Primerdesign™ Ltd

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

CE IVD

Instructions for Use (IFU)

Issue 6.00

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
Combined Nasal/Oropharyngeal swabs	CE IVD Extraction System, suitable for the directed sample types exsig® Mag extraction kit	Applied Biosystem® 7500 (Thermofisher) CFX Opus (Bio-Rad) Lightcycler 480 II (Roche) genesig® q32 (Primerdesign™, Novacyt)

96 tests

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1 Intended Use

The genesig® COVID-19 3G Real-Time PCR is a CE marked, *in vitro* diagnostic real-time reverse transcriptase PCR (RT-PCR) assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in combined nasal/oropharyngeal swabs. 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® COVID-19 3G Real-Time PCR (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 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 to the sustained risk of further spread (2). The virus transmission occurs largely from infected droplets produced by a carrier through coughing, sneezing, and breathing in close proximity of another person, although surface transmission has also been suspected in certain cases as the virus is known to survive on inanimate objects for a few days. By the 30th March 2021, SARS-CoV-2 had infected 128 million and claimed 2.79 million lives, 81% of whom were 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 combined nasal/oropharyngeal swabs. 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 combined nasal/oropharyngeal swabs 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 primers/probe mix, which contains the SARS-CoV-2 specific probes labelled with the FAM, ROX and Cy5 fluorophores. The primers/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	110 µl	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	
DNase/RNase 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 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.

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 five 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.
- Primers/probe mix, the enzyme Mastermix, Positive Control Template and RNA 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 primers/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 combined nasal/oropharyngeal swabs.

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 –20 °C 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 –20 °C.
- Freeze/thaw cycles should be minimised and not exceed five freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at –20 °C.

10.2 genesig® COVID-19 3G Primers/ Probe mix preparation

- Upon receipt, the dried primers/probe can be stored at –20 °C 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 primers/probe in 110 µl (per each vial) of Template Preparation Buffer, vortex to mix.
- Resuspended primers/probe are stable for up to one month when stored at –20 °C.
- Freeze/thaw cycles should be minimised and not exceed five freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at –20 °C.
- 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 -20 °C 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 -20°C.

- 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.
- Freeze/thaw cycles should be minimised and not exceed five freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20 °C.
- 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 -20 °C 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. A volume of 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 -20 °C.
- Freeze/thaw cycles should be minimised and not exceed five freeze/thaws. The reagent, once resuspended, can be aliquoted into smaller volumes if required and stored at -20 °C.

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 DNase/RNase 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)

- DNase/RNase 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 **Sections 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 DNase/RNase free tube:

Reaction Mix Component	1 x Volume Required (µl)
OneStep Lyophilised Mastermix	10*
genesig® COVID-19 3G primers/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 8 µl 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 (Primerdesign, Novacyt Group, software version 1.5.0 or greater)

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		

For the genesig® q32 please use the genesig® Multiplex module and program the instrument as described in the genesig® q32 Instrument Guide.

The tables below contain the information required when performing Step 5 of the "Multiplex Set-Up Guide".

Use	Dye	Color	Name
Yes	FAM	User Preference	ORF1ab
Yes	HEX	User Preference	IEC
Yes	ROX	User Preference	M gene
Yes	Cy5	User Preference	S gene

13 Interpretation of Results

13.1 Acceptance criteria of controls

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 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	
ORF1ab FAM (465-510)	M gene ROX (533-610)	S gene Cy5 (618-660)	HEX/VIC (533-580)	Result †
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 health care professional in the context of patient medical history and clinical symptoms.

**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. Upper respiratory specimens, 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, upper respiratory 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 three 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 (100% 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/µl)	Detection rate (%)	Mean Cq (STDV)	Detection rate (%)	Mean Cq (STDV)	Detection rate (%)	Mean Cq (STDV)
187.5	1.5	100%	32.1 (1.2)	100%	33.3 (1.6)	100%	31.5 (1.2)
125.0	1.0	100%	32.6 (0.8)	100%	33.8 (1.2)	100%	32.4 (0.2)
62.5	0.5	100%	33.4 (0.7)	85%	34.4 (1.1)	100%	33.1 (0.8)

31.3	0.25	95%	34.3 (0.8)	85%	34.2 (0.3)	100%	33.5 (0.6)

The data above demonstrates that the genesig® COVID-19 3G assay detects 1 copy/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 Biosystems® ABI 7500 Real-Time PCR System (Thermofisher)	10	0.5	100	20/20	35.35 (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 were collected from five donors and extracted with the KingFisher™ Flex Purification System in conjunction with exsig™ Mag Extraction System. Thirty samples were contrived at 5 x 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 genesig® 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	95%
PPA	100%
NPA	90%

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 15th of December 2021, in silico analysis confirms the COVID-19 assay primers and probe still show 99.8%, 99.7% and 99.9% detection with the 5,371,630, 5,383,006 and 5,286,317 full length, good quality SARS-CoV-2 sequences at the ORF1ab, S gene and M 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 primers/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, five 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

15.4. Precision (repeatability and reproducibility)

Summary: This study assesses the repeatability (intra-run) and reproducibility (inter-run) of the genesig® COVID-19 3G kit on the genesig® q32, and was performed on three different batches with varying operators, instruments, and testing days. Samples were contrived with three contrivance levels determined from the Limit of Detection (LoD) of 1 copy/µl in the PCR reaction:

- A high viral load sample (15x LoD).
- A medium viral load sample (10x LoD).
- A low viral load sample (5x LoD).

The samples were contrived with TwistBio Synthetic SARS-CoV-2 whole-genome RNA verification material and extracted using the KingFisher™ Flex extraction platform. Two operators performed the study on two days, with three different sets of q32 instruments. A total of 10 replicates were obtained for each contrivance level. The PCT must have produced a Cq between 14-25 in the FAM, ROX and Cy5 channels, and the NTC should have been free of amplification in the FAM, HEX, ROX, and Cy5 channels. The precision was measured by reporting the % Coefficient of Variance (Mean Cq/SD x 100).

Coefficient of variance (%) for genesig® COVID-19 3G kit on the genesig® q32 assay Batch 1

Contrivance level	Channel	Intra-run	Inter-machine	Inter-operator	Inter-day
15x LoD	FAM	0.76%	1.09%	0.97%	0.48%
	HEX	4.03%	1.38%	1.40%	0.93%
	ROX	3.19%	0.89%	0.50%	1.17%
	Cy5	0.75%	0.98%	0.84%	0.86%
10x LoD	FAM	0.56%	0.56%	0.89%	1.77%
	HEX	2.82%	0.42%	1.29%	2.77%
	ROX	1.75%	0.76%	1.40%	2.15%
	Cy5	0.54%	0.54%	0.72%	1.59%
5x LoD	FAM	1.07%	1.57%	0.42%	1.38%
	HEX	3.02%	3.99%	1.28%	2.06%
	ROX	2.47%	1.54%	2.48%	2.65%
	Cy5	0.92%	1.55%	0.58%	1.95%

Coefficient of variance (%) for genesig® COVID-19 3G kit on the genesig® q32 assay Batch 2

Contrivance level	Channel	Intra-run	Inter-machine	Inter-operator	Inter-day
15x LoD	FAM	0.47%	2.36%	0.44%	0.95%
	HEX	1.09%	1.66%	1.18%	2.61%
	ROX	0.76%	1.76%	0.96%	0.68%
	Cy5	0.40%	2.33%	0.55%	1.10%
10x LoD	FAM	0.34%	0.84%	0.57%	1.74%
	HEX	0.80%	1.41%	3.36%	4.08%
	ROX	0.95%	0.61%	1.08%	1.80%
	Cy5	0.62%	0.75%	0.56%	1.52%
5x LoD	FAM	1.38%	5.29%	0.88%	0.64%
	HEX	1.03%	1.69%	2.42%	1.86%
	ROX	3.50%	5.41%	2.68%	1.11%
	Cy5	0.76%	5.83%	1.10%	0.90%

Coefficient of variance (%) for genesig® COVID-19 3G kit on the genesig® q32 assay Batch 3

Contrivance level	Channel	Intra-run	Inter-machine	Inter-operator	Inter-day
15x LoD	FAM	1.04%	0.71%	1.47%	0.76%
	HEX	1.58%	1.95%	2.73%	1.42%
	ROX	1.46%	1.02%	1.58%	1.10%
	Cy5	1.17%	1.09%	1.34%	1.06%
10x LoD	FAM	1.57%	1.52%	0.79%	0.96%
	HEX	3.43%	1.83%	1.25%	1.47%
	ROX	0.84%	1.47%	0.91%	0.83%
	Cy5	1.37%	1.30%	1.03%	0.99%
5x LoD	FAM	1.17%	1.10%	0.59%	1.57%
	HEX	2.45%	3.27%	1.90%	3.99%
	ROX	2.40%	1.42%	3.76%	1.54%
	Cy5	1.03%	1.45%	1.22%	1.55%

Conclusions: The results show that the genesig® COVID-19 3G produces reproducible and repeatable results across all channels. The Cq variance produced within a run and between operators, machines, and days was never more than 5.83%, demonstrating the assay can consistently reproduce data over all three batches.

15.5. Interfering Substances

Summary: The objective of this study was to evaluate the effects of potential exogenous and endogenous interfering substances on the genesig® COVID-19 3G Real-Time PCR detection kit. Changes in the performance of the assay were analysed by Cq values of samples containing the potential interfering substances at relevant clinical concentrations.

For each interfering substance, a sample negative of COVID-19 was collected from the same donor. The interfering substances were spiked into each sample. The samples were extracted using the KingFisher™ Flex extraction platform in conjunction with the exsig™ Mag extraction system. Prior to extraction, each sample was contrived with 2 µl SARS-CoV-2 synthetic viral RNA at 5 x the LoD reported. The contrivance in this study was 5 copies/µl in the PCR reaction. After amplification, each interfering substance was evaluated against its relevant control (water, ethanol or NaOH) and given a pass/fail based on the acceptance criteria: The % call agreement for each interfering substance vs the control is ≥95% for each target channel, or a mean Cq value for each interfering substance sample is within 2 Cq of the mean control value for at least two out of three target channels; and the overall mean Cq value over all target channels is within 2 Cq of the mean control Cq value over all target channels.

Interfering substances tested

Substance	Tested concentration
Blood (Haemoglobin)	0.2 g/ml
α-Amylase	7.92 mg/ml
Oseltmavir (Antiviral medication)	0.00798 mg/ml
Oxymetazoline	0.0000012 mg/ml
Nasacort	10% v/v
Dymista Allergy Nasal Spray (Corticosteroids – Azelastine hydrochloride & Fluticasone)	6.85 mg/ml Az 2.5 mg/ml Fl
Tobramycin (antibacterial eyedrops)	0.03 mg/ml
Strepsils (throat lozenge)	1% w/v
Dexamethasone (Corticosteroid)	1.52 µmol/L
Fluticasone (Corticosteroid)	0.1 mg/ml
Guaifenesin (Antiviral medication)	0.9 mg/ml
Mupirocin (Antibacterial medication)	5 µg/ml
Mucin	0.2 mg/ml

None of the 13 interfering substances screened was found to have a significant effect on the genesig® COVID-19 3G Real-Time PCR detection kit. Fluticasone had a Cq difference greater than 2 for the M gene target compared to its relevant control (10% ethanol). A t-test was carried out, which proved that there was a statistically significant difference between the interferant sample and its control. However, both ORF1ab and the S gene

fulfilled the acceptance criteria, and the mean Cq of the IEC of Fluticasone showed no significant difference when compared to the mean Cq of the IEC of its control. This could indicate that the result was not representative of assay interference from the substance itself.

16. Clinical Performance Evaluation

An initial clinical performance validation aimed to evaluate the in vitro diagnostic performance of the genesig® COVID-19 3G assay compared with a comparator assay, TaqPath (ThermoFisher). Combined nasal/oropharyngeal swab samples were previously collected from patients suspected of having COVID-19 at the Queen Elizabeth Hospital, NHS Gateshead, both symptomatic and asymptomatic. These were analysed by TaqPath assay, and the eluates frozen at -70 °C prior to the commencement of this study.

493 frozen patient sample eluates were tested, with 415 giving a concordant result with the original TaqPath assay result. The 78 discordant samples were then analysed by a resolver assay, the genesig® Real-Time PCR COVID-19 (CE-IVD) assay. 1 sample was excluded due to a lack of eluate for the resolver assay, 20 samples were excluded as they were below the LoD of the assay under investigation (Cq>34). All other samples were determined to be True Positive (2), True Negative (54), or False Negative (1).

The contingency table below illustrates the total positives and negatives that were used to calculate the Diagnostic Sensitivity (PPA), Diagnostic Specificity (NPA), and the 95% confidence interval (CI) for sensitivity and specificity. Of the 472 samples, 157 gave true positive results and 314 gave true negative results. This resulted in diagnostic sensitivity of 99.4% (95% CI 96.5% - 100%) and diagnostic specificity of 100% (95% CI 98.8%-100%).

Contingency table for genesig® COVID-19 3G Clinical Performance Evaluation – Queen Elizabeth Hospital - NHS Gateshead

		Comparator assay (TaqPath) / Resolver assay (genesig® COVID-19)		
		Positive	Negative	Total
genesig® COVID-19 3G	Positive	157	0	157
	Negative	1	314	315
	Total	158	314	472

Clinical performance of genesig® COVID-19 3G

	%	95% CI
Diagnostic Sensitivity	**99.4%**	**96.5% - 100%**
Diagnostic Specificity	**100%**	**98.8% - 100%**

A second retrospective Clinical Performance Study was conducted at The Scientists Laboratory (TSL) Ltd, London. This study compared the genesig® COVID-19 3G assay to a composite reference standard consisting of the following component tests:

- VIASURE SARS-CoV-2 Real-Time PCR Detection Kit
- genesig® Real-Time PCR Coronavirus (COVID-19) CE-IVD

Agreement between the component tests was considered to represent the true diagnostic status of a sample. If the two component tests were not in accordance, the diagnostic status of the sample was determined to be inconclusive and the sample excluded from the study.

The samples included in the study represented fresh or archived combined nasal/oropharyngeal samples. Fresh samples were assayed with genesig® Real-Time PCR Coronavirus (COVID-19) CE-IVD immediately after extraction with ThermoFisher MagMAX. The remaining eluates were frozen at -20 °C and thawed within 48 hours prior to analysis with genesig® COVID-19 3G assay and VIASURE SARS-CoV-2 Real-Time PCR Detection Kit. Archived samples had been stored at -20 °C in transport medium for a maximum of 1 month. Following thawing, these samples were extracted with the ThermoFisher MagMAX kit and all three assays performed on each specimen in parallel.

All assays were performed according to their respective instructions for use (IFUs):

- genesig® COVID-19 3G Real Time PCR CE STED IFU Issue 4.0 (19th August 2021)
- genesig® Real Time PCR COVID-19 (CE IVD) IFU version 6.00 (11th June 2021)
- VIASURE SARS-CoV-2 Real-Time PCR Detection Kit IFU (IU-NCO212enes1120 rev.02 November 2020)

In total, 549 clinical specimens were submitted to the study. From these samples, 42 were not accepted as the diagnostic status of the sample was determined to be inconclusive. The initial clinical performance characteristics of the genesig® COVID-19 3G assay were a diagnostic sensitivity of 99.5% (95% CI 97.07-99.99%) and a diagnostic specificity of 97.5% (95% CI 95.12-98.91%). Out of the 507 included samples:

- 187 positives were concordant between genesig® COVID-19 3G and the composite reference standard
- 311 negatives were concordant between genesig® COVID-19 3G and the composite reference standard
- 8 genesig® COVID-19 3G-positives were discordant relative to the composite reference standard
- 1 genesig® COVID-19 3G negative was discordant relative to the composite reference standard.

Further investigation of the relative analytical sensitivities of the assays demonstrated that seven of the eight discordant samples that were positive with genesig® COVID-19 3G were likely to be true positives. This is because they were determined to be operating at a viral load beyond the analytical sensitivity of the composite reference standard. Based on these findings, these seven samples were classified as ‘inconclusive discordant samples’ and excluded from the final analysis:

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

		Agreed result of VIASURE SARS-CoV-2 Real-Time PCR and genesig® Real Time PCR COVID-19 assays		
		Positive	Negative	Total
genesig® COVID-19 3G	Positive	187	1	188
	Negative	1	311	312
	Total	188	312	500

Clinical performance of genesig® COVID-19 3G across full viral range

	%	95% CI
Diagnostic Sensitivity	99.5%	97.07 - 99.99%
Diagnostic Specificity	99.7%	98.23 - 99.99%

From the positive samples, the FAM reading in the VIASURE SARS-CoV-2 assay can be used to divide the cohort of samples into low, low-medium, medium-high, and high Cq ranges. Consequently, the sensitivity of the genesig® COVID-19 3G assay in each Cq range can also be determined:

Clinical sensitivity of genesig® COVID-19 3G in different Cq ranges

Cq in comparator	% of samples	% sensitivity of genesig® COVID-19 3G	95% CI
<25	22.83	100.0%	91.59% - 100.00%
≥25 to <30	25.54	100.0%	92.45% - 100.00%
≥30 to <35	29.35	100.0%	93.40% - 100.00%
≥35	22.28	97.6%	87.14% - 99.94%

17. 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.

18. Technical Support

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

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

19. 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

EU Authorized Representative

Single Use

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