Primerdesign™ Ltd

Primerdesign Ltd

genesig®

COVID-19 3G

HT Assay

CE IVD

Instructions for Use (IFU)

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GENESIG

Kits by Primerdesign

genesig® Coronavirus (COVID-19) 3G HT Assay

In vitro Real-Time PCR diagnostic test for the detection of SARS-CoV-2 in human samples

Validated For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	exsig® Mag extraction kit	Applied Biosystem® 7500 Fast (Thermofisher) CFX Opus (Bio-Rad®) Roche® LightCycler 480 II genesig® q32 (Primerdesign™, Novacyt)
Oropharyngeal Swabs		
Saliva		

1536 tests

IVD

CE

REF Catalogue number: D00062

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1. Intended Use

The High Throughput genesig® Real-Time PCR COVID-19 3G HT (CE IVD) assay is an *in vitro* diagnostic real-time reverse transcriptase PCR (RT-PCR) assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs and saliva specimens. The assay provides rapid screening of individuals suspected of SARS-CoV-2 infection and aids the diagnosis of suspected COVID-19 disease in patients.

The genesig® Real-Time PCR COVID-19 3G HT (CE IVD) 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.

2. Summary and Explanation

The COVID-19 pandemic was caused by a coronavirus named SARS-CoV-2. The first human cases were identified in Wuhan, China and had reported onset of symptoms around 1st 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 HT (CE-IVD) assay has been developed to target three genes to ensure the accuracy of the genesig® assay.

3. Principles of the Procedure

For optimum product performance and sensitivity, genesig® Coronavirus (COVID-19) 3G HT (CE IVD) should be used with RNA which is isolated and purified from nasopharyngeal swabs, oropharyngeal swabs or saliva using an appropriate 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 HT (CE-IVD) 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 and probe to amplify and detect the RNA Internal Extraction Control (IEC) template in the genesig® COVID-19-3G HT kit. The IEC specific probe is labelled with the HEX/VIC fluorophore. The genesig® COVID-19-3G HT RNA IEC template is not related to the SARS-CoV-2 viral sequence. The genesig® COVID-19-3G HT (CE-IVD) assay channel allocations are described in the table below.

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

4. Materials Provided

The genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay consists of 3 individual packs of components. Each pack is designed to be used independently for each stage of the Real-Time PCR set-up. Pack 1 consists of the reagents for the Real-Time PCR reaction mix, Pack 2 consists of the reagents for the COVID-19 positive and negative controls, and Pack 3 contains the RNA Internal extraction control (IEC) which should be added during specimen RNA extraction (refer to Section 14.1):

Reagent label	Number of Vials 1536 tests	Volume (ml per vial)	Lid colour	Resuspended with?
Pack 1				
OneStep HT 2X Mastermix	1*	19	Orange	n/a
COVID-19 3Gprimer/probe mix (including IEC primer/probe mix)	1*	3.8	Green	n/a
Pack 2				
genesig® COVID-19 3G Positive Control Template	1	0.8**	Red	Template Preparation Buffer
Template Preparation Buffer	1	1.5	Yellow	n/a
Water DNase/RNase Free	1	1.5	Clear or white	n/a
Pack 3				
genesig® RNA Internal Extraction Control (IEC)	16*	2.6	Green	n/a

*Sterile DNase/RNase free falcon tubes
** The projected volume once resuspended.

The COVID-19 primer/probe mix contains the primers and FAM, ROX and Cy5 labelled probes specific to SARS-CoV-2, as well as the primers and HEX/VIC labelled probe specific to the genesig® RNA IEC.

The genesig® COVID-19 3G Positive control template is provided lyophilised. The table above indicates which buffer to use, as well as the volume to add, to resuspend the positive control template prior to use.

Pack 1, 2 and 3 are shipped frozen on dry ice and must be stored at -20°C upon receipt. Refer to Section 10 for storage, handling, and stability of reagents.

5. Required Equipment and Consumables (Not Provided)

- PCR hood
- Benchtop microcentrifuge
- Vortex mixer
- 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)
- DNA/RNA remover
- 384-well and 96-well Real-Time PCR reaction plates, e.g. White Roche® LightCycler Multiwell plate
- Plate seal
- PCR molecular grade RNase/DNase free water

6. Real-Time PCR instruments

The genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay has been validated for use with the CFX Opus (Bio-Rad®), Applied Biosystems® ABI 7500 Fast Real-Time PCR System (Thermofisher®), Roche® LightCycler 480 II Real-Time PCR instrument and genesig® q32 (Primerdesign™, Novacyt).

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

7. Extraction Kits/Instruments

The genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay has been validated using the exsig® Mag extraction kit (Primerdesign™ Ltd).

Please consult the IFU of the chosen Extraction System for full usage details.

8. 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

9. Warnings and Precautions

9.1. General

- For *in vitro* diagnostic use (IVD) only.
- Handle all specimens as if infectious using safe laboratory procedures. Specimen 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) biological safety cabinet (refer to the guidance detailed in Section 8).
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use PPE such as (but not limited) 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® Coronavirus (COVID-19) 3G HT (CE IVD) assay primers and probes and IEC are supplied in a buffer that contains Ethylenediaminetetraacetic Acid (EDTA). These components should be handled according to the SDS. In the event of damage to protective packaging, contact Primerdesign™ for instructions.

9.2. Preventing Contamination

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by the amplification product (amplicon).

- The genesig® COVID-19 3G positive control template is provided in a sealed foil envelope and contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.

- Maintain separate areas for handling of specimen preparation, pre-PCR assay set-up, and post-PCR amplified nucleic acids.

- Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen 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.

- Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.

- Change aerosol barrier pipette tips between all manual liquid transfers.
- During 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.
- 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. DNA/RNA remover, ethanol, 10% bleach) to minimise risk of nucleic acid contamination.
- RNA samples and the RNA control should be maintained on a cold block or on ice during preparation and used to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the PCR plate seal is not broken.
- Dispose of unused kit reagents and human specimens according to national regulations (refer to guidance detailed in Section 8).

9.3. Prevent DNase/RNase contamination

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

10. Reagent Storage, Handling and Stability Conditions

10.1. Storage conditions

- Pack 1, 2 and 3 are shipped in one box, frozen on dry ice and must be stored at -20°C upon receipt. The reagents can be stored for up to 6 months at -20°C or until the expiry date is reached, whichever occurs first, after which the kit should be discarded. Refer to the guidance detailed in Section 8. Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.

- If the kit's protective packaging is damaged upon receipt, please contact Primerdesign™ for instructions. Attention should be paid to the "use by" date specified on the pack label and individual tube labels. On this date, the kit should be discarded following the guidance detailed in Section 8.

- Always check the expiration date prior to use. Do not use expired reagents.

- The genesig® COVID-19 3G positive control template is 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.

- It should be noted that the Onestep HT 2X Master Mix and the primers/probe should not be mixed before setting up the PCR and that both components should be stored separately. A new mixture of the Master Mix and primers/probe should be made each time a new PCR test is conducted.

10.2. In Use Stability

- Primer/probe mix, positive control template, RNA IEC and OneStep HT 2X Master Mix are stable for up to 6 months when stored **individually** at -20°C.

- The kit should not be used past the "use by" date as indicated on the pack label and individual tube labels.

- When *in use*, the time the kit components are stored at room temperature should be minimised.

11. Specimen Collection, Handling and Storage

11.1. Compatible Samples

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

11.2. Collecting the Specimen

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 specimen collection, storage and transport are likely to yield false test results, for more information, refer to Section 8.

11.3. Transporting Specimens

Specimens 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 specimens.

11.4. Storing Specimens

- Extracted nucleic acid should be stored at -70ºC or lower.
- Refer to Section 8 weblinks for guidance.

12. Reagent and Controls Preparation

12.1. COVID-19 3G Primers/probe mix

- **Precautions: this reagent should only be handled in a clean area and not exposed to light.**
- Primers/probe mix is stable for up to 6 months when stored at -20ºC.
- Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
- The OneStep HT 2X Master Mix and the Primers/Probe mix should not be mixed before setting up the PCR and that both components should be storage separately.

12.2. genesig® COVID-19 3G Positive Control Template preparation

- The genesig® COVID-19 3G Positive Control Template (PCT) is provided in a sealed foil envelope and contains a high copy number of the template. It should be opened and processed away from clinical specimens and kit components to avoid cross-contamination.
- The PCT tube contains synthetic DNA representing the SARS-CoV-2 genomic region of interest. Following resuspension, this will be at a concentration of $1.25x10^5$ copies per µl. This yields a total of 10^6 copies per reaction in the PCR tube.
- Caution: This reagent contains a high copy number of positive control material and should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination of other kit reagents and clinical specimens.
- Upon receipt, the dried PCT can be stored at -20ºC for up to 6 months or until the expiry date is reached, whichever occurs first.
- Using aseptic technique, resuspend the dried PCT in 800 µl of Template Preparation Buffer, pulse vortex to mix. Resuspended PCT is stable for up to 6 months when stored at -20ºC.
- The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC. Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
- To ensure PCR run validity, the PCT should produce amplification in the FAM, ROX and Cy5 channels.

12.3. genesig® RNA Internal extraction control (IEC) preparation

- The genesig® RNA Internal extraction control (IEC) can be added to the nucleic acid extraction system (not provided) to provide an RNA template control, detect PCR inhibition and confirm the integrity of the PCR run.
- Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination.

- Upon receipt, the IEC can be stored at -20ºC for up to 6 months or until the expiry date is reached, whichever occurs first.

- The reagent can be stored at -20ºC. Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.

12.4. Negative Extraction Control (NEC) preparation

- Prepare at least 1 negative extraction control (NEC) each time RNA is extracted from a clinical specimen or sample.

- The NEC is an extraction with no clinical specimen/sample added, it is prepared by extracting RNase/DNase free water. The genesig® RNA Internal extraction control is added to the NEC sample during extraction as directed by the manufacturer's IFU. This NEC will serve as the negative control for the entire testing system and the detection of contamination during PCR plate set-up.

12.5. No Template Control (NTC)

- DNase/RNase free water is provided to use as a No Template control (NTC) if required in addition to the NEC (refer to Section 12.4)

- The NTC is used to check for contamination during PCR plate set-up.

13. 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 and DNA/RNA remover, to minimise the risk of nucleic acid contamination.

- Performance of the genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay is dependent upon the amount and quality of RNA purified from samples. The genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay has been validated for recovery and purity of RNA using the exsig® Mag extraction kit in conjunction with the KingFisher™ Flex Purification System.

14. Assay Set Up

14.1. RNA Extraction

The genesig® COVID-19 3G HT assay results are dependent upon the amount and quality of template RNA purified from samples. If performing a nucleic acid purification step prior to RT-PCT, follow the steps below:

a. Consult the IFU of the extraction system for full usage details.

b. 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.

c. The genesig® COVID-19 IEC is supplied in a wet format. It should be incorporated in the extraction as directed by the extraction system IFU. Primerdesign™ recommends 20µl is added per sample, directly into the lysis stage of the extraction.

d. The IEC **should not** be added directly to the clinical samples before RNA extraction, i.e., not before the clinical samples is mixed with a lysis buffer of the nucleic acid extraction kit. Doing so may compromise testing.

e. Where the IFU provides no specific guidance for the addition of an IEC or where an automate system does not support the addition of 20µl IEC, please contact Primerdesign™ for guidance.

14.2. Mastermix Set-up

Plate set-up configuration can vary with the number of samples. A NEC must be included in each plate set-up (refer to **Sections 12.4 and 14.1** on how to prepare NEC). NTCs should be included in each plate set-up. A PCT well must be included in each plate set-up.

f. The genesig® COVID-19 3G PCT will be added after all other reagents and samples have been added to the plate. This will be in an area for handling nucleic acid and away from the NEC, NTC and any clinical specimen/samples. This is to prevent plate set-up, reagent, and specimen contamination with the PCT. Resuspend the genesig® COVID-19 3G PCT in 800 µl of Template Preparation Buffer as detailed in **Section 4**.

g. Briefly centrifuge the OneStep HT 2X Mastermix and Primers/probe mix in pack 1.

h. Calculate the amount of Master Mix and Primers/Probe required for PCR set-up and mix the reagents into a sterile falcon tube and mix thoroughly by pulse vortexing. The OneStep HT 2X Mastermix and Primers/Probe mix should not be mixed before setting up the PCR and that both components should be storage separately.

Reaction mix Component	Volume required/sample (µl)
OneStep HT 2X Master Mix	10
Primers & Probe mix	2

i. Dispense 12µl of this reaction mix into the number of wells required, in an appropriate PCR plate. Include 1 well for the PCT, 1 well for the NEC and 1 well for the NTC for each PCR plate.

j. Add 8µl of the following into the appropriate wells according to your plate set-up.

k. NEC (please refer to **Sections 12.4 and 14.1**)

l. NTC (please refer to **Section 12.5**)

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

n. Gently vortex nucleic acid sample tubes for approximately 5 seconds.

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

p. Change gloves often to avoid contamination.

q. Add 8µl of PCT (please refer to **Section 12.2**) into the appropriate well according to your plate set up. Seal the plate with an appropriate seal.

r. Briefly vortex the plate and centrifuge for 10 seconds to collect contents at the bottom of the tube before placing it in the Real-Time PCR instrument.

14.3. Programming 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

a) Enter the following amplification program:

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

*Acquisition must be performed at the end of this stage. When using Roche® LightCycler 480 II please select the following detection format: *Dual Color Hydrolysis Probe / UPL Probe*. *When*

using the ABI 7500 please select 'none' for the dye to use as a passive reference dye in the plate set up.

b) Ensure wells loaded with clinical sample(s) are designated as "Sample Type – Unknown"; the software will automatically calculate quantities for these wells if amplification occurs.

c) Ensure the well loaded with PCT is designated as "Sample Type - Standard" and assigned the appropriate concentration (see **Section 12.2**)

15. Interpretation of Results

15.1. Acceptance criteria of controls included in the genesig® Coronavirus (COVID-19) 3G HT (CE IVD) 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 amplification is Cq>35 in all channels.

b. NEC produces positive amplification Cq<35 in the HEX/VIC (533-580) channel (this is the detection of the 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.

15.2. 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 (+) / (-)	SARS-CoV-2 Positive*
Cq (-)	Cq (+)	Cq (+)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (+)	Cq (-)	Cq (-)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (-)	Cq (+)	Cq (-)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (-)	Cq (-)	Cq (+)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (-)	Cq (-)	Cq (-)	Cq (+)	SARS-CoV-2 Negative**
Cq (-)	Cq (-)	Cq (-)	Cq (-)	Result invalid, repeat testing of sample

*All instances of SARS-CoV-2 target amplification indicate a SARS-CoV-2 positive sample. Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification. All results should be interpreted by a healthcare professional in the context of patient medical history and clinical samples.

**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., if the NEC IEC Cq 28 then the sample 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.

16. Limitations of The Procedure

- The procedures in this IFU 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. 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 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 handbook.
    - 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 healthcare 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 SARS-CoV-2 infection.

17. Performance Evaluation

The genesig® COVID-19 3G HT 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® ABI 7500 Fast Real-Time PCR System (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.

17.1. Analytical Sensitivity

17.1.1 Tentative of the LoD

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 5 contrivance levels: 30, 25, 20, 15 and 10 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.

17.1.2 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 PCR 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%	33.89 (0.47)	100%	36.46 (0.89)	100%	34.05 (0.50)
156.3	25	1.25	20	100%	34.32 (0.48)	95%	36.95 (0.93)	100%	35.01 (0.93)
125.0	20	1.0	20	100%	34.77 (0.96)	95%	37.33 (1.23)	100%	34.87 (1.04)
93.8	15	0.75	20	95%	35.44 (1.05)	50%	38.32 (2.37)	90%	35.83 (1.02)
62.5	10	0.5	20	100%	35.32 (0.95)	80%	37.86 (1.02)	90%	35.93 (0.77)

The data above demonstrates that the limit of detection for the genesig® COVID-19 3G HT assay is 1 copy/ul of SARS-CoV-2 whole viral genome RNA ≥95% across all samples.

17.1.3 Alternative Instrument Testing

The LoD was further confirmed by testing on three other PCR platforms: Applied Biosystems® ABI 7500 Fast 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. In this bridging study, the LoD of 20 copies/reaction was tested alongside two other contrivance levels, i.e., 30 and 10 copies/reaction in the final PCR reaction. Overall, the bridging study demonstrates a consistent detection rate of 1 copy/µl, or 1000 copies/ml across all four PCR platforms. The LoD was calculated using SARS-CoV-2 whole-genome RNA provided by Twist BioScience. The results are summarised below:

genesig ® COVID-19 3G HT- 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®)	10	0.5	100	20/20	35.32 (0.95)
Lightcycler 480 Instrument (Roche®)	10	0.5	95	19/20	32.65 (0.7)
Applied Biosystems® ABI 7500 Fast Real-Time PCR System (Thermofisher™)	10	0.5	100	20/20	35.72 (0.6)
genesig® q32 (Primerdesign™, Novacyt)	20	1.0	100	20/20	31.99 (0.6)

genesig ® COVID-19 3G HT- 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	1.0	95	19/20	37.33 (1.23)
Lightcycler 480 Instrument (Roche®)	20	1.0	100	20/20	33.78 (0.3)
Applied Biosystems® ABI 7500 Fast Real-Time PCR System (Thermofisher™)	20	1.0	100	20/20	34.62 (0.4)
genesig® q32 (Primerdesign™, Novacyt)	20	1.0	100	20/20	33.11 (0.3)

genesig® COVID-19 3G HT- 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®)	20	1.0	95	19/20	34.87 (1.04)
Lightcycler 480 Instrument (Roche®)	20	1.0	100	20/20	32.76 (0.3)
Applied Biosystems® ABI 7500 Fast Real-Time PCR System (Thermofisher™)	10	0.5	100	20/20	34.36 (0.6)
genesig® q32 (Primerdesign™, Novacyt)	10	0.5	100	20/20	32.55 (0.5)

17.2. Accuracy

Diagnostic accuracy of the genesig® COVID-19 3G HT 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 HT and compared with the contrivance status (28 positive and 28 negative) to produce the percentage agreements.

Alongside the genesig® COVID-19 3G HT accuracy study, a comparison study was performed between genesig® COVID-19 3G HT and an alternative COVID-19 assay: genesig® COVID-19 HT (CE-IVD). The PPA, NPA and OPA of each kit were calculated and compared to the alternative kit. Briefly, 56 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. 28 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 28 samples were not contrived and remained negative. Overall, the results show that the genesig® COVID-19 3G HT assay meets the performance criteria, obtaining OPA, PPA and NPA above 90% for all gene targets. The below tables show the result summary:

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

		Randomised contrived samples		
		Positive	Negative	Total
Candidate Method (genesig® COVID-19 3G HT assay)	Positive	28	0	28
	Negative	0	28	28
	Total	28	28	56

Agreement	Level
OPA	100%
PPA	100%
NPA	100%

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

		Randomised contrived samples		
		Positive	Negative	Total
Comparative Method (genesig® COVID-19 HT assay)	Positive	28	0	28
	Negative	0	28	28
	Total	28	28	56

Agreement	Level
OPA	100%
PPA	100%
NPA	100%

17.3. Analytical Specificity

This study aims to assess the Analytical Specificity, i.e., inclusivity and exclusivity for the genesig® COVID-19 3G HT assay. Two methods assessed exclusivity (cross-reactivity). 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 HT assay. In addition, the *in silico* analysis also evaluated assay inclusivity.

17.3.1 Inclusivity

To ensure the COVID-19 primer/probe remains specific to detect SARS-CoV-2 genomes, Primerdesign's™ Bioinformaticians review the SARS-CoV-2 sequence submissions daily 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 lengths, good quality SARS-CoV-2 sequences at ORF1ab and S gene, respectively, as published on the GISAID EpiCoV database.

17.3.2 Exclusivity

Related pathogens and pathogens that are likely to be present in the clinical sample have been evaluated as part of genesig® COVID-19 3G assay analytical specificity to identify the homology between the primer/probe of the assay and the pathogens. These include:

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)

Upon wet testing, the data demonstrates that the 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 tubes in appropriate channels. Overall, this data confirms that the COVID-19 primer/probe maintains the expected inclusivity and exclusivity criteria outlined in the study's Design Inputs.

17.4. Interfering Substances

The effects of potential exogenous and endogenous interfering substances present within target saliva samples on the genesig® COVID-19 3G HT Real-Time PCR detection kit was assessed. The appropriate volume of the interfering substance at the relevant concentration (Table below) was then spiked into each saliva sample to make a total volume of 100µl. Changes in the assay's performance were analysed by Cq values of samples containing the potential interfering substances.

Each substance's results are listed alongside its relevant control's results. Overall, interfering substance samples gave positive amplification for each replicate and all controls passed the relevant acceptance criteria on both plates. The results from this study show that none of the interfering substances that were tested had any significant interference with the genesig® COVID-19 3G HT assay. Although one substance showed slight effect on one of the gene targets, it was not a homogenous interference on overall assay to be deemed a potential interfering substance. The results from the interfering substance screen can be seen in table below.

Table of Interfering Substances and concentrations.

Interfering Substance	Stock concentration	Tested concentration	% Volume of total sample	Relevant control
Blood (Haemoglobin)	Aqueous solution 10g/ml	0.2g/ml	5%	5% H_2O
Nasacort Allergy Nasal Spray (Triamcinolone acetonide)	Aqueous solution	10% v/v	10%	10% H_2O
Dymista Allergy Nasal Spray (Corticosteroids – Azelastine hydrochloride & Fluticasone)	Aqueous solution	6.85 mg/ml Az 2.5mg/ml Fl	5%	5% H_2O
Corticosteroid – Dexamethasone	Aqueous solution 30.6µmol/L	1.52µmol/L	5%	5% Ethanol
Corticosteroids – Fluticasone	Neat powder 200mg	0.1mg/ml	10%	10% Ethanol
Antiviral medication – Guaifenesin	Aqueous solution 0.9 mg/ml	0.9mg/ml*	5%	5% Ethanol
Antiviral medication – Oseltamivir	Aqueous solution 0.00798 mg/ml	0.00798 mg/ml*	5%	5% H_2O

Antibacterial medication – Mupirocin	100% ethanol solution 30µg/ml	5µg/ml	16.6%	16.6% Ethanol
Antibacterial eye drops – Tobramycin	Aqueous solution 0.6mg/ml	0.03mg/ml	5%	5% H_2O
Throat lozenge (Strepsils – 2,4-Dichlorobenzyl Alcohol, & Amylmetacresol)	Tablet 20mg	1% w/v	5%	5% H_2O
Mucin	Neat powder 100g	0.2mg/ml	5%	5% 1M NaOH
α-Amylase	100% ethanol solution 158.4mg/ml	7.92mg/ml	5%	5% H_2O
Oxymetazoline	Aqueous solution 0.0000012 mg/ml	0.0000012 mg/ml*	5%	5% H_2O

17.5. Precision

Assessment of repeatability (intra-run) and reproducibility (inter-run) of the genesig® COVID-19 3G HT assay has been performed by contriving SARS-CoV-2 negative saliva samples with a known copy number of synthetic RNA template representing the SARS-CoV-2 genomic region of interest. Precision was performed on three batches of the assay, each at three contrivance levels, reproducing a high, medium and low viral load samples:

- High viral load sample: 1.5 x 10^4 copies/ml (15x LoD*)
- Medium viral load sample: 1.0 x 10^4 copies/ml (10x LoD)
- Low viral load sample: 5.0 x 10^3 copies/ml (5x LoD)

*Contrivance level concentrations were based on the analytical sensitivity of the assay from Section 17.1.

Samples were extracted with the exsig® Mag Extraction System on the KingFisher™ Flex extraction platform and tested on the Applied Biosystems® ABI 7500 Fast Real-Time PCR system.

Variance was assessed from operators, instruments and day of testing. Two different operators performed the study over two days with two Applied Biosystems® ABI 7500 Fast Real-Time PCR instruments.

A total of 10 replicates were obtained for each contrivance level.

The precision was measured by reporting the % Coefficient of Variance which is required to be below 9%, for which the assay achieved for all 3 batches and all variances.

Summary of Repeatability and Reproducibility for the genesig® COVID-19 3G HT assay (FAM, VIC ROX, Cy5 channels, 3 batches).

		Coefficient of variance (%) for genesig® COVID-19 3G HT assay Batch 1			
Sample concentration (copies /ml)	Target channel	Repeatability	Inter-Instrument	Inter-operator	Inter-day
1.5×10^4	FAM	2.32	6.97	7.22	7.24
	VIC	1.10	6.12	8.83	7.14
	ROX	2.23	6.96	6.99	7.23
	Cy5	2.35	7.12	7.39	7.42
1.0×10^4	FAM	1.18	6.70	5.86	4.78
	VIC	0.46	3.46	7.29	4.66
	ROX	1.24	6.33	5.88	4.77
	Cy5	0.70	6.62	5.67	4.38
0.5×10^3	FAM	0.72	5.91	8.78	8.41
	VIC	0.26	6.50	4.03	6.87
	ROX	0.80	6.00	8.60	7.81
	Cy5	0.52	6.22	8.50	7.81

		Coefficient of variance (%) for genesig® COVID-19 3G HT assay Batch 2			
Sample concentration (copies /ml)	Target channel	Repeatability	Inter-Instrument	Inter-operator	Inter-day
1.5×10^4	FAM	0.99	5.01	4.57	4.73
	VIC	0.67	5.57	6.13	4.11
	ROX	1.20	5.26	5.05	5.12
	Cy5	0.89	4.77	4.73	4.75
1.0×10^4	FAM	0.35	6.76	4.76	6.53
	VIC	1.45	6.78	8.08	6.76
	ROX	0.54	6.17	5.41	6.20
	Cy5	0.53	6.66	5.05	6.57
0.5×10^3	FAM	0.68	5.59	4.53	4.48
	VIC	0.63	5.66	5.79	6.80
	ROX	0.69	6.29	4.47	4.09
	Cy5	0.62	5.90	4.74	3.70

Sample concentration (copies /ml)	Target channel	Coefficient of variance (%) for genesig® COVID-19 3G HT assay Batch 3			
		Repeatability	Inter-Instrument	Inter-operator	Inter-day
1.5×10^4	FAM	0.74	4.43	4.48	4.88
	VIC	0.93	7.60	5.76	5.99
	ROX	0.47	5.94	4.79	5.10
	Cy5	1.14	5.25	4.05	4.55
1.0×10^4	FAM	0.51	3.74	3.91	4.21
	VIC	0.60	3.71	4.05	3.69
	ROX	0.44	3.90	3.74	4.30
	Cy5	0.37	3.54	3.84	4.40
0.5×10^3	FAM	1.04	5.39	6.65	6.65
	VIC	1.44	5.52	8.71	7.83
	ROX	0.99	5.75	7.48	7.49
	Cy5	0.57	5.60	7.95	8.25

18. Clinical Performance Evaluation

This clinical performance validation aimed to evaluate the *in* vitro diagnostic performance of the genesig® COVID-19 3G HT assay. Samples were previously collected from patients suspected of having COVID-19 at the Queen Elizabeth Hospital, NHS Gateshead, both symptomatic and asymptomatic.

The contingency table below illustrates the total positives and negatives that were used to calculate the Diagnostic Sensitivity (PPA), Diagnostic Specificity (NPA), Positive Predictive Value, (PPV) and Negative Predictive Value (NPV) and the 95% confidence interval (CI) for sensitivity and specificity. Of the 476 samples, 197 gave true positive results, 278 gave true negative results and 1 sample gave false positive result. This resulted in diagnostic sensitivity of 100% (95% CI 98.1%-100%) and diagnostic specificity of 99.6% (95% CI 98.0%-99.9%)

Contingency table for genesig® COVID-19 3G HT Clinical Performance Evaluation

		Comparator assay (TaqPath™) / Resolver assay (genesig® COVID-19 3G)		
		Positive	Negative	Total
genesig® COVID-19 3G HT	Positive	197	1	198
	Negative	0	278	278
	Total	197	279	476

Clinical performance of genesig® COVID-19 3G HT

	%	95% CI
Diagnostic Sensitivity	100%	98.1%-100%
Diagnostic Specificity	99.6%	98.0%-99.9%
PPV	99.5%	
NPV	100%	

19. Disposal

Dispose of unused kit reagents, human specimens, and sealed post-amplification plates as laboratory clinical waste according to national regulations. Refer to Section 8 for guidance weblinks.

20. Primerdesign™ Ltd Quality Control

In accordance with Primerdesign™ Ltd ISO 13485 certified Quality Management System, each batch of the Primerdesign™ Ltd genesig® Coronavirus (COVID-19) 3G HT (CE IVD) assay is tested against predetermined specifications to ensure consistent product quality.

Primerdesign™ Ltd perform weekly in silico analysis of all published SARS-CoV-2 genomes (GISAID EpiCoV and NCBI databases) to identify if the virus mutates in the COVID-19 primer and probe target region.

21. Technical Support

For Technical support, please contact our dedicated technical support team on: Phone: +44 (0) 800 0156 494

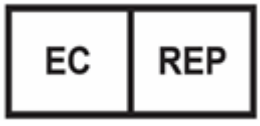
Email: support@primerdesign.co.uk

22. Trademarks and Disclaimers

Trademarks: genesig® and the Primerdesign™ logo.

All other trademarks that appear in this IFU are the property of their respective owners.

23. Explanation of Symbols

Symbol	Explanation
	In vitro diagnostics
	Manufacturer
	Catalogue number
	Suffices for
	Use by Date
	Temperature limit
	Consult Electronic Instructions for Use
	Batch Code
	Keep away from sunlight (primer/probe mix)
	Positive Control
	EU Authorized Representative
	Single Use

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