PCR plate with adhesive optical foil (e.g. **inno-train**, Art. No.: 00207KF10) and close by pressing firmly (use disposable lab gloves and an applicator, e.g. FluoApp by **inno-train**, Art No.: 002 07F A01). Avoid finger prints and dirtying the foil.
- ⇨ Centrifuge the PCR plate shortly (2 min at min. 1000g).
- ⇨ Transfer the PCR plate to the real-time cycler (note the correct orientation!); if needed, cover the plate with a clean PCR pressure pad (e.g. FluoPad, **inno-train**, Art. No.: 002 07C P01) and start PCR program.

3.4 Temperature Profile (for all real-time devices)

Step	Stage	Cycles	Acquisition/ Data collection	Temp	Time
Reverse Transcription	Hold	1	-	50 °C	10 min
Initial Denaturation	Hold	1	-	95 °C	2 min
Denaturation	Cycling	45	-	95 °C	5 sec
Annealing/ Extension			yes	60 °C	30 sec

The test system was validated with the following real-time instruments:

- FluoQube (**inno-train** Art. No. 005 010 000) (software version 3.4)
- Q-Tower (Analytic Jena) (software version 3.4)
- GeneAmp® System 7500 (Applied Biosystems) (7500 Software 2.3)
- Quantstudio 6 pro (Applied Biosystems) (Design & Analysis Software 2.4.3)

Please ensure that all instruments used have been installed, calibrated and maintained according to the manufacturer's instruction and recommendations.

The use of other instruments has to be validated by the user.

3.5 Real-time Settings ABI7500

Define the following settings:

Settings	
Reaction Volume	20 µL
Ramp Rate	Default
Passive Reference	(none)

Define the fluorescence detectors:

Target	Reporter Dye	Quencher Dye
SARS-CoV-2 N1 + E gene	FAM	(none)
Human RNase P gene	HEX (/JOE/VIC)	(none)
SARS-CoV-2 BA.1 specific deletion	ROX	(none)
SARS-CoV-2 BA.2 specific deletion	Cy5	(none)

3.6 Real-time Settings Quantstudio 6 Pro

Define the following settings:

Settings	
Run Mode	Fast
Analysis Mode	Standard Curve
Reaction Volume	20 µL
Heated Cover Temperature	105.0 °C
Ramp Rate	2.5°C/ s (except cooling phase during cycling: 2 °C/s)
Passive Reference	None

Define the fluorescence detectors as described below.

Target	**Reporter Dye**	**Quencher Dye**
SARS-CoV-2 N1 + E gene	FAM	NFQ-MGB
Human RNase P gene	HEX (/JOE/VIC)	NFQ-MGB
SARS-CoV-2 BA.1 specific deletion	ROX	NFQ-MGB
SARS-CoV-2 BA.2 specific deletion	Cy5	NFQ-MGB

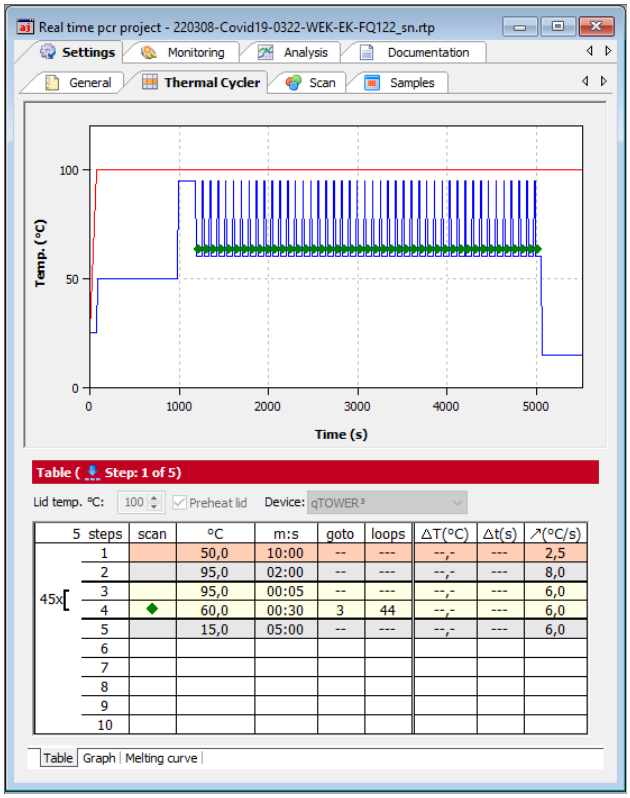
3.7 Real-time Settings FluoQube und QTower

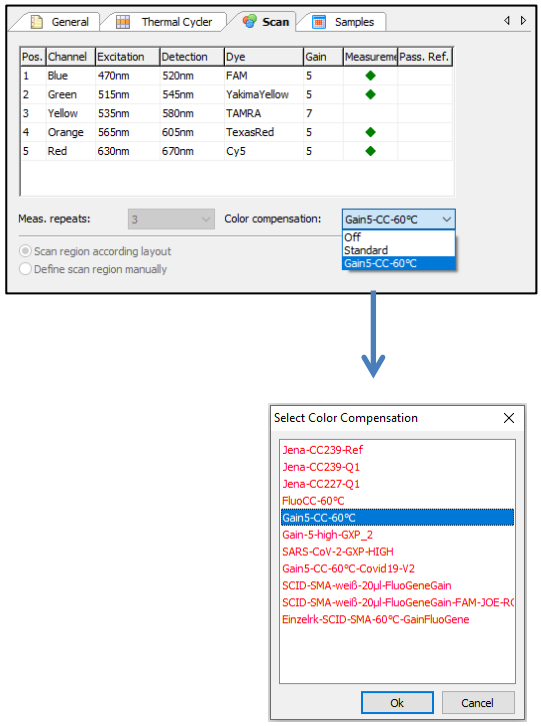
If one of these devices is used, please contact support@inno-train.de, we will supply you with the appropriate template. Or define the following settings (see below).

Please note:

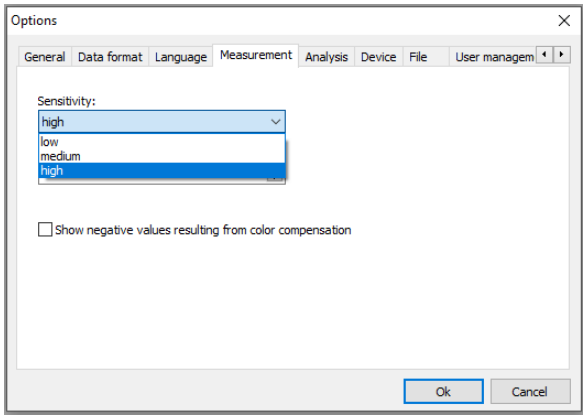
The test detects two different SARS-CoV-2 targets. If FluoQube or QTower are used, the FAM signal shows a slight cross irradiation into the JOE/VIC/HEX channel, while the JOE/VIC/HEX channel shows a strong cross irradiation into the ROX channel. As a result, it might be difficult to evaluate the reactivity of the different independent from each other. Therefore, we recommend using at least the qPCR Color Compensation Kit from Analytik Jena. https://www.analytik-jena.de/produkte/kits-assays-reagenzien/reagenzien/reagenzien-fuer-real-time-pcr/qpcr-color-compensation-kit/

Once you used the color compensation kit, in the field "Select Color Compensation" it will appear and can be selected.





In order to adjust the measurement sensitivity, refer to the menu bar, go to "Extras" → "Options…" → "Measurement" and choose sensitivity "high" and click "Ok":



4 EVALUATION

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

4.1 Interpretation of Results

The use of positive and negative controls in each run is mandatory. Validate the reactions by checking the absence of signal in the negative control well and the presence of signal for SARS-CoV-2 and for Human RNase P in the positive control well. Check Internal Control signal to verify the correct functioning of the amplification mix. The analysis of the samples is done by the software of the used real-time PCR equipment itself according to manufacturer´s instructions.

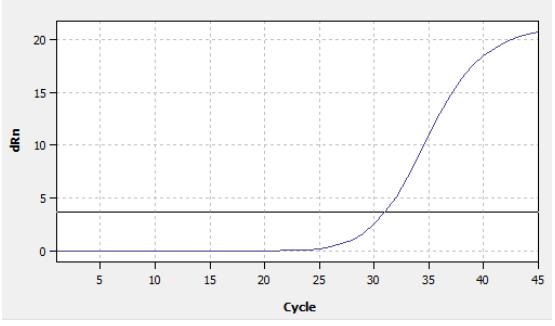
Using the following table read and analyze the results:

CoV-2Genes N1 & E	**Human RNase P**	**Omicron BA.1**	**Omircon BA.2**	**Result**	**Action**
FAM	**JOE/HEX/VIC**	**ROX**	**Cy5**		
Positive	Positive / Negative	Positive	Negative	SARS-CoV-2 **BA.1 variant** detected	Report Result
Positive	Positive / Negative	Negative	Positive	SARS-CoV-2 **BA.2 variant** detected	Report Result
Positive	Positive / Negative	Negative	Negative	SARS-CoV-2 detected **Unknown variant**	Report Result
Negative	Positive	Negative	Negative	SARS-CoV-2 **not detected**	Report Result
Negative	Negative	Negative	Negative	**Invalid Test**	**Repeat**

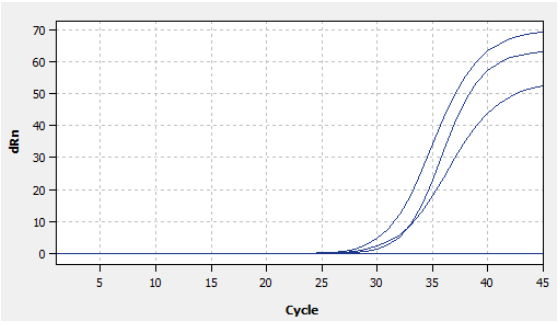
Please adjust the threshold of your qPCR instrument for positive detection to be at 10% of the signal intensity of the maximum value of the positive control. Samples that reach less than 10% of the signal intensity of the positive control shall be regarded as negative.

A sample is considered **positive** if the Ct value is ≤ 38. The internal control may or may not show an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids.

A sample is considered **negative**, if the sample shows no amplification signal in the detection system but the internal control is positive. An inhibition of the PCR reaction can be excluded by the amplification of internal control.



Negative Control



Positive Control

In case of absence of any signal in sample wells we recommend to repeat the assay diluting the sample 1:10 or to repeat the extraction to check for possible problems of inhibition.

Attention:

In case of a doubtful interpretation result, it is recommended to verify the correct performance of each of the steps and review the parameters and the shape of the curve. If the situation is not solved, it is recommended to repeat the assay, preferably in duplicate. The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

5 GENERAL WARNINGS AND PRECAUTIONS

- ⇨ In samples of human origin there is still a potential risk of infection even after DNA/RNA extraction. Therefore gloves and lab coats should be worn when performing the FluoGene method and all recommendations on handling infectious material should be followed.
- ⇨ Inadequate patient material may affect the results of analysis.
- ⇨ The fluorophores are photosensitive. Therefore the PCR plates should be processed quickly.
- ⇨ Reagents should not be used after their expiry date.

5.1 Limitations of Use

Covid-19 FluoQube Q has been validated for use with sputum, oropharyngeal swab and nasopharyngeal swab samples run on the Applied Biosystem® 7500 Real-Time PCR System, inno-train FluoQube, Analytik Jena Q-Tower, Applied Biosystem® Quantstudio 6 pro.

The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results.

Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.

All samples should be handled as if they are infectious following proper biosafety precautions.

Interpretation of results must account for the possibility of false negative and false positive results.

False negative results may be caused by:

- Unsuitable collection, handling and/or storage of samples.
- Sample outside of viraemic phase.
- Failure to follow procedures in this handbook.
- Use of unauthorized extraction kit or PCR platform.

False positive results may be caused by:

- Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
- Unsuitable handling of amplified product.

All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.

This test cannot rule out diseases caused by other pathogens.

A negative result for any PCR test does not conclusively rule out the possibility of infection.

6 PERFORMANCE DATA/PERFORMANCE ASSESSMENT

6.1 Quality control

Covid-19 FluoGene Q contains a positive and a negative control that must be included in each run in order to interpret the results correctly. Also, the internal control (IC) in each well confirms the correct performance of the technique.

In accordance with inno-train´s ISO 13485 certified Quality Management System, each batch of Covid-19 FluoGene Q is tested against predetermined specifications to ensure consistent product quality.

6.2 Clinical sensitivity and specificity

The clinical performance of Covid-19 FluoGene Q was tested using 100 respiratory specimens (nasopharyngeal swab) from symptomatic patients with suspicion of COVID-19. These results were compared with those obtained with another CE marked test. The results were as follows:

	CE marked SARS-CoV-2 Detection Kit			
Covid-19 FluoGene Q		**+**	**-**	**Total**
	+	5	0	5
	-	0	95	95
	Total	5	95	100

6.3 Analytical specificity

Covid-19 FluoGene Q assay has been designed to detect all publicly available SARS-CoV-2 viral RNA sequences. This was assessed with *in silico* sequence comparison analyses and in vitro specimen testing. Upon *in silico* analysis the assay design was found to detect all SARS-CoV-2 virus strains and exhibited no cross reactivity with non-SARS-CoV-2 species.
The results of the in silico analysis showed that Covid-19 FluoGene Q does not falsely positive detect the specimen listed below:

Influenza A H1N1	No overlap/ possible priming
Influenza A H3N2	No overlap/ possible priming
Influenza B Victoria	No overlap/ possible priming
Influenza B Yamagata	No overlap/ possible priming
RSV A	No overlap/ possible priming
RSV B	No overlap/ possible priming
Coronavirus NL63	No overlap/ possible priming
Coronavirus 229E	No overlap/ possible priming
Coronavirus HKU	No overlap/ possible priming
Coronavirus OC43	No overlap/ possible priming
MERS-CoV	No overlap/ possible priming

6.4 Analytical sensitivity

Covid-19 FluoGene Q has a detection limit of ≥10 RNA copies per reaction for *N1* and *E* genes.

7 EXPLANATION OF SYMBOLS

⚠	Note accompanying documents	⌛	Expiry date
LOT	Lot number	REF	Article number
📖	Follow Instructions for Use	🏭	Manufacturer
🌡	Observe upper temperature limit	IVD	In vitro diagnosticum
BM	Buffer Mix	PPM	Primer/Probe mix
EM	Enzyme Mix		
Σ	Contents sufficient for <n> tests		

8 LITERATURE

Toptan T, Hoel S, Westhaus S, Bojkova D, Berger A, Rotter B, Hoffmeier K, Cinatl J, Ciesek S, Widera M: Optimized qRT-PCR approach for the detection of intra- and extra-cellular SARS-CoV-2 RNAs. *Int. J. Mol. Sci.* **2020**, *21*(12), 4396; https://doi.org/10.3390/ijms21124396

Vogels, C.B.F., Brito, A.F., Wyllie, A.L. *et al.* Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. *Nat Microbiol* **5,** 1299–1305 (2020). https://doi.org/10.1038/s41564-020-0761-6

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