Primerdesign™ Ltd

genesig® COVID-19 3G Real-Time PCR assay

CE IVD

Instructions for Use (IFU)

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GENESIG
Kits by Primerdesign

genesig® COVID-19 3G

Real-Time PCR Assay

In vitro Real-Time PCR diagnostic test for COVID-19

Validated For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	CE IVD Extraction System, suitable for the directed sample types QIAamp® Viral RNA Mini kit (Qiagen extraction system) exsig ® Mag extraction kit	Applied Biosystem® 7500 (Thermofisher) CFX Opus (Bio-Rad) Lightcycler 480 II (Roche) genesig® q32 (Primerdesign™, Novacyt)
Oropharyngeal Swabs		
Saliva		

96 tests

IVD

REF D00063

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1 Intended Use

The genesig® COVID-19 3 Gene (3G) assay is a CE marked, *in vitro* diagnostic, real-time-reverse transcriptase PCR (Real-Time PCR) multiplex assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 (targeting the ORF1ab, M gene and S gene), in nasopharyngeal swabs, oropharyngeal swabs and saliva samples. This multiplex assay provides rapid screening of individuals suspected of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and aids the diagnosis of suspected COVID-19.

The assay has been designed to be used with the Real-Time PCR instruments capable of detecting FAM (Max Absorption 499nm, Maximum Emission 519nm), HEX/VIC (Max Absorption 538nm, Maximum Emission 559nm), ROX (Max Absorption 575nm, Maximum Emission 602nm) and Cy5 (Max Absorption 643nm, Maximum Emission 667nm) fluorophores.

The genesig® COVID-19 3G assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of Real-Time PCR and *in vitro* diagnostic procedures.

Positive results are indicative of the presence of SARS-CoV-2. Positive results do not rule out co-infection with other bacteria or other viruses. Positive and negative results must be combined with clinical observations, patient history and epidemiological information.

Sample test results are available to interpret in under three hours using the genesig® COVID-19 3G assay. This time includes the time to extract nucleic acid from a sample, PCR set-up, PCR run time and availability of results.

2 Summary and Explanation

The COVID-19 pandemic is caused by a coronavirus named SARS-CoV-2. The first human cases were identified in Wuhan, China and had reported onset of symptoms around 1 December 2019 (1). By 11th March 2020, cases positive for SARS-CoV-2 had been recognised in 110 countries and the WHO declared COVID-19 a pandemic due the sustained risk of further spread (2). Globally the SARS-CoV-2 has infected 128 million as of 30th March 2021, and has claimed 2.79 million lives, 81% of whom are above 65 years of age (3). As with most viruses the SARS-CoV-2 also mutates, and the changes in the genomic code have resulted in the emergence of the virus variants. These variants are suspected to have altered the transmissibility rate, impact on the body's immune response and, possibly have effects on vaccine efficacy (4). Timely and accurate diagnostics are thus crucial for clinical treatment of patients, public health decision-making and contact tracing, infection control practices and personal protective equipment (PPE) use and avoid overwhelming our health-care system.

Recent prevalence of mutations with potential biological significance within the Spike protein of SARS-CoV-2 have raised concern over the most effective targets in COVID-19 for Real-Time PCR based diagnostic methods (5-7), suggesting the need to test for more than one target at a time. The genesig® COVID-19 3G assay has been developed to target three genes to ensure the accuracy of the genesig® assay.

The genesig® COVID-19 3G assay is a molecular *in vitro* diagnostic test for the detection of the SARS-CoV-2 ribonucleic Acid (RNA) in nasopharyngeal swabs, oropharyngeal swabs, and saliva samples. The viral RNA is released from the sample during incubation with a viral inactivation/ lysis agent. Following the RNA sample extraction process, an aliquot of the resulting sample is tested using well-established nucleic acid amplification technology with the genesig® COVID-19 3G assay. The supplied primers/probes are designed for the specific detection of SARS-CoV-2 RNA, including ORF1ab, the M gene and S gene.

3 Principles of the Procedure

RNA is isolated and purified from nasopharyngeal swabs, oropharyngeal swabs and saliva using a CE IVD nucleic acid extraction system. Using PCR technology, the RNA is reverse transcribed to cDNA and subsequently amplified using forward and reverse primers. A fluorescent labelled probe is used to detect the amplicon. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology and the probes are labelled with fluorescent reporter and quencher dyes.

During PCR cycling, the probe anneals to a specific target sequence located between the forward and reverse primers. The probe is cleaved by the 5' nuclease activity of the Taq polymerase during the extension phase of the PCR cycle, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each PCR cycle, additional reporter dye molecules are released from the probe, increasing the fluorescence intensity. Fluorescence intensity is recorded at each cycle of the PCR by the Real-Time PCR machine.

The genesig® COVID-19 3G assay includes primer/probe mix, which contains the SARS-CoV-2 specific probes labelled with the FAM, ROX and Cy5 fluorophores. The primer/probe mix also includes primers/probe to amplify and detect the RNA internal extraction control (IEC) template in the genesig® COVID-19 3G kit. The IEC specific probe is labelled with the HEX/VIC fluorophore. The genesig® COVID-19 3G RNA IEC template is not related to the SARS-CoV-2 viral sequence.

The genesig® COVID-19 3G assay channel allocations are described in the table below.

Reagent Label	FAM	HEX/VIC	ROX	Cy5
genesig® COVID-19 3G primer/probe mix	ORF1ab region	Internal Extraction Control (IEC)	M gene region	S gene region

4 Materials Provided

The genesig® COVID-19 3G assay contains:

Reagent Label	Number of Vials per pack	Lid Colour	Volume (µl per vial)	Resuspended with
genesig® COVID-19 3G Primer/probe mix (including Internal Extraction Control) *	2	Amber, vial stored in a sealed silver foil pouch	110µl	Template Preparation Buffer
OneStep Lyophilised Mastermix*	2	Gold cap, vial stored in a sealed silver foil pouch	525 µl	Mastermix Resuspension Buffer
genesig® COVID-19 3G Internal Extraction Control (IEC)*	2	Blue, vial stored in a sealed blue foil pouch	1000 µl	Template Preparation Buffer
genesig® COVID-19 3G Positive Control Template*	1	Red, vial stored in a sealed red foil pouch	800 µl	Template Preparation Buffer
Mastermix Resuspension Buffer	2	Blue, vial stored in a loose box	750 µl	NA
Template Preparation Buffer	3	Yellow, vial stored in a loose box	1500 µl	
RNase/Dnase Free Water	1	White, vial stored in a loose box	1500 µl	

* Provided lyophilised. They should be resuspended in the buffer and volume provided in the table.

5 Required Equipment and Consumables (Not Provided)

- PCR hood
- Vortex mixer
- Microcentrifuge
- Adjustable micropipettes (2 or 10 µl, 200 µl and 1000 µl)
- Aerosol barrier pipette tips with filters
- Disposable gloves
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- RNase/DNase remover
- PCR reaction plates compatible with the Real-Time PCR instrument to be used)
- Plate seal (compatible with the PCR plate to be used)

6 Facilities/Training Requirements

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:

- Refer to the UK Government guidance on handling and processing potential COVID-19 samples in laboratories: www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens
- Refer to the World Health Organization Laboratory biosafety guidance related to coronavirus disease (COVID-19): Interim guidance, 28th January 2021: https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1
- Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

7 Warnings and Precautions

7.1 General

- Handle all samples as infectious material using safe laboratory procedures. Sample processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) microbiological safety cabinet (refer to the guidance detailed in **Section 6**).
- Follow necessary precautions when handling samples. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited to) gloves, eye protection and lab coats when handling kit reagents while performing this assay and handling materials, including samples, reagents, pipettes and other equipment and reagents.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
- The genesig® COVID-19 3G assay Template Preparation Buffer contains Ethylene Glycol Tetraacetic Acid (EGTA). This component should be handled according to the Safety Datasheet. In the event of damage to protective packaging, contact Primerdesign™ for instructions.

7.2 Preventing Contamination

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical sample or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
- The genesig® COVID-19 3G Positive Control is provided in a sealed foil envelope and contains a high copy number of synthetic DNA templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
- Maintain separate areas for handling of sample preparation, pre-PCR assay set-up, and post-PCR amplified nucleic acids.
- Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. sample tubes, pipette tips) for handling sample preparation, pre-PCR assay set-up, and post-PCR amplified nucleic acids.
- Wear a clean lab coat and disposable gloves when setting up assays.
- Change gloves regularly and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.

- Change aerosol barrier pipette tips between all manual liquid transfers.
- During the preparation of samples, compliance with good laboratory techniques is essential to minimise the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. A good aseptic technique should always be used when working with nucleic acids.
- **DO NOT** substitute or mix reagent from a different kit from other manufacturers. Use the appropriate buffers (provided with the kit) as instructed in the table in **Section 4.**
- Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g. 10% bleach and DNA/RNA remover) pre- and post-PCR set-up to minimise the risk of nucleic acid contamination.
- RNA samples should be maintained on a cold block or on ice during preparation to ensure sample stability.
- Handle post-amplification PCR plates/tubes with care to ensure that the seal is not broken.
- Dispose of unused kit reagents and human biological samples according to national regulations (refer to guidance detailed in **Section 6**).

7.3 Prevent DNase/RNase contamination

- Use RNase/DNase free disposable plasticware and pipettes reserved for DNA/RNA work to prevent cross-contamination with RNases/DNases from shared equipment.
- Use RNase/DNase free filter tips throughout the procedure to prevent aerosol and liquid contamination.

8 Reagent Storage, Handling and Stability Conditions

- The genesig® COVID-19 3G assay is shipped at ambient temperatures but must be stored at -20°C upon arrival.
- The genesig® COVID-19 3G assay should be stored in the original packaging and is stable for up 18 months once stored at -20°C.
- Always check the expiration date prior to use. The kit should not be used past the "use by" date as indicated on the pack label and individual tube labels. Once the "use by" date has been reached, the kit components should be discarded following the disposal instructions in **Section 6**.
- If the kit's protective packaging is damaged upon receipt, please contact Primerdesign™ for instructions.
- All resuspended reagents are stable for one month when stored at -20°C.
- Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze/thaw cycles. Once resuspended, components may be aliquoted into smaller volumes, if required.
- When in use, the kit components should be returned to the freezer promptly after use to minimise the time at room temperature.
- Primer/probe mix, the enzyme Mastermix, Positive Control Template and IEC are all delivered lyophilised and must be resuspended in the appropriate supplied buffer to the correct volume as detailed in the table in **Section 4.**
- It is important to protect the fluorogenic primer/probe mix from light as this reagent is photosensitive.

9 Sample Collection, Handling and Storage

9.1 Compatible Samples

The assay has been designed to be used with the extraction systems using samples obtained from nasopharyngeal swabs, oropharyngeal swabs, saliva, and anterior nasal swab samples.

9.2 Collecting the Samples

Swab samples should be collected using swabs with a synthetic tip, such as nylon or Dracon® and with an aluminium or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport medium.

Inadequate or inappropriate sample collection, storage and transport are likely to yield false test results, for more information, refer to **Section 6**.

9.3 Transporting Samples

Samples must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 samples.

9.4 Storing Samples

- Extracted nucleic acid should be stored at -70˚C or lower.
- Refer to **Section 6** weblinks for guidance.

10 Reagent and Controls Preparation

10.1 OneStep Lyophilised Mastermix preparation

- Upon receipt, the dried Mastermix can be stored at -20ºC for up to 18 months or until the expiry date, whichever occurs first.
- Using aseptic technique, resuspend in 525 µl of Mastermix Resuspension Buffer, gently swirl to mix.
- The resuspended Mastermix is stable for up to one month when stored at -20ºC.
- Freeze/thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20ºC.

10.2 Genesig® COVID-19 3G Primer/ Probe mix preparation

- Upon receipt, the dried primer/probe can be stored at -20ºC for up to 18 months or until the expiry date, whichever occurs first.
- Precaution: The reagent should only be handled in a clean area and not exposed to light.
- Using aseptic technique, resuspend the dried primer/probe in 110 µl (per each vial) of Template Preparation Buffer, vortex to mix.
- Resuspended primer/probe are stable for up to one month when stored at -20ºC.
- Freeze/thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20ºC.
- Store aliquots in the dark and keep away from sunlight.

10.3 Genesig® COVID-19 3G Positive Control Template (PCT) preparation

- The genesig® COVID-19 3G PCT is provided in a red sealed foil envelope and contains a high copy number of synthetic DNA material. It should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination of other kit reagents and clinical samples.
- Upon receipt, the dried PCT can be stored at -20ºC for up to 18 months or until the expiry date. Do not use it after the expiry date (see product label).
- Using aseptic technique, resuspend the dried PCT in 800 µl of Template Preparation Buffer, vortex to mix. Resuspended PCT is stable for up to one month when stored at -20ºC. Following resuspension, the concentration is 10^6 copies per µl.

- Freeze/thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20ºC.
- To ensure PCR run validity, the PCT should produce amplification in the FAM, ROX and Cy5 channel.

10.4 Genesig® COVID-19 3G Internal Extraction Control (IEC) preparation

- The genesig® COVID-19 3G IEC is an RNA control for detecting RNA inhibition and confirm the integrity of the PCR run.
- Upon receipt, the dried IEC can be stored at -20ºC for up to 18 months or until the expiry date, whichever occurs first.
- Precaution: The reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination.
- Using aseptic technique, resuspend the dried IEC in 1000 µl of Template Preparation Buffer, vortex to mix. 20 µl of IEC needs to be added per sample in the lysis state of the extraction.
- Resuspended IEC is stable for up to one month when stored at -20ºC.
- Freeze/thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20ºC.

10.5 Negative Extraction Control (NEC) preparation

- Prepare at least 1 NEC each time RNA is extracted from a sample.
- The NEC preparation has no sample added. It is prepared by extracting from RNase/DNase free water. The IEC is added to the NEC sample during extraction as directed in the manufacturer's IFU. This NEC is used to check for contamination during the extraction stage.

10.6 No Template Control (NTC)

- RNase/DNase free water is provided to use as a NTC if required in addition to the NEC.
- The NTC is used to check for contamination during PCR plate set-up.

11 General Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges and other equipment prior to use.
- Decontamination agents should be used, such as 10% bleach, 70% ethanol RNase/DNase remover to minimise the risk of nucleic acid contamination.
- Performance of the genesig® COVID-19 3G assay is dependent on the amount and quality of RNA purified from samples. This study has been validated for recovery and purity of RNA for use with the exsig® Mag extraction kit using KingFisher™ Flex Purification System.

12 Assay Set-up

12.1 Sample extraction procedure

The genesig® COVID-19 3G assay results are dependent upon the amount and quality of template RNA purified from samples.

- Consult the IFU of the extraction system for full usage details.
- Prepare at least 1 NEC each time extraction is performed (i.e. an extraction with no clinical sample added). This NEC is used to check for contamination during the extraction stage.
- The genesig® COVID-19 3G IEC should be resuspended in a 1000 µl Template Preparation Buffer. It should be incorporated in the extraction as directed by the extraction system IFU. Primerdesign™ recommends 20 µl is added per sample, directly into the lysis stage of the extraction.
- The IEC should not be added directly to the clinical sample before RNA extraction (i.e. not before the clinical sample is mixed with a lysis buffer of the nucleic acid extraction kit/system). Doing so may compromise testing.
- Where the IFU provides no specific guidance for the addition of an IEC or where an automated system does not support the addition of 20 µl IEC, please contact Primerdesign™ for guidance.

12.2 Mastermix Set-up

a) Resuspend the dried primer/probe in 110 µl (per each vial) of Template Preparation Buffer, vortex to mix.

b) Resuspend the OneStep Lyophilised Mastermix in 525 µl Mastermix Resuspension Buffer, gently swirl to mix.

c) Plate set-up configuration can vary with the number of samples. An NEC must be included in each plate set-up (refer to **Section 10.5 and 10.6** on how to prepare NEC). An NTC and PCT should be included in each plate set-up.

    - The PCT will be added after all other reagents and samples have been added to the plate.
    - This will be an area for handling nucleic acid and away from the NEC, NTC and any clinical samples.
    - This is to prevent plate set-up, reagent, or sample contamination with the PCT.

d) Determine the number of reactions (n) to set-up per assay (including NEC, PCT and any NTCs for each plate). It is necessary to make an excess reaction mix to allow for

pipetting error. Use the following guide to determine volume of reagents to add to the reaction mix:

- If number of samples (n) is ≤ 10, then N = n+1
- If number of samples (n) is > 10 and ≤ 20, then N = n+2
- If number of samples (n) is > 20, then N = n+ 10% of total number of samples

e) Prepare a reaction mix of the following reagents from resuspended components in a 1.5 ml RNase/DNase free tube:

Reaction Mix Component	1 x Volume Required (µl)
OneStep Lyophilised Mastermix	10*
genesig® COVID-19 3G primer/probe mix (including Internal Control)	2*

*Multiply all numbers by (N). Refer to step (d) above to ensure there is a sufficient reaction mix for all samples, NEC, PCT and NTCs to be tested.

f) Add the 12 µl into the number of wells required for your testing in an appropriate PCR plate for your chosen PCR platform. Reserve one well each for the PCT, NEC and NTC for every PCR plate.

g) Add 8 µl of the following into the appropriate wells according to your plate set-up:
   a. NEC (please refer to **Sections 10.5**)
   b. NTC (please refer to **Sections 10.6**)

h) Cover the entire reaction plate and move the reaction plate to the nucleic acid handling area.

i) Gently vortex nucleic acid sample tubes for approximately 5 seconds.

j) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.

k) Change gloves often to avoid contamination.

l) Add 8 µl of the RNA/nucleic acid extracted from clinical specimen/sample(s) into the appropriate wells according to your plate set-up.

m) Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

n) Add 8µl of PCT into the appropriate well according to your plate set up. Seal the plate with an appropriate seal and place it in the instrument.

12.3 Programming of the Real-Time PCR Instrument

Please refer to one of the following manuals for additional information on using the instrument:

- Bio-Rad CFX Opus Real-Time PCR Instrument Guide https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000119983.pdf
- Applied Biosystems® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (as per Applied Biosystems manual (2010)).
- genesig® q32 Instrument Guide (2020) software version 1.2.2

Enter the following amplification program:

Stage	Steps	Time	Temperature	Cycles	Detection Format
Hold	Reverse Transcription	10 min	55°C	1	FAM (465-510) HEX/VIC (533-580) ROX (533-610 Cy5 (618-660)
	Initial Denaturation (Taq Activation)	2 min	95°C	1	
Cycling	Denaturation	10 sec	95°C	45	
	Annealing and Extension	60 sec	60°C		

13 Interpretation of Results

13.1 Acceptance criteria of controls included in the genesig® COVID-19 3G assay

Before interpreting sample results, it is necessary to verify the success of the run. If the following criteria are not satisfied, then testing needs to be repeated:

a) NTC is free from amplification in all channels.
b) NEC produces positive amplification in the HEX/VIC (533-580) channel (this is the detection of the genesig® COVID-19 3G RNA IEC).
c) PCT produces a Cq of between 14-22 in the FAM (465-510), ROX (533-610) and Cy5 (618-660) channels for ORF1ab, M gene and S gene, respectively.

For instrument specific guidance on correctly assigning Cq values, follow manufacture instructions.

Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

13.2 Interpretation of Patient Sample Results

If all the control acceptance criteria are fulfilled, then each sample can be assessed with the following metric:

SARS CoV-2 Targets			IEC	Result †
ORF1ab FAM (465-510)	M gene ROX (533-610)	S gene Cy5 (618-660)	HEX/VIC (533-580)	
Cq (+)	Cq (+)	Cq (+)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (+)	Cq (+)	Cq (-)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (+)	Cq (-)	Cq (+)	Cq (+) / (-)	Secondary test required**
Cq (-)	Cq (+)	Cq (+)	Cq (+) / (-)	Secondary test required**
Cq (+)	Cq (-)	Cq (-)	Cq (+) / (-)	Secondary test required**
Cq (-)	Cq (+)	Cq (-)	Cq (+) / (-)	Secondary test required**
Cq (-)	Cq (-)	Cq (+)	Cq (+) / (-)	Secondary test required**
Cq (-)	Cq (-)	Cq (-)	Cq (+)	SARS-CoV-2 Negative***
Cq (-)	Cq (-)	Cq (-)	Cq (-)	Result invalid, repeat testing of sample

*All instances of test sample amplification in the FAM and ROX channels indicate a SARS-CoV-2 positive sample. Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

**If there is no amplification in the FAM, ROX and Cy5 channels for a test sample, to confirm the result is valid as SARS-CoV-2 negative, there should be amplification in the HEX/VIC channel. This confirms the PCR run is valid and the genesig® COVID-19 3G IEC added to the test sample during the RNA extraction process has been detected. The following acceptance criteria should be applied for FAM, ROX and Cy5 negative samples:

- The IEC Cq value produced by the patient sample should be < 36 and should not exceed the NEC IEC Cq value + 6, i.e. sample RNA IEC Cq 28 NEC RNA IEC Cq 34. Failure to satisfy this criterion indicates a compromised sample extraction and an invalid result; testing of the sample must be repeated.

14 Limitations of The Procedure

- The procedures in this IFU must be followed as described. Any deviations may result in assay failure or erroneous results.
- Good laboratory practice is required to ensure the performance of the kit. 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.
- As with any molecular test, mutations within the target sequence of SARS-CoV-2 could affect the genesig® COVID-19 3G primer and/or probe binding, resulting in failure to detect the presence of the virus.
- 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 IFU.
    - Use of unauthorised 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 the patients' medical history and clinical symptoms.
- This test cannot rule out infections caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of SARS-CoV-2 infection.

15 Performance Evaluation

The genesig® COVID-19 3G assay performance evaluation was performed on the CFX Opus Real-Time PCR instrument (Bio-Rad). A set of additional testing at the LoD level was performed on the Applied Biosystems® 7500 Real-Time PCR instrument (Thermofisher), Lightcycler 480 II (Roche) and genesig® q32 (Primerdesign, Novacyt) instruments for analytical sensitivity. Saliva samples, negative for SARS-CoV-2 were extracted using the KingFisher™ Flex Purification System in conjunction with the exsig™ Mag extraction kit.

15.1 Analytical Sensitivity

The limit of detection (LoD) is defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. Briefly, samples were contrived in the lysis stage of the extraction with SARS-CoV-2 RNA provided by Twist BioScience™. The tentative LoD was tested at 3 contrivance levels: 20, 10 and 5 copies/reaction in the final PCR reaction. Each contrivance level was tested on 5 replicates for tentative LoD using the CXF Opus Real-Time PCR (Bio-Rad). The LoD of an assay was considered if all targets reached 95% confident, i.e. ORF1ab, M and S genes, respectively.

15.1.1 Verification of the LoD

Once the tentative LoD was established (95% positive call rate) for all targets, it was verified by testing the samples and contrivance in the same way as the tentative assay. Contrivance was diluted to required levels around the tentative LoD at each assay, giving a total of 20 replicates per target.

Results for the analytical sensitivity study using CFX Opus Real-Time PCT instrument:

Target concentrations/replicates				ORF1ab (FAM)		M gene (ROX)		S gene (Cy5)	
Initial conc. Of Twist used (copies/µl)	Conc. of Twist in the PCR reaction (copies/rxn)	Conc. of Twist in the PCR reaction (copies/µl)	Total replicates	Detection rate (%)	Mean Cq (STDV)	Detection rate (%)	Mean Cq (STDV)	Detection rate (%)	Mean Cq (STDV)
187.5	30	1.5	20	100%	32.1 (1.2)	100%	33.3 (1.6)	100%	31.5 (1.2)
125.0	20	1	20	100%	32.6 (0.8)	100%	33.8 (1.2)	100%	32.4 (0.2)
62.5	10	0.5	20	100%	33.4 (0.7)	85%	34.4 (1.1)	100%	33.1 (0.8)
31.3	5	0.25	20	95%	34.3 (0.8)	85%	34.2 (0.3)	100%	33.5 (0.6)

This data above demonstrates that the genesig® COVID-19 3G assay detects 1 copies/µl of SARS-CoV-2 whole viral genome RNA ≥95% across all samples. This is therefore the limit of detection of the assay.

15.1.2 Alternative Instrument Testing

The LoD was further confirmed by testing on three other PCR platforms: Applied Biosystems® 7500 Real-Time PCR Instrument (Thermofisher), Lightcycler 480 II (Roche) and genesig® q32 Real-Time PCR Instrument (Primerdesign, Novacyt). The LoD for each platform was determined as the copies/µl in the contrivance level which produced a 95% call rate. Overall, the genesig® COVID-19 3G assay is defined as 1 copies/µl, or 1000 copies/ml in the PCR reaction. The LoD was calculated using SARS-CoV-2 whole genome RNA provided by Twist BioScience®. The results are summarised below:

genesig ® COVID-19 3G - ORF1ab (FAM)					
PCR Instrument	Conc. of Twist in the PCR reaction (copies/rxn)	Conc. of Twist in the PCR reaction (copies/µl))	Positive Calls (%)	Positive calls/Total no. results included on analysis	Mean Cq (STDV)
CFX Opus Real-Time PCR (Bio-Rad)	5	0.25	100	19/20	34.3 (0.8)
Lightcycler 480 Instrument (Roche)	5	0.25	100	20/20	34.9 (0.5)
Applied Biosysterms® ABI 7500 Real-Time PCR System (Thermofisher)	10	0.5	100	20/20	35.3 (1.3)
genesig® q32	5	0.25	95	19/20	34.6 (0.8)

genesig® COVID-19 3G - M gene (ROX)					
PCR Instrument	Conc. of Twist in the PCR reaction (copies/rxn)	Conc. of Twist in the PCR reaction (copies/µl))	Positive Calls (%)	Positive calls/Total no. results included on analysis	Mean Cq (STDV)
CFX Opus Real-Time PCR (Bio-Rad)	20	1	100	20/20	33.8 (1.2)
Lightcycler 480 Instrument (Roche)	20	1	100	19/20	33.5 (0.7)
Applied Biosysterms® ABI 7500 Real-Time PCR System (Thermofisher)	20	1	100	19/20	34.0 (1.5)
genesig® q32	20	1	100	20/20	34.0 (1.4)

genesig® COVID-19 3G - S gene (Cy5)					
PCR Instrument	Conc. of Twist in the PCR reaction (copies/rxn)	Conc. of Twist in the PCR reaction (copies/µl))	Positive Calls (%)	Positive calls/Total no. results included on analysis	Mean Cq (STDV)
CFX Opus Real-Time PCR (Bio-Rad)	**5**	**0.25**	**100**	**20/20**	**33.5 (0.6)**
Lightcycler 480 Instrument (Roche)	**10**	**0.5**	**100**	**19/20**	**33.4 (0.7)**
Applied Biosysterms® ABI 7500 Real-Time PCR System (Thermofisher)	**10**	**0.5**	**100**	**19/20**	**34.8 (1.7)**
genesig® q32	**10**	**0.5**	**100**	**20/20**	**33.3 (1.0)**

15.2. Accuracy

Diagnostic accuracy of the genesig® COVID-19 3G assay was determined by generating a Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA). Samples were tested blind with genesig® COVID-19 3G and compared with the contrivance status (30 positive vs 30 negative) to produce the percentage agreements.

Alongside the genesig® COVID-19 3G accuracy study, a comparison study was performed between genesig® COVID-19 3G and an alternative COVID-19 assay: genesig® COVID-19 (CE-IVD). The PPA, NPA and OPA of each kit was calculated and compared to the alternative kit. Briefly, 60 negatives for SARS-CoV-2 saliva samples were collected from 5 donors and extracted with the KingFisher™ Flex Purification System in conjunction with exsig™ Mag Extraction System. 30 samples were contrived at 5x the LoD, as defined in Analytical Sensitivity. Samples were contrived with synthetic SARS-CoV-2 RNA provided by Twist BioScience™. The remaining 30 samples were not contrived and remained negative. The below tables show the result summary:

genesig® COVID-19 3G: Results for the blind contrivance accuracy study using genesig® COVID-19 3G.

		Randomised contrived samples		
		Positive	Negative	Total
Candidate Method (genesig® COVID-19 3G assay)	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60

Agreement	Level
OPA	100%
PPA	100%
NPA	100%

genesig® COVID-19 (CE-IVD): Result for the blind contrivance accuracy study using PROmate™ COVID-19 (CE-IVD).

		Randomised contrived samples		
		Positive	**Negative**	**Total**
Comparative Method (genesig® COVID-19 1G assay)	**Positive**	30	3	33
	Negative	0	27	27
	Total	30	30	60

Agreement	Level
OPA	100%
PPA	100%
NPA	100%

15.3. Analytical Specificity

The objective of this study is to assess the Analytical Specificity, i.e. inclusivity and exclusivity for the genesig® COVID-19 3G assay. Exclusivity (cross-reactivity) was assessed by two methods. The first was via comprehensive *in silico* analysis, and the second was to 'wet' test inactivated viruses and bacteria from related organisms using the genesig® COVID-19 3G assay. The *in silico* analysis also evaluated assay inclusivity.

15.3.1 Latest in silico Specificity Analysis

To ensure the COVID-19 primer/probe remain specific to detect SARS-CoV-2 genomes, Primerdesign's Bioinformaticians review daily the SARS-CoV-2 sequence submissions on the GISAID EpiCoV database. As of 25th of March 2021, in silico analysis confirms the COVID-19 assay primers and probe still show 99.9% and 99.8% detection with the 685,681 and 685,682 full length, good quality SARS-CoV-2 sequences at ORF1ab and S gene respectively, as published on the GISAID EpiCoV database.

15.3.2 Wet testing

Related pathogens and pathogens that are likely to be present in the clinical sample have been evaluated *in silico* to identify the homology between the primer/probe of the assay and the pathogens. Upon *in silico* analysis, the genesig® COVID-19 3G the data presented in this report demonstrates that the genesig® COVID-19 3G assay exhibits no cross reactivity with any of the panel members chosen for this study. None of the Coronavirus strains were detected in the Qnostics and Zeptometrix panels, whereas extracted SARS-CoV-2 strain was detected across all tested genesig® COVID-19 3G tubes in appropriate channels. Overall, this data confirms that the genesig® COVID-19 3G assay maintains the expected inclusivity and exclusivity criteria outlined in the study's Design Inputs.

In vitro testing:

For in vitro testing, 5 panels were sourced:
NATtrol™ Pneumonia panel (ZeptoMetrix)
NATrol™ Coronavirus-SARS Stock (ZeptoMetrix)
Respiratory Evaluation Panel (Qnostics, Scotland, UK)
QCMD from the 2019 Coronavirus EQA programme (Qnostics)
QCMD from the 2019 MERS Coronavirus EQA Programme (Qnostics)

The samples from these panels are representative of true clinical samples and evaluated by the genesig® COVID-19 3G Real-Time PCR assay. The results of the *in vitro* cross-reactivity testing are presented below:

		genesig® COVID-19 3G Cq			
Sample number	Panel member	FAM	HEX/VIC	ROX	Cy5
1	A.baumannii	N/A	25.5	N/A	N/A
2	E.cloacae	N/A	21.5	N/A	N/A
3	E. coli	N/A	22.4	N/A	N/A
4	H.influenzae	N/A	24.5	N/A	N/A
5	K.oxytoca	N/A	22.7	N/A	N/A
6	P.aeruginosa	N/A	23.3	N/A	N/A
7	P.mirabilis	N/A	23.9	N/A	N/A
8	S.agalactiae	N/A	23.8	N/A	N/A
9	S.marcescens	N/A	19.6	N/A	N/A
10	S.pneumoniae	N/A	24.0	N/A	N/A
11	S.pyogenes	N/A	24.9	N/A	N/A
12	K.pneumoniae	N/A	24.3	N/A	N/A
13	K.pneumoniae	N/A	23.7	N/A	N/A
14	K.pneumoniae	N/A	23.6	N/A	N/A
15	Coronavirus-SARS	N/A	24.5	N/A	N/A
16	INF A H1N1	N/A	23.9	N/A	N/A
17	INF A H3N2	N/A	24.4	N/A	N/A
18	INF B Victoria	N/A	23.9	N/A	N/A
19	INF B Yamagata	N/A	24.1	N/A	N/A
20	RSV A	N/A	24.1	N/A	N/A
21	RSV B	N/A	23.4	N/A	N/A
22	Coronavirus- NL63	N/A	24.1	N/A	N/A
23	Coronavirus- 229E	N/A	23.8	N/A	N/A
24	Coronavirus- HKU	N/A	24.6	N/A	N/A
25	Coronavirus- OC48	N/A	24.0	N/A	N/A
26	MERS Coronavirus	N/A	23.9	N/A	N/A
Positive extraction control	Extracted SARS-CoV-2 Medium Q Control (mean)	31.2	24.8	29.6	28.2
PCT	genesig® COVID-19 3G Positive control	17.2	NA	15.2	14.7
NTC	NTC	N/A	N/A	N/A	N/A

16. Disposal

Dispose of unused kit reagents, clinical samples, and sealed post-amplification plates as laboratory clinical waste according to local, state and federal regulations. Refer to **Section 6** for guidance weblinks.

17. Technical Support

For Technical support, please contact our dedicated technical support team on:

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

18. Explanation of Symbols

Symbol	Explanation
	In vitro diagnostics
	Manufacturer
	Catalogue number
	Sufficient for number of tests
	Use by Date
	Temperature limit
	Consult Electronic Instructions for Use
	Batch Code
	Keep away from sunlight (primer/probe mix)
	Positive Control

20. References

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