Primerdesign™ Ltd

PROmate® COVID-19 2G

96 reactions

Total workflow solution for the qualitative detection of SARS-CoV-2 viral RNA. For use with anterior nasal samples collected as dry swabs.

NOT FOR USE WITH SWABS STORED IN GUANIDINIUM THIOCYANATE-CONTAINING MEDIA

Instructions for Use (IFU)

Issue 2.01

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Primerdesign™ Ltd

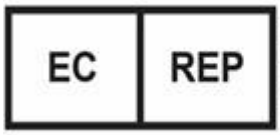
PROmate® COVID-19 2G

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

For Use with:

Sample Types	Extraction Platforms	PCR Platform
Anterior Nasal Swabs	PROmate® COVID-19 2G (Direct to PCR)	genesig® q32 (Primerdesign™, Novacyt)

96 tests



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1. Intended Use

PROmate® COVID-19 2G is a total workflow solution, inclusive of sample preparation, polymerase chain reaction (PCR) amplification and analysis on the genesig® q32 Real-Time PCR instrument, specifically for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This assay is based on the PROmate® COVID-19 assay with an additional second target in the gene encoding Nsp16, one of the nonstructural proteins of SARS-CoV-2, which increases the specificity and accuracy of this assay.

PROmate® COVID-19 2G is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal samples acquired on dry swabs. The assay provides rapid screening of individuals for SARS-CoV-2 infection and aids the diagnosis of suspected COVID-19 in patients. The assay is intended for use with the validated genesig® q32 Real-Time PCR platform.

The PROmate® COVID-19 2G Sample Preparation Buffer provides total viral inactivation. As such, there are two discrete protocols to follow for users both with and without a Class II Microbiological Safety Cabinet. This allows total flexibility for the use of the product depending on the workflow set-up of the user.

SARS-CoV-2 is generally detectable in samples during the acute phase of infection and asymptomatic infection. Positive results are indicative of the presence of SARS-CoV-2 Ribonucleic Acid (RNA). Positive results do not rule out co-infection with other bacteria or viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Positive and negative results must be interpreted alongside clinical observations, patient history and epidemiological information.

PROmate® COVID-19 2G is intended for use by personnel specifically instructed and trained in handling SARS-CoV-2 positive samples and the techniques of Real-Time PCR and *in vitro* diagnostic procedures.

Sample test results are available to interpret in under 95 minutes using PROmate® COVID-19 2G. This time includes the processing of the sample, the PCR set-up and run time.

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 reported onset of symptoms around 1st December 2019 (1). By 11th March 2020, positive cases 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). Globally SARS-CoV-2 had infected 195 million as of 28th July 2021 and had claimed 4.18 million lives (3). As with most viruses, the SARS-CoV-2 also mutates,and the changes in the genomic code have resulted in the emergence of different virus variants. These variants are suspected of having altered transmissibility rate, and impact the body's immune response and, possibly, 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 avoidance of overwhelming healthcare systems.

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

PROmate® COVID-19 2G workflow is a combination of a 'direct to PCR' sample processing method and molecular *in vitro* diagnostic test for the detection of the SARS-CoV-2 RNA from anterior nasal swabs and for interpretation on the genesig® q32 instrument. The viral RNA is released from the swab sample during incubation with a viral inactivation/lysis agent. Following the swab sample preparation process, an aliquot of the resulting sample is tested using well-established nucleic acid amplification technology in the genesig® Coronavirus (COVID-19) assay. The PCR assay contains oligonucleotide primers and dual-labelled hydrolysis probes, as well as control material, for use in Real-Time PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA.

3. Principles of the Procedure

Viral RNA is extracted from anterior nasal swabs using 'direct to PCR' technology which only includes one pipetting step. Using a one-step reaction, the viral 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 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 instrument.

PROmate® COVID-19 2G includes SARS-CoV-2 specific primers and probes that are labelled with the FAM and Cy5 fluorophores. The primer/probe mix also includes primers and probes to amplify and detect an Internal Control (IC) RNA template. This is simultaneously amplified along with any target SARS-CoV-2 RNA and provides a process control for the confirmation of a successful PCR. The IC specific probe is labelled with the HEX fluorophore.

The oligonucleotide primers and probes for the detection of SARS-CoV-2 were selected from the ORF1ab genomic region of SARS-CoV-2 labelled with a FAM fluorophore and the gene encoding the Nsp16 viral nonstructural protein of SARS-CoV-2 labelled with a Cy5 fluorophore.

PCR amplification using the included chemistry and plasticware in PROmate® COVID-19 2G is designed specifically for use on the genesig® q32 instrument. The reagents are provided in pre-loaded magazines which will slot into the instruments to facilitate ease of use. For users operating PROmate® COVID-19 2G where turnaround requires incomplete runs, separate controls and magazines are available for total flexibility with sample numbers.

PROmate® COVID-19 2G is designed to preclude the need for a Class II Microbiological Safety Cabinet, if required. By enabling the release of viral RNA from the sample directly in closed tubes without creating aerosols, PROmate® COVID-19 2G guarantees protection of the operator and prevents contamination of the work area. The use of PROmate® COVID-19 2G with or without Class II Microbiological Safety Cabinet depends upon the protocol followed. For further information, please refer to **Section 12**.

4. Materials Provided

PROmate® COVID-19 2G is presented in a format that is suitable for running 96 reactions over 3 separate runs (3x32). PROmate® COVID-19 2G is supplied in 2 separate pack types for the ease of transportation and storage.

PROmate® COVID-19 2G (Catalogue: D00074)

The reagents necessary for each run come in two different packs; a box, shipped at ambient (Pack 1) and a frozen pouch (Pack 2) shipped on dry ice.

PROmate® COVID-19 2G Pack 1 contains 6 bags (3 foils and 3 zip-lock bags) and should be stored at ambient temperature. This is shown below in Table 1.

Table 1: Pack 1 (D00074) PROmate® COVID-19 2G Box
(3x foil pouches and 3x clear plastic zip-lock bags)

Reagent label	Number of vials per 32 test pack	Volume (µl per vial)	Lid colour
PROmate® COVID-19 2G RNase Inhibitor with IC	30	n/a	n/a
PROmate® COVID-19 2G Sample Preparation Buffer	30	1000	Blue

PROmate® COVID-19 2G Pack 2 is shipped on dry ice and should be stored frozen (-25°C to -15°C). Upon opening, the pack contains 3 PROmate® COVID-19 2G Positive Controls, 3 PROmate® COVID-19 2G q32 reaction magazines (including lids) and 3 negative controls as below in Table 2.

Table 2: Pack 2 (D00074) PROmate® COVID-19 2G Pouch
(3x foil pouches with positive control, 3x foil pouches with magazine and 3x negative control)

Reagent label	Number of tubes per 32 test pack	Volume (µl per vial)	Lid/foil colour
PROmate® COVID-19 2G Positive Control	1	20	Gold foil
PROmate® COVID-19 2G Master Mix q32 magazine	1	15	White Foil
PCR tube lids	31	n/a	Clear
PROmate® COVID-19 2G Negative Control Solution	1	50	Red

5. Required Equipment and Consumables (Not Provided)

- Vortex
- Microcentrifuge (optional)
- Adjustable 10 µl or 20 µl micropipette, or a fixed 5 µl micropipette
- Aerosol barrier pipette tips with filters
- 0.1 ml PCR tubes (for balancing of lid for partial runs)
- Disposable gloves
- Scissors (optional)
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- 70% Alcohol (either ethanol or isopropanol)
- DNase/RNase remover
- Barcode reader (optional)

6. Real-Time PCR instruments

The PROmate® COVID-19 2G assay has been validated with the following Real-Time PCR instruments:

- genesig® q32 Real-Time PCR instrument (Primerdesign™, Novacyt Group, software version 2G PROmate q32 v1.2.2 (2nd gen))

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

7. 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 Interim guidance on laboratory biosafety: https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1 from 28th January 2021.

- Refer to the Centers for Disease Control and Prevention (CDC) guidelines: Interim Laboratory Biosafety Guidelines for Handling and Processing Samples Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

8. Warnings and Precautions

8.1. General

- Handle all clinical samples assuming they are infectious using safe laboratory procedures. Sample processing should be performed in accordance with national biological safety regulations.

- PROmate® COVID-19 2G can be performed in the presence or absence of a Class II Microbiological Safety Cabinet. When following the PROmate® COVID-19 2G workflow that requires work in a Class II Cabinet, perform all manipulations of potential live virus samples within a Class II (or higher) Cabinet (refer to the guidance detailed in **Section 7**).

- Follow necessary precautions when handling clinical 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 and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes and other equipment.

- Please consult the safety data sheet (SDS) before using this kit, which is available on request.

- The PROmate® COVID-19 2G Sample Preparation Buffer contains Triton X-100 Reduced. This component should be handled according to the SDS. This product is hazardous to the environment and should be disposed of as detailed in the SDS. In the event of damage to protective packaging, contact Primerdesign™ for instructions.

8.2. 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 sample or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).

- The PROmate® COVID-19 2G Positive Control is provided in a sealed foil pouch and contains a high copy number template. This foil pouch should not be opened until samples and NTC have been loaded. The sealed lid of the PCR reaction tube for the positive control should not be removed to avoid cross-contamination.

- **DO NOT** open reaction tubes once PCR has been completed.

- **Never** open the positive control reaction tube, before or after PCR run.

- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuge) and supplies (e.g., microcentrifuge tubes, pipette tips) for handling of clinical 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 reagents 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 kits 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 sample preparation procedure. Good aseptic technique should always be used when working with nucleic acids.
- Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g., 10% bleach) to minimise risk of nucleic acid contamination.
- RNA samples should be maintained on a cold block or on ice during preparation to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with 10% bleach.
- Handle post-amplification PCR tubes with care to ensure that the seal is not broken.
- Dispose of human biological samples according to national and international regulations (refer to guidance in **Section 17**).

8.3. 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 procedure to prevent aerosol and liquid contamination.

9. Reagent Storage, Handling and Stability Conditions

9.1. Storage conditions

- The PROmate® COVID-19 2G Pack 1 is shipped at ambient temperatures and can be stored at ambient temperature upon arrival.
- The PROmate® COVID-19 2G Pack 2 is shipped frozen, on dry ice and needs to be stored at -20°C upon arrival.
- Pack 2 is stable at -20°C for up to 12 months following manufacture. Always check the expiration date prior to use. Do not use expired reagents.
- Once the "use by" date has been reached, the kit components should be discarded following the disposal instructions in **Section 17.**
- If the kits protective packaging is damaged upon receipt, please contact Primerdesign™ for instructions.

9.2. In-use Stability

PROmate® COVID-19 2G is pre-dispensed, with the operator selecting the number of reactions and controls required as appropriate to complete their run. As such, there is no necessity to make bulk reagents and dispense, facilitating ease of use for the operator. Reagents should be stored at their specified temperatures and removed from storage prior to use only.

When operating partial runs, the excess number of Master Mix reaction tubes not required can be removed and saved for subsequent runs. In this scenario, the magazine should be removed from frozen storage, the desired number of reaction tubes removed from the magazine (to be used on a later run) and then immediately returned to the specified storage conditions. Avoid repeated freeze-thaw cycling, when possible. If tubes are removed, they need to be replaced with empty tubes (not provided) for balancing the lid during the run.

10. Clinical Sample Collection, Handling and Storage

10.1. Compatible Samples

- This product is intended for use with dry swabs only. Use of media not supplied as part of this product will impact the detection limit of the device.

- Samples that present with obvious blood or other particulate matter are NOT compatible with PROmate® COVID-19 2G and should be discarded.

10.2. Collecting the Compatible Clinical Samples

Sampling should be conducted with the correct swab type and collected following the correct sampling technique. CDC guidance on collection of anterior nasal swabs can be found here: How To Collect Your Anterior Nasal Swab (cdc.gov).

Inadequate or inappropriate clinical sample collection, storage and transport are likely to yield false test results. Training of personnel in clinical sample collection is highly recommended. CLSI MM13 (Clinical and Laboratory Standards Institute) may be referenced as an appropriate resource.

Anterior Nasal swabs	
Collection	Swabs: Dry Dacron® or polyester flocked swabs in a sterile container
Transport temperature	2-8°C ≤ 72hrs* (dry swabs) <4°C ≤ 24hrs (resuspended swabs) 4-20°C ≤ 4hrs (resuspended swabs)
Short-term storage (pre-extraction)	2-8°C ≤ 72hrs* (dry swabs) <4°C ≤ 24hrs (resuspended swabs) 4-20°C ≤ 4hrs (resuspended swabs)
Long-term storage (pre-extraction)	≤ -70°C for longer periods* (dry swabs)

* CDC recommendations: CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Samples Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Local regulations pertaining to sample handling may also apply.

- Swab samples should be collected and placed in a clean, dry, sterile transport prior to testing.

- For dry swabs for use inside a Class II Microbiological Safety Cabinet, swab samples must be transported within 24 hours and tested as soon as possible after collection. If this is not possible, the following storage recommendations should be followed:

    - If the swab is stored at 2-8°C, the clinical sample must be tested within 72 hours.

    - If testing cannot be conducted within 72 hours, the swab clinical sample should be frozen at -70°C or colder until testing is conducted.

- For resuspended samples for use inside a Class II Microbiological Safety Cabinet,

swab samples must be transported within 4 hours and tested as soon as possible after collection. If this is not possible, the following storage recommendations should be followed:

  - Swab samples must be transported within 4 hours or stored refrigerated.

- For further guidance on clinical samples please refer to the following:

  - UK Government guidance on handling and processing potential COVID-19 samples in laboratories: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens

  - World Health Organization Interim guidance on laboratory biosafety from 28 January 2021: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: https://www.who.int/publications/i/item/WHO-WPE-GIH-2021.1

  - Interim Guidelines for Collecting, Handling and Testing Clinical Samples from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19): https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

- The PROmate® COVID-19 2G Sample Preparation Buffer contains Triton X-100 Reduced (Triton X-100 replacement) and has been utilised for the inactivation of SARS-CoV-2 by Public Health England. Therefore, samples should be handled according to these revised national guidelines for sample management prior to inactivation.

- Follow clinical sample collection instructions by device manufacturer for proper collection methods.

- Swab samples should be collected using swabs with a synthetic tip, such as nylon or Dracon® and with an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.

10.3. Transporting Clinical Samples

Clinical 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 UN3373 Biological Substance, Category B when sending potential SARS-CoV-2 samples.

11. General Preparation

11.1. Equipment preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use.
- Decontamination agents such as 10% bleach should be used to minimise the risk of nucleic acid contamination.

12. Assay Set Up

12.1. Procedure Caution

The following instructions should be adhered to when handling clinical samples and reagents during procedure set-up with PROmate® COVID-19 2G in order ensure consistent, accurate results:

- Do not handle reactions tubes by their base as this may affect optical reading. Reaction tubes should be held by the rim/lid to avoid any contact with the sides of the tube.
- Tubes must not rest directly on the workbench; use racks for holding tubes wherever possible.
- Ensure the contents of the tubes are at the bottom of the wells prior to loading samples and prior to running on the instrument.
- Ensure absence of any bubbles in reaction tube prior to running the instrument. Failure to do so could result in errors in signal interpretation by the instrument.
- **Before** opening the positive control foil pouch, visually inspect for leakage. If there is evidence of leakage inside the bag, **DO NOT OPEN** and use a replacement positive control to avoid contamination.
- Visually inspect all reaction tubes for air bubbles prior to running PROmate® COVID-19 2G. Failure to do so could result in errors in signal interpretation by the instrument.

For total user flexibility there are **two separate** workflows for using PROmate® COVID-19 2G:

- Processing of samples **outside** of a Class II Microbiological Safety Cabinet – protocol in Section 12.2.
- Processing of samples **inside** a Class II Microbiological Safety Cabinet– protocol in Section 12.3.

Please follow the appropriate protocol for your workflow stream as listed below. It is important that the correct workflow is followed to ensure user safety and total viral inactivation before sample manipulation prior to PCR set-up.

12.2. Swab Sample Processing Outside of a Category II Biosafety Cabinet

Sample collection

a. Add one lyophilised RNase inhibitor with IC and red bead to the Sample Preparation Buffer tube to be used. The bead within is coloured red; this provides easy identification that the inhibitor has been added.
b. Place patient swab in assigned Sample Preparation Buffer tube and break to fit inside the tube (**optional:** use scissors to cut swab to length appropriately, if necessary). Close lid with swab inside. **If using scissors, ensure they are decontaminated with 70% alcohol (ethanol or isopropanol) in between each sample.**
c. Once all samples have been resuspended, leave the Sample Preparation Buffer tubes at room temperature for at least **5 minutes**. This is necessary for total SARS-CoV-2 inactivation.
d. The sample resuspension and inactivation (a-c) must take place at the point of sample taking.

Sample processing

e. Label the sample appropriately to ensure patient sample traceability.
f. Repeat step e for all patient samples to be processed.
g. Open frozen PROmate® Pack 2 and defrost:
    - Magazine with PCR reaction tubes. Ensure liquid content is at the bottom of the tubes.
    - Positive control tube. DO NOT open pouch.
    - Negative control tube.
h. Vortex each Sample Preparation Buffer tube thoroughly to release swab contents into solution.
i. Once ambient incubation is complete, remove the foil on a PCR reaction tube and transfer 5 µl of the relevant patient Sample Preparation Buffer tube into the designated PCR reaction tube. Seal with provided cap.
j. Repeat step (h) and (i) for all samples.
k. Add 5 µl of the Negative Control (red lid) into the PCR reaction negative control tube (position N) and seal.
l. Inspect the Positive Control pouch, and if no leakage present, open, remove the tube and ensure the liquid content is at bottom of the tube. Place in position "P" of the magazine. DO NOT open the Positive control PCR tube.
m. **Flick the magazine to remove any bubbles or use microcentrifuge**.
n. Place magazine in correct orientation to genesig® q32. When operating with a partial run (i.e., NOT complete magazines), use blank 0.1 ml PCR tubes to fill gaps and balance the lid on the instrument.
o. Start the run protocol using the provided template file.

12.3. Swab Sample Processing with a Category II Biosafety Cabinet

a. Add one lyophilised RNase inhibitor with IC and red bead to each Sample Preparation Buffer tube to be used, close and invert to mix. The bead within is coloured red; this provides easy identification that the inhibitor has been added.
b. Inside of the category II class biosafety cabinet, place patient swab in assigned Sample Preparation Buffer tube and twirl to release the swab contents into suspension. Upon removal of swab, squeeze head against side of tube to release liquid. Close lid and vortex thoroughly.
c. Label the sample appropriately to ensure patient sample traceability.
d. Repeat steps (b) and (c) for all patient samples to be processed.
e. Once all samples have been resuspended, leave the Sample Preparation Buffer tubes at room temperature for at least **5 minutes**. This is necessary for total SARS-CoV-2 inactivation.
f. Open frozen PROmate® Pack 2 and defrost:
    - Magazine with PCR reaction tubes. Ensure liquid content is at the bottom of the tubes.
    - Positive control tube. DO NOT open pouch.
    - Negative control tube.
g. Once ambient incubation is completed samples can be handled outside of the Category II biosafety cabinet. Remove the foil on a PCR reaction tube and transfer 5 µl of the relevant patient sample from Sample Preparation Buffer tube into the designated PCR reaction tube. Seal with provided cap.
h. Repeat step (g) for all samples.
i. Add 5 µl of the Negative Control (red lid) into the PCR reaction negative control tube (position N) and seal.
j. Inspect the Positive Control pouch, and if no leakage present, open, remove the tube and ensure the liquid content is at bottom of the tube. Place in position “P” ofthe magazine. DO NOT open the Positive control PCR tube.
k. **Flick to remove any bubbles in the q32 magazine or use microcentrifuge.**
l. Place magazine in correct orientation into the genesig® q32. When operating with a partial run (i.e., NOT complete magazines), use blank 0.1 ml PCR tubes to fill gaps and balance the lid on the instrument.
m. Start the run protocol using the provided template file.

12.4. Programming the Real-Time PCR Instrument

Please refer to the following manual for additional information on using the instrument: genesig® q32 gen 2 (Primerdesign™, Novacyt, software version 1.2.2 (2nd gen)). Cycling conditions are provided in template run file: [2G PROmate q32 template]

13. Interpretation of Results Using PROmate® COVID-19 2G

13.1. Acceptance Criteria of Controls on genesig® q32

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:

- Negative Control is free from amplification in the FAM, HEX and Cy5 channels*.
- PCT produces a Cq of between 14-25 in the FAM and Cy5 channels.

* If the Negative Control does produce positive amplification in the FAM/HEX/Cy5 channels, the FAM/HEX/Cy5 Cq value produced by the patient sample should be >5 Cq earlier than the FAM/HEX/Cy5 Cq value of the Negative Control (i.e., if patient sample FAM/HEX/Cy5 Cq = 30, Negative Control FAM/HEX/Cy5 Cq ≥35 is acceptable) in order to proceed with the interpretation of patient sample results using the genesig® q32.If the patient sample produces a FAM/HEX/Cy5 Cq <5 Cq earlier than the Negative Control Cq (e.g if patient sample FAM/HEX/Cy5 Cq = 30, Negative Control FAM/HEX/Cy5 Cq = 32) then the results should not be analysed due to contamination.

13.2. Interpretation of results using genesig® q32

If all the control acceptance criteria are fulfilled, then each sample can be assessed with the below criteria if using PROmate® COVID-19 2G with the genesig® q32.

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

ORF1ab FAM (465-510)	Nsp16 Cy5 (618-660)	IC HEX (533-580)	Result
Cq < 38.14	Cq (+)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq < 38.14	Cq (-)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (-) or Cq > 38.14	Cq (+)	Cq (+) / (-)	SARS-CoV-2 Positive*
Cq (-) or Cq > 38.14	Cq (-)	Cq ≤35	SARS-CoV-2 Negative**
Cq (-) or Cq > 38.14	Cq (-)	Cq (-) or Cq>35	Result invalid, repeat testing of sample

* This assay has a Limit of Blanks (LoB) established for the FAM channel at Cq 38.14 (see Section 15.2). For a sample to be considered positive for SARS-CoV-2, a Cq below 38.14 should be observed in FAM channel and/or any Cq should be observed in Cy5 channel.

** If there is no amplification in the FAM or Cy5 channels for a test sample, to confirm the result is valid as SARS-CoV-2 negative, there should be an amplification Cq ≤35 in the HEX channel for the IC. This confirms that amplification has not been inhibited.

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 PROmate® COVID-19 2G primer and/or probe binding, resulting in failure to detect the virus.
- False negative results may be caused by:
    - Unsuitable collection, handling and/or storage of samples.
    - Sample outside of viremic phase.
    - Failure to follow procedures in this handbook.
- False positive results may be caused by:
    - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or Positive Control Template.
    - Unsuitable handling of amplified product.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection with SARS-CoV-2.

15. Performance Evaluation

15.1. Analytical Sensitivity

The limit of detection (LoD) is defined as the lowest concentration of the analyte that can be reliably detected with >95% confidence using PROmate® COVID-19 2G on the genesig® q32 Real-Time PCR system. The tentative LoD was established by contriving Sample Preparation Buffer tubes with extracted SARS-CoV-2 whole viral genome RNA at 5 concentrations: 5 copies/µl, 1 copy/µl, 0.5 copies/µl, 0.25 copies/µl and 0.05 copies/µl in the qPCR tube. This was done over two batches of PROmate® COVID-19 2G over three days. Each contrivance level was tested in replicates of 5 and the tentative LoD was established as the lowest concentration where all replicates gave positive amplification for both batches over all three days.

The tentative LoD was verified by running 20 replicates of the tentative LoD concentration and two concentrations slightly below. The LoD would then be confirmed as the lowest concentration that could be detected with >95% confidence. The results can be seen in the table below.

Target concentrations/replicates			ORF1ab (FAM)		Nsp16 (Cy5)		IC (HEX)
Conc. in Sample Prep Buffer (copies/µl)	Conc. in PCR reaction (copies/µl)	Total replicates	Detection rate (%)	Mean Cq (STDV)	Detection rate (%)	Mean Cq (STDV)	Mean Cq (STDV)
1	0.25	20	100%	34.41 (0.65)	100%	33.86 (0.45)	19.75 (0.26)
0.6	0.15	20	100%	35.02 (0.65)	100%	34.80 (0.49)	19.81 (0.19)
0.4	0.1	20	100%	35.82 (0.68)	100%	35.77 (0.51)	19.73 (0.44)

The data above demonstrates that the PROmate® COVID-19 2G assay detects 0.4 copies/µl in Sample Preparation tube ≥95% across all samples. This is therefore the limit of detection of the assay.

15.1.1 Bridging to samples in VTM

Although the PROmate® COVID-19 2G assay is designed to be used with dry anterior nasal swabs, we sought to ensure that clinical performance studies could be undertaken on samples stored in VTM, as these are commonly found in clinical labs. As such, SARS-CoV-2 genomic RNA was contrived into VTM. 40 µl of this VTM was then mixed with sample preparation buffer, left to incubate for 5 min, and then 5 µl of this mixture added into the PROmate cassette containing 15 µl of mastermix in each well.

As outlined in the table below, when 40 ul of contrived VTM was added to yield a contrivance level equivalent to 0.1 copies/ul in the PCR reaction, the LoD obtained with dry swabs was maintained.

Target concentrations/replicates				ORF1ab (FAM)	Nsp16 (Cy5)	IC (HEX)
Conc. in Sample PrepBuffer (copies/µl)	Conc. in PCR reaction (copies/µl)	Total replicates	Detection rate (%)	Mean Cq (STDV)	MeanCq (STDV)	Mean Cq (STDV)
0.6	0.15	20	100%	35.35 (0.78)	34.80 (0.53)	20.90 (0.15)
0.4	0.1	20	100%	36.20 (0.53)	35.57 (0.91)	19.58 (0.25)
0.2	0.05	20	75%	36.54 (0.90)	36.24 (1.20)	19.39 (0.17)

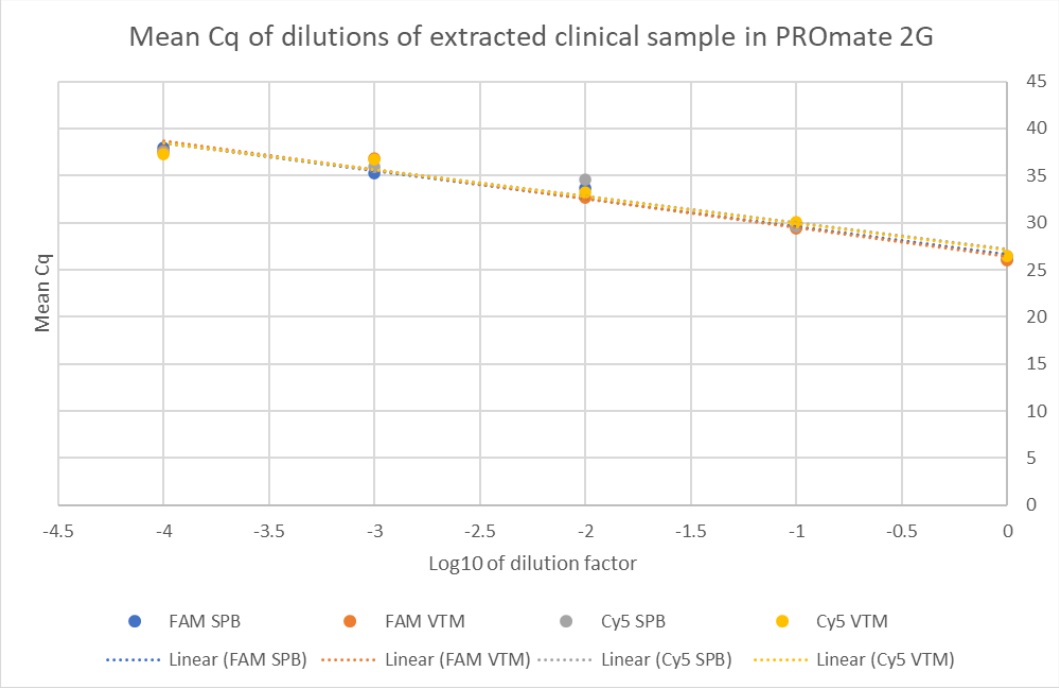
Using SARS-CoV-2 genomic RNA extracted from a known positive clinical sample, the potential effect of the presence of 40 µl of VTM on assay performance was further tested across 5 orders of magnitude. Firstly, 45 anterior nasal swabs were swirled in 1 ml of Sample Preparation Buffer containing the RNase inhibitor/IC bead, whilst 15 anterior nasal swabs were swirled in Viral Transport Media (VTM). 500 µl of these samples were contrived with

5 µl of the neat extracted SARS-CoV-2 genomic RNA, or 5 µl of a set of four ten-fold dilutions. 40 µl of each sample (SPB or VTM) was then added into 1 ml of SPB, also prepared with the RNase inhibitor/IC bead. 5 µl of these mixtures were then loaded into the q32 magazine and the assay run on the q32 instrument.

The Cq of the replicate of each dilution was checked, with those on the FAM channel >38.14 removed from analysis as they are over the Limit of Blank (see section 15.2). The ability of the assay to determine a positive sample was the same whether prepared fully in SPB or in the presence of 40 µl of VTM.

Conc. in PCR reaction (copies/µl)	Detection rate in SPB only	Detection rate in samples with 40 µl of VTM
Neat extracted RNA	100%	100%
1:10 dilution	100%	100%
1:100 dilution	100%	100%
1:1,000 dilution	100%	100%
1:10,000 dilution	66.7%	66.7%

Furthermore, plotting the Mean Cq from the two assay channels of each dilution shows equivalent assay performance across 5 orders of magnitude, confirming that 40 µl of patient samples in VTM are suitable for the testing of clinical performance of the PROmate® COVID-19 2G assay (section 16).



15.2. Limit of Blank

The Limit of Blank (LoB) is the highest apparent analyte concentration that is expected to be detected in the FAM channel only when replicates of blank sample containing no analyte are tested. Positive amplification of Cq higher than the LoB in the FAM channel will be concluded as negative.

The LoB was determined by analysing 30 negative samples each in two batches of PROmate® COVID-19 2G and the Cq values generated were used to determine what level of FAM amplification could be considered insignificant.

The LoB of the assay is 38.14 Cq and any Cq higher than this value in the FAM channel should be concluded as negative.

15.3. Analytical Specificity

This study aims to assess the inclusivity and exclusivity for the PROmate® COVID-19 2G 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. In addition, the *in silico* analysis also evaluated assay inclusivity.

Inclusivity

To ensure the COVID-19 primers/probe remain 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 15th of December 2021, *in silico* analysis confirms the COVID-19 assay primers and probe still show 99.8% and 99.9% detection with the 5,371,630 and 5,409,824 full length, good quality SARS-CoV-2 sequences at the ORF1ab and Nsp16 gene, respectively, as published on the GISAID EpiCoV database.

Exclusivity

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. This was done by using the NATtrol™ Respiratory Verification Panel.

Overall, the data demonstrates that the assay exhibits no cross-reactivity with any of the panel members chosen for this study and maintains the expected inclusivity and exclusivity criteria outlined in the Design Inputs of this study. *M pneumoniae* was reported as negative due to the Cq being >38.14 (above the LoB).

Panel Member	FAM Cq	HEX Cq	Cy5 Cq
Influenza AH1	N/A	26.36	N/A
Influenza AH3	N/A	26.05	N/A
Influenza A H1N1pdm	N/A	26.17	N/A
Influenza B	N/A	26.95	N/A
RSV A	N/A	25.91	N/A
Parainfluenza 1	N/A	26.03	N/A
Parainfluenza 2	N/A	26.36	N/A
Parainfluenza 3	N/A	25.91	N/A
Parainfluenza 4	N/A	26.01	N/A
Adenovirus 3	N/A	25.16	N/A
Metapneumovirus	N/A	31.83	N/A
Rhinovirus	N/A	27.90	N/A
Coronavirus OC43	N/A	29.89	N/A
Coronavirus 229E	N/A	25.28	N/A
Coronavirus NL63	N/A	25.95	N/A
Coronavirus HKU-1	N/A	25.44	N/A
B. pertussis	N/A	26.17	N/A
C. pneumoniae	N/A	26.43	N/A
M. pneumoniae	38.30*	25.86	N/A
Negative Control	N/A	25.71	N/A
SARS-CoV-2	25.64	26.98	25.06

*Reported as negative due to Cq > 38.14 (LoB)

15.4. Accuracy

Diagnostic accuracy of the PROmate® COVID-19 2G assay was determined by generating a Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA). Samples were tested blind with PROmate® COVID-19 2G and compared with the contrivance status (30 positives and 30 negatives in total) to produce the percentage agreements and 95% Confidence Intervals (CI). Samples were contrived with SARS-CoV-2 whole genome extracted RNA at 5x Limit of Detection (LoD). The OPA, PPA, NPA and the 95% CIs are detailed in the tables below.

Results for the blind contrivance accuracy study using PROmate® COVID-19 2G.

		Contrivance Status		
		Positive	Negative	Total
Candidate method (PROmate® COVID-19 2G)	Positive	30	1	31
	Negative	0	29	29
	Total	30	30	60

Agreement	Level	95% CI
OPA	98.3%	91.2% - 99.7%
PPA	100%	88.7% -100.0%
NPA	96.7%	83.3% - 99.4%

Overall, the percentage agreements for the PROmate® 2G kit are above the required specification of 90% on the genesig® q32 Real-Time PCR instrument.

15.5. Interfering Substances

The effects of potential exogenous and endogenous interfering substances present within anterior nasal samples on PROmate® COVID-19 2G was assessed. Changes in the assay performance were analysed by comparing Cq values of samples containing the potential interfering substances and samples without them. From all the substances analysed (see table below), only blood significantly interfered with PROmate® COVID-19 2G assay, with a delay in the Cy5 channel. Therefore, it is recommended that if a swab has visible blood on it, it should be discarded, and a new sample taken.

Table of Interfering Substances and the tested concentrations

Interfering Substance	Tested concentration
Nasal Corticosteroid (Dynamista)	2.38 mg/ml
Nasacort	4.739%
Blood	0.007 g/ml
Tobramycin	0.028 mg/ml
Guaifenesin	0.004 mg/ml
Dexamethasone	1.450 µmol/L
Oseltamivir	$3.8x10^{-4}$ mg/ml
Oxymetazoline	5.7×10^{-9} mg/ml
Mulpirocin	1.422 µg/ml
Fluticasone	0.047 mg/ml
Mucin	0.190 mg/ml

15.6. Precision

Assessment of repeatability (intra-run) and reproducibility (inter-run) of PROmate® COVID-19 2G was performed by contriving with a known copy number of extracted whole viral genome SARS-CoV-2 RNA. Precision was performed on three batches of the assay, each at three contrivance levels, reproducing a high, medium and low viral load sample:

- High viral load sample: 6 copies/µl (15x LoD*)
- Medium viral load sample: 4 copies/µl (10x LoD*)
- Low viral load sample: 2 copies/µl (5x LoD*)

*Contrivance level concentrations are copies/µl in the sample prep buffer and were based on the analytical sensitivity of the assay from
Section 15.1.

Variance was assessed from operators, instruments and day of testing. Two different operators performed the study over two days with two genesig® q32 Real-Time PCR instruments and a total of 10 replicates were obtained for each contrivance level.

The precision was measured by reporting the % Coefficient of Variance (%CV) for the SARS-CoV-2 targets in the FAM and Cy5 channels and the IC in the HEX channel. The assay achieved an accepted industrial standard of below 9% CV for the SARS-CoV-2 targets for all 3 batches and all variances. Although two batches showed higher variability for the IC in the HEX channel, this can be attributed to potential inhibition from the extracted SARS-CoV-2 viral RNA template that was contrived into the Sample Preparation tube. Level of inhibition could vary between samples, causing higher variation in the Cq of the internal control and is not a direct indication of the precision of the assay itself.

Summary of repeatability and reproducibility for the PROmate® COVID-19 2G assay (FAM, HEX, Cy5 channels, 3 batches)

		Coefficient of variance (%) for PROmate® COVID-19 2G Batch 1			
Conc. in Sample Prep Buffer (copies/µl)	Target channel	Repeatability	Inter-Instrument	Inter-operator	Inter-day
6	FAM	1.66	5.81	5.68	5.33
	HEX	1.82	9.44	8.90	7.15
	Cy5	0.66	4.18	4.30	3.63
4	FAM	0.72	4.60	4.41	4.82
	HEX	0.93	6.68	6.16	6.15
	Cy5	0.85	4.87	4.69	4.99
2	FAM	1.58	5.48	5.90	5.66
	HEX	1.25	6.43	6.92	8.14
	Cy5	1.07	5.03	4.86	4.88

Conc. in Sample Prep Buffer (copies/µl)	Target channel	Coefficient of variance (%) for PROmate® COVID-19 2G Batch 2			
		Repeatability	Inter-Instrument	Inter-operator	Inter-day
6	FAM	0.47	3.26	3.52	4.41
	HEX	1.21	6.92	7.31	8.04
	Cy5	0.66	3.91	3.90	4.70
4	FAM	1.22	5.39	5.17	5.34
	HEX	2.37	10.13	9.49	9.96
	Cy5	1.38	5.67	5.25	5.56
2	FAM	1.21	4.97	5.12	5.42
	HEX	1.30	7.18	7.35	7.20
	Cy5	1.07	5.04	4.81	4.88

Conc. in Sample Prep Buffer (copies/µl)	Target channel	Coefficient of variance (%) for PROmate® COVID-19 2G Batch 3			
		Repeatability	Inter-Instrument	Inter-operator	Inter-day
6	FAM	0.68	4.49	4.55	4.53
	HEX	0.79	5.65	5.66	5.11
	Cy5	0.56	4.33	4.82	4.54
4	FAM	0.78	4.35	4.67	4.23
	HEX	0.87	6.73	5.86	7.31
	Cy5	0.88	4.77	4.99	4.60
2	FAM	1.61	5.97	5.84	6.31
	HEX	0.46	5.45	4.99	7.10
	Cy5	1.36	6.28	5.58	5.92

16. Clinical Performance Evaluation

An initial clinical performance validation study sought to evaluate the direct-to-PCR workflow of the PROmate® COVID-19 2G assay. This was a retrospective study using 465 archive samples, stored as VTM suspensions, from the laboratory at the Queen Elizabeth Hospital,NHS Gateshead. The samples were collected from patients suspected of having

COVID-19,both symptomatic and asymptomatic.

Without access to the original swabs, it was necessary to use 40 µl of VTM as a replacement for the 'swab head'. Using archived samples in this fashion required demonstrating the commutability of adding 40 µl VTM to the PROmate Sample Preparation Buffer tube, versus an equivalent volume of fresh Sample Preparation Buffer. Once commutability was established (section 15.1.1), the comparison of methods proceeded.

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 465 samples, 181 gave true positive results, 282 gave true negative results whilst 2 gave false negative results. This gave diagnostic sensitivity of 98.9% (95% CI 96.2%-99.9%) and diagnostic specificity of 100% (95% CI 98.7%-100%).

Contingency table for PROmate® COVID-19 2G Clinical Performance Evaluation - VTM samples

		Comparator assays (TaqPath™ and Xpert®)/Resolver assay (genesig® Real-Time PCR COVID-19 (CE-IVD))		
		Positive	Negative	Total
PROmate® COVID-19 2G	Positive	181	0	181
	Negative	2	282	284
	Total	183	282	465

Clinical performance of PROmate® COVID-19 2G assay

	%	95% CI
Diagnostic Sensitivity	98.9	96.2-99.9
Diagnostic Specificity	100.0	98.7- 100

A second clinical performance study was undertaken to assess the sampling workflow without adaptations. In this workflow, following the inactivation of the dry swab in Sample Preparation Buffer containing RNase inhibitor and IC, specimens were either tested directly in PROmate mastermix as per the IFU, or 100 was extracted using the exsig™ Mag Extraction System on the Kingfisher Flex Purification System using the standard exsig™ Mag protocol and eluting in 100 µl to keep the input and output volumes the same. The extracted specimens were tested on the VIASURE SARS-CoV-2 assay in parallel with the genesig® Real-Time PCR Coronavirus (COVID-19) CE-IVD assay. Using these two comparators as a composite reference standard allowed for a greater degree of certitude in determination of diagnostic status for each sample.

Overall, this clinical performance study tested 316 clinical specimens. 33 samples were excluded due to discordant comparator results, and 5 were excluded where there was no available result for one or more assay, or due to technical IC/IEC failure on one or more assays.

This study has therefore allowed the assessment of 278 unique samples with the PROmate® COVID-19 2G assay across the full viral range. Of the 278 samples, 103 gave true positive results, 168 gave true negative results, and there were 5 false positive results, and 2 false negative results. Together they confirm an overall diagnostic sensitivity of 98% (95% CI 93.3%-99.8%) and diagnostic specificity of 97% (93.4%-99.1%).

Contingency table for PROmate® COVID-19 2G assay Clinical Performance Evaluation - dry anterior nasal swabs

		Composite Reference Standard		
		Positive	Negative	Total
genesig® COVID-19 3G	Positive	103	5	108
	Negative	2	168	170
	Total	105	173	278

Overall clinical performance of PROmate® COVID-19 2G assay

	%	95% CI
Diagnostic Sensitivity	98.1%	93.3% to 99.8%
Diagnostic Specificity	97.1%	93.4% to 99.1%

From the positive samples the FAM reading in the VIASURE assay (or the alternative SARS-CoV-2 target reading in ROX when no FAM Cq available), can be used to divide the cohort of samples into low, low-medium, medium-high, and high Cq ranges. Consequently, the sensitivity of the PROmate® COVID-19 2G assay in each Cq range can also be determined:

Clinical sensitivity of PROmate® COVID-19 2G assay in different Cq ranges

Cq in comparator	Number of samples	% of samples	% sensitivity of genesig® COVID-19 3G	95% CI
<25	15	14.29	100.0%	78.2% to 100.0%
≥25 to <30	42	40.00	100.0%	91.6% to 100.0%
≥30 to <35	27	25.71	100.0%	87.2% to 100.0%
≥35	21	20.00	90.5%	69.6% to 98.8%

17. Disposal

Dispose the unused kit reagents, human samples, and sealed post-amplification plates as clinical laboratory waste according to national regulations. Refer to **Section 7** for guidance weblinks.

The PROmate® COVID-19 2G Sample Preparation Buffer contains Triton X 100 Reduced and is very toxic to aquatic life. Do not let product enter drains and any discharge into the environment must be avoided.

18. Primerdesign™ Ltd Quality Control

In accordance with Primerdesign™ Ltd ISO 13485 certified Quality Management System, each batch of the PROmate® COVID-19 2G assay is tested against predetermined specifications to ensure consistent product quality.

Primerdesign™ Ltd performs 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.

19. Technical Support

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

Phone: +44 (0) 800 0156 494

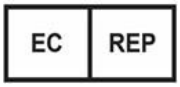
Email: support@primerdesign.co.uk

20. Trademarks and Disclaimers

Trademarks: PROmate®, genesig® and the Primerdesign™ logo.

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

21. Explanation of Symbols

	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)
	Single Use Only
	EU Authorized Representative
	Positive Control

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