Primerdesign™ Ltd

exsig® COVID-19 Direct

96 reactions

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

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

CE IVD

Instructions for Use (IFU)

Issue 3.00

15th December 2020

EXSIG

Workflow Solutions by Primerdesign

exsig® COVID-19 Direct

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

For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	exsig® Direct (Extraction Only)	Applied Biosystem® 7500 Bio-Rad CFX Connect™ Roche® LightCycler 480 II FluoroCycler®XT (Bruker Hain Lifescience) QuantStudio 5 (Thermofisher Scientific) genesig® q16/ q32 (Primerdesign, Novacyt)
Anterior Nasal Swabs		

96 tests

CE IVD

REF Z-exsig™ COVID-19 direct

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1. Intended Use

The exsig® COVID-19 Direct assay is a CE marked, *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from SARS-CoV-2 from nasopharyngeal and anterior nasal specimens prepared on dry swabs. The assay provides rapid screening of individuals suspected of SARS-CoV-2 infection and aids the diagnosis of suspected COVID-19 in patients. The assay is intended for use with the designated PCR platforms listed in **Section 7**.

SARS-CoV-2 is generally detectable in specimens during the acute phase of infection and during asymptomatic infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out co-infection with other bacteria or other 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 combined with clinical observations, patient history, and epidemiological information.

The exsig® COVID-19 Direct (CE IVD) assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in handling SARS-CoV-2 positive samples and the techniques of real-time PCR and *in vitro* diagnostic procedures.

Specimen test results are available to interpret in under 2 hours using the exsig® COVID-19 Direct (CE IVD) assay. This time includes the processing of the swab sample, the PCR set-up and the PCR run time.

2. Summary and Explanation

An outbreak of pneumonia of unknown aetiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) in December 2019. Chinese authorities identified a novel coronavirus SARS-CoV-2 (previously called 2019-nCoV) which has resulted in confirmed human infections worldwide and cases of COVID-19 disease. Symptoms of COVID-19 disease include severe respiratory illness and has resulted in the death of patients. Patients can become infected with SARS-CoV-2 virus by person-person contact (through contact with a contaminated environment or person).

The exsig® COVID-19 Direct (CE IVD) assay is a combination of a 'direct to PCR' sample processing method and a molecular *in vitro* diagnostic test for the detection of the SARS-CoV-2 RNA from nasopharyngeal and anterior nasal dry swabs. The viral RNA is released from the swab sample via the addition of a viral inactivation / lysis agent. Following the sample preparation process, the resulting lysate is tested using well-established nucleic acid amplification technology. The PCR assay contains oligonucleotide primers and dual-labeled hydrolysis probes, as well as control material, for use in Real-Time RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA.

3. Principles of the Procedure

Viral RNA is released from nasopharyngeal and anterior nasal dry swabs using the 'direct to PCR' exsig® COVID-19 Direct technology. Using polymerase chain reaction (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 (CE IVD) assay is supplied as part of the exsig® COVID-19 Direct (CE IVD) product. The genesig® COVID-19 (CE IVD) assay includes primers and probe mix which contains a SARS-CoV-2 specific probe labelled with the FAM fluorophore. The primer/probe mix also includes primers and probes to amplify and detect the internal extraction control RNA template supplied (genesig® Easy RNA Internal Extraction control (IEC)). The IEC specific probe is labelled with the HEX fluorophore.

The genesig® Easy RNA Internal Extraction control template is added to the sample during the exsig® COVID-19 Direct (CE IVD) sample preparation to provide an RNA template control, detect PCR inhibition and confirm the integrity of the PCR run. The genesig® Easy RNA Internal Extraction control template is not related to the SARS-CoV-2 viral sequence. The purpose of the internal control is to monitor the integrity of the PCR run and inhibition in patient samples, it does not confirm the presence of human material.

The oligonucleotide primers and probe for the detection of SARS-CoV-2 were selected from the orf1 ab genomic region. The supplied primer/probe mix is designed for the specific detection of SARS-CoV-2 RNA (probe labelled with FAM fluorophore) and the supplied genesig® Easy RNA Internal Extraction control (IEC specific probe is labelled with HEX fluorophore).

PCR amplification has been validated using the following Real-Time PCR instruments: Applied Biosystems® 7500 Real-Time PCR System (software version 2.3), Roche® LightCycler 480 II (software version 1.5.1.62 SP3), Bio-Rad CFX Connect™ Real-time PCR Detection System (software 1.1), FluoroCycler® XT (FC XT 101, Bruker Hain Lifescience), QuantStudio 5 (ThermoFisher Scientific, software v 1.4.3) the genesig® q16 (Primerdesign, Novacyt Group, software version 2.10.1) and the genesig® q32 (Primerdesign, Novacyt, software version 1.2.2).

4. Materials Provided

The exsig® COVID-19 Direct (CE IVD) assay is comprised of the following two packs:

exsig® COVID-19 Direct sample preparation pack

Reagent label	Number of Vials 96 tests	Volume (ml per vial)	Lid colour	Resuspended with:
exsig® Sample Preparation Buffer (small)	1	9.45	Green	n/a
exsig® Sample Preparation Buffer (large)	1	113.4	White	n/a
Viral Inactivation Buffer	1	1.12	Yellow	n/a
PCR Optimiser	1	0.22	Red	n/a

genesig® Real Time PCR Coronavirus (COVID-19) CE IVD assay

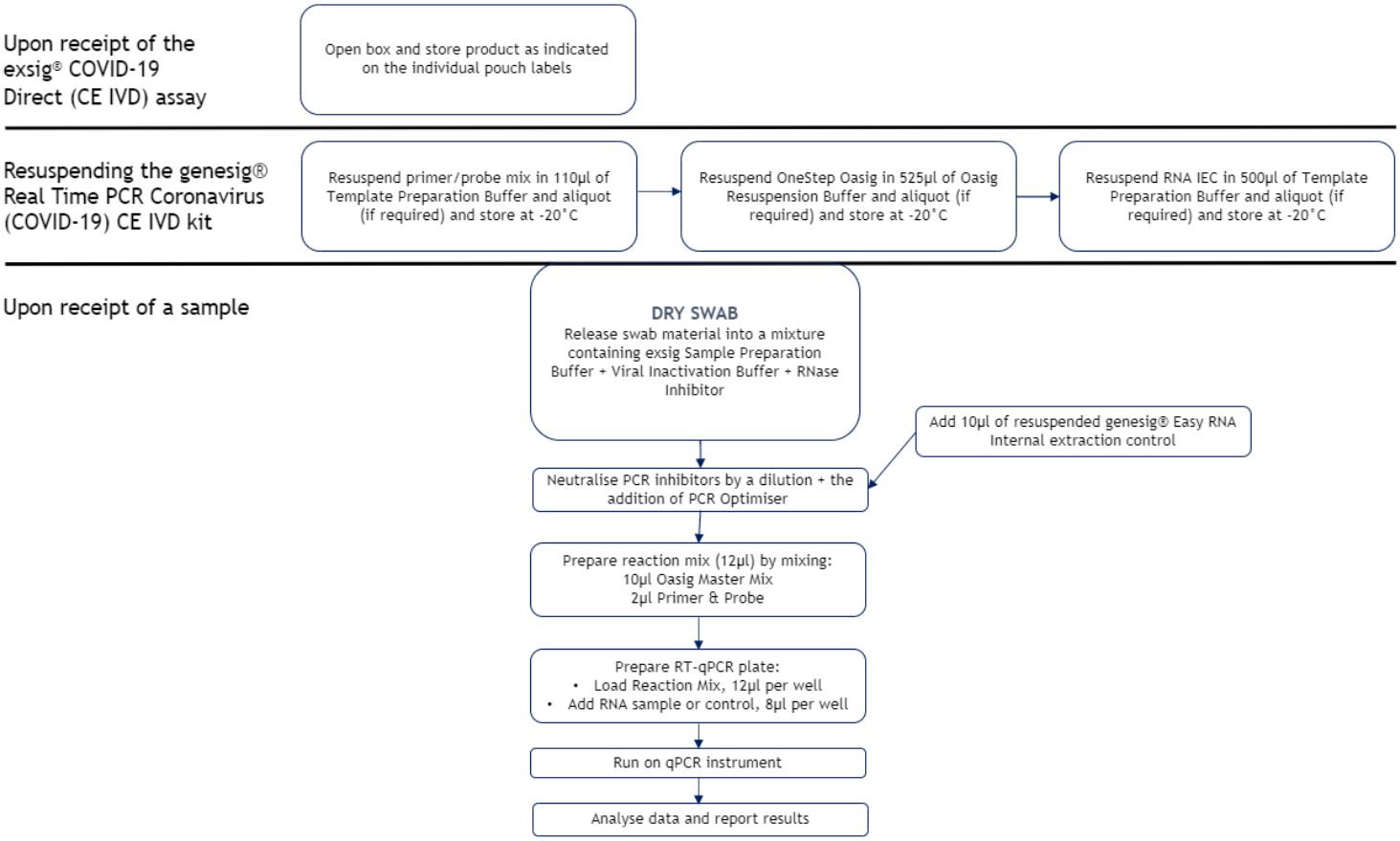
Reagent label	Number of Vials 96 tests	Volume (µl per vial)	Lid colour	Resuspended with:
Oasig™ OneStep 2X RT-qPCR Master Mix Lyophilised	2	525*	Red	Oasig™ resuspension buffer
COVID-19 Primer & Probe Mix (including IEC primer/probe mix)	2	110*	Amber	Template preparation buffer
Oasig™ resuspension buffer	2	750	Blue	n/a
Template preparation buffer	2	1500	Yellow	
Water RNase/DNase Free	1	1500	White	
genesig® COVID-19 Positive control template	1	600*	Red, vial stored in sealed foil pouch	Template preparation buffer
genesig® Easy RNA Internal extraction control (IEC)	2	500*	Blue, vial stored in sealed foil pouch	Template preparation buffer

*The projected volume once resuspended

The COVID-19 Primer & Probe Mix contains the primers and FAM labeled probe specific to SARS-CoV-2, and includes the primers and HEX labeled probe specific to the genesig® Easy RNA Internal extraction control (IEC).

The Oasig™ OneStep 2X RT-qPCR Master Mix, COVID-19 Primers & Probes Mix, genesig® COVID-19 Positive control template and genesig® Easy RNA Internal extraction control (IEC) are all provided lyophilised. The table above indicates which buffer to use, as well as the volume to add, to resuspend these reagents.

5. Summary of Preparation and Testing Process



6. Required Equipment and Consumables (Not Provided)

- PCR hood
- Benchtop microcentrifuge
- Vortex mixer
- Adjustable micropipettes (2 or 10µl, 20 µl, 200µl and 1000µl)
- Aerosol barrier pipette tips with filters
- Disposable gloves
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNA/RNA remover
- qPCR reaction plates compatible with the Real time PCR instrument to be used
- RNase/DNase free water
- Plate seal
- 1.5 ml microcentrifuge tubes or deep well microtiter plate
- Dry heating block, capable of heating 1.5 ml tubes at 56˚C±1˚
- Ice (or cold block capable of holding 1.5ml tubes)
- RNase Inhibitor*

*RNase inhibitor is not provided but is highly recommended to be used during sample preparation. Contact your local Primerdesign representative for a compatible RNase Inhibitor, Catalogue no:
Z-RNase-inhibitor

7. Real-Time PCR instruments

The exsig® COVID-19 Direct (CE IVD) assay has been validated with the following Real-Time PCR instruments:

- Applied Biosystems® 7500 Real-Time PCR System (software version 2.3)
- Roche® LightCycler 480 II (software version 1.5.1.62 SP3)
- Bio-Rad CFX Connect™ Real-Time PCR Detection System (Maestro™ software version 1.1)
- FluoroCycler® XT (FC XT 101, Bruker Hain Lifescience)
- QuantStudio 5 (ThermoFisher Scientific, QuantStudio Design & Analysis Software v 1.4.3)
- genesig® q16 (Primerdesign, Novacyt Group, software version 2.10.1)
- genesig® q32 (Primerdesign, Novacyt Group, software version 1.2.2)

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

8. Facilities/Training Requirements

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures:

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

Refer to the World Health Organization Interim guidance on laboratory biosafety: www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance from 13 May 2020.

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

9. Warnings and Precautions

9.1 General

- For in vitro diagnostic use (IVD) only.
- Handle all specimens as if infectious using safe laboratory procedures. Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) microbiological safety cabinet (refer to the guidance detailed in **Section 8**).
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited) gloves, eye protection and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes and other equipment and reagents.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
    - The exsig® COVID-19 Direct (CE IVD) assay components "exsig® Sample Preparation Buffer" and "Template Preparation Buffer" contain EGTA. This component should be handled according to the SDS. In the event of damage to protective packaging, contact Primerdesign for instructions.
    - The exsig® COVID-19 Direct (CE IVD) assay component "Viral Inactivation 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.

9.2 Preventing Contamination

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
- The genesig® COVID-19 positive control template is provided in a sealed foil envelope and contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
    - Maintain separate areas for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
    - Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
    - Wear a clean lab coat and disposable gloves when setting up assays.
    - Change gloves regularly and whenever contamination is suspected.
    - Keep reagent and reaction tubes capped or covered as much as possible.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimise the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the sample preparation procedure. Good aseptic technique should always be used when working with nucleic acids.
    - When mixing reagents by pipetting up and down, this should be done with a volume roughly equal to 50% of the total component volume.
    - **DO NOT** use water to resuspend the kit components. 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, ethanol, DNA/RNA remover) 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 a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.
- Dispose of human specimens according to national and international regulations (refer to guidance detailed in **Section 8**).

9.3 Prevent DNase/RNase contamination

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

10. Reagent Storage, Handling and Stability Conditions

10.1 Storage conditions

- The exsig® COVID-19 Direct (CE IVD) assay is shipped at ambient temperatures but must be opened upon arrival to allow the individual components to be stored as indicated on the pack & tube labels.
- The exsig® COVID-19 Direct components should be stored in the original packaging at the temperatures indicated on the individual tube labels and are stable until the "use by" date indicated on the tubes.
    - The genesig® Real-Time PCR COVID-19 (CE IVD) pack should be stored in the original packaging at -20°C and is stable until the "use by" date indicated on the pack label.
    - Once resuspended, the genesig® Real-Time PCR COVID-19 (CE IVD) pack components may be aliquoted into smaller volumes, if required, and are stable for up to six months if stored at -20°C.
    - Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
    - Primer/probe mix, the enzyme master mix, positive control template and RNA internal extraction control 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.**
- If the kit's protective packaging is damaged upon receipt, please contact Primerdesign for instructions. Attention should be paid to the "use by" date specified on the individual tube and pack labels. On this date, the kit should be discarded following the disposal instructions in **Section 19.**
- Always check the expiration date prior to use. Do not use expired reagents.

10.2 In Use Stability

- When *in use* all exsig® COVID-19 Direct (CE IVD) assay kit components should be promptly returned, after each usage, to their appropriate storage conditions to minimise the time at room temperature.
- The exsig® COVID-19 Direct (CE IVD) components should be stored according to conditions listed on individual vials and are stable until the "use by" date indicated on the tube labels.
    - The PCR Optimiser can be stored at 2-8°C but, once opened, should be stored at -20°C. When stored at -20°C, it must not be subjected to > 5 freeze-thaw cycles.
- The genesig® Real-Time PCR COVID-19 (CE-IVD) pack should be stored in the original packaging and is stable for up to six months once resuspended and stored at -20°C.
    - Repeated thawing and freezing of the resuspended genesig® Real-Time PCR COVID-19 (CE-IVD) pack should be kept to a minimum and should not exceed 5 freeze-thaw cycles. Components may be aliquoted into smaller volumes after resuspension, if required.

11. Specimen Collection, Handling and Storage

11.1 Compatible Specimens

- This product is intended for use with dry swabs only
- This product is not intended for use with swab material stored in viral transport media, guanidinium thiocyanate-containing media or any other liquid collection solution. Use of media not supplied as part of this product will impact the limit of detection of the device
- **Samples that present with obvious blood or other particulate matter are NOT compatible with PROmate™ COVID-19 and should be discarded**

11.2 Collecting the Specimen Compatible Specimens

Sampling should be conducted with the correct swab type and collected by a trained professional, following the correct sampling technique. CDC guidance on collection of anterior nasal swabs can be found here: https://www.cdc.gov/coronavirus/2019-ncov/downloads/community/COVID-19-anterior-self-swab-testing-center.pdf

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

- Swab specimens should be collected and placed in a clean, dry, sterile transport tube.
    - **note: to achieve the highest levels of sensitivity the swab should be stored dry.**

- Swab specimens 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:

    - Swab samples must be transported within 24 hours or stored refrigerated
    - If the swab is stored at 2-8°C, the specimen must be tested within 72 hours.

- If testing cannot be conducted within 72 hours, the swab specimen should be frozen at -70˚C or colder until testing is able to be conducted.

- For further specimen guidance 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 13 May 2020: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
  - Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)

  https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

- The Viral Inactivation Buffer supplied in the exsig® COVID-19 Direct (CE IVD) product contains Triton X-100 reduced (Triton X-100 replacement) which has been utilised for use in the inactivation of other highly pathogenic agents (eg. General Procedures for Inactivation of Potentially Infectious Samples with Ebola Virus and Other Highly Pathogenic Viral Agents, WHO 2014). The use of Triton X-100 has been validated by Public Health England (HCM-CoV-2-006-Version3). Therefore, samples should be handled according to these revised national guidelines for sample management prior to inactivation.

- Follow specimen collection devices manufacturer instructions for proper collection methods.

- Swab specimens 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.

11.2 Transporting Specimens

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

11.3 Storing Specimens

- During sample processing, specimens should be tested immediately. If this is not possible, samples may be stored, after the dilution in exsig® Sample Preparation Buffer, for a maximum of 7 days at –70˚C.

12. genesig® Real Time PCR Coronavirus (COVID-19) CE IVD Reagent Preparation

12.1 Oasig™ OneStep 2x RT-qPCR Master Mix (lyophilised) preparation

- Upon receipt, the dried master mix can be stored at -20ºC. Do not use after expiry date.
- Using aseptic technique, resuspend in 525µl of oasig™ resuspension buffer, gently swirl to mix.
- Store at -20ºC. Resuspended master mix is stable for up to six months when stored at -20ºC.
- Freeze/ thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.

12.2 COVID-19 and IEC Primer/Probe mix preparation

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

12.3 genesig® COVID-19 Positive control template preparation

- The genesig® COVID-19 Positive control template (PCT) is provided in a sealed foil envelope and contains a high copy number of templates. It should be opened and processed away from clinical specimens and kit components to avoid cross-contamination.
- The PCT tube contains synthetic DNA representing the SARS-CoV-2 genomic region of interest. Following resuspension, this will be at a concentration of 1.7 x 10^5 copies per µl.
- Caution: This reagent contains a high copy number of positive control material and should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination of other kit reagents and clinical specimens.
- Upon receipt, the dried PCT can be stored at -20ºC for up to 18 months or the expiry date, whichever occurs first.
- Using aseptic technique, resuspend the dried PCT in 600µl of Template preparation buffer, vortex to mix. Resuspended PCT is stable for up to six months when stored at -20ºC.
- Freeze/ thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.
- To ensure PCR run validity, the PCT should produce amplification in the FAM channel.

12.4 genesig® Easy RNA Internal extraction control (IEC) preparation

- The genesig® Easy RNA Internal extraction control (IEC) can be added during the sample preparation to provide an RNA template control, detect PCR inhibition and confirm the integrity of the PCR run.
- Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination.
- Upon receipt, the dried IEC can be stored at -20ºC for up to 18 months or the expiry date, whichever occurs first.
- Using aseptic technique, resuspend the dried IEC in 500µl of Template preparation buffer, vortex to mix. Resuspended IEC is stable for up to six months when stored at -20ºC.
- Freeze/ thaw cycles should be minimised and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.

12.5 Negative Extraction Control (NEC) preparation

- Prepare at least 1 negative extraction control (NEC) every time a cohort of swab samples are processed with the exsig® COVID-19 Direct (CE IVD) assay. For example, each PCR run should include a minimum of 1 NEC, prepared in parallel with the clinical samples.
- The NEC will serve as the negative control for the entire testing system and to check for contamination during PCR plate set-up.
- The NEC is prepared by processing 1ml of RNase/DNase free water as if it were a sample containing swab specimen material. This NEC is processed in parallel to the clinical samples and the IEC is added as normal.

13. General Preparation

13.1 Equipment Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use.
- Decontamination agents should be used such as 10% bleach, 70% ethanol, and an RNA/DNA remover to minimise the risk of nucleic acid contamination.

14. Assay Set Up

14.1 Sample Preparation Procedure

Refer to **Section 11** for appropriate specimen collection, storage and transport conditions.

	Nasopharyngeal and anterior nasal swabs
Collection	Swabs: Dacron or polyester flocked swabs in a sterile container (either dry or in PBS)
Transport temperature*	2-8ºC ≤ 72hrs
Short-term storage (pre-extraction) *	2-8ºC ≤ 72hrs
Long-term storage (pre-extraction) *	≤ -70˚C for longer periods

*These are CDC recommendations: CDC 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, Local regulations pertaining to sample handling may also apply.

Prepare at least 1 negative extraction control (NEC) every time a cohort of swab samples are processed with the exsig® COVID-19 Direct (CE IVD) assay. For example, each PCR run should include a minimum of 1 NEC, prepared in parallel with the clinical sample being tested. This NEC will serve as the negative control for the entire testing system.

14.2 Swab Specimen Processing

14.2.1 Dry Swab

a) Warm a heating block to 56˚C, prior to starting this procedure.
b) Prepare a sample suspension mix of the following reagents in a DNase/RNase free falcon tube (or equivalent):
    a. Determine the number of samples (n) to be processed. Remember to include the NEC in your calculation. It is necessary to make excess reaction mix to allow for pipetting error. Use the following guide to determine volume of reagents to add to the reaction mix:
        i. If number of samples (n) is ≤ 10, then **N = n+1**
        ii. If number of samples (n) is > 10 and ≤ 20, then **N = n+2**
        iii. If number of samples (n) is > 20, then **N = n+10% of total number of samples**

Sample Suspension Mix Component	Volume required for 1 sample (µl)	Volume required for n samples (µl)
exsig® Sample Preparation Buffer	1000	1000 x N
Viral Inactivation Buffer*	1	1 x N
RNase Inhibitor (40U/µl)**	1	1 x N

*A *minimum of 10 samples should be processed to avoid pipetting < 10µl of Viral Inactivation Buffer*

*** RNase Inhibitor is not provided with the kit. Refer to **section 6***

c) Pulse vortex the sample suspension mix 3 times.
d) Add 1000µl of the sample suspension mix to the required number of tubes (1 for the NEC and 1 per swab specimen).
e) Place each swab into the tube/well designated for that sample and vigorously twirl for 10 seconds to release the specimen into suspension. **Perform the following sample processing stages inside a microbiological safety cabinet. Do not add swab material to the NEC tube.**
f) As the swab is removed from each tube, press the swab head against the inside wall to release any excess solution from the swab. These are the **Sample Suspension** tubes.
g) Dispose of the used swab in biohazard waste.
h) Heat the **Sample Suspension** tubes at 56 ± 1°C for 2 minutes.
i) Place **Sample Suspension** tubes on ice immediately.
j) Dispense 45µl **exsig® Sample Preparation Buffer** into a fresh set of tubes (label 1 for the NEC and 1 for each swab specimen being processed). These are the **Sample Dilution** tubes.
k) Place the **Sample Dilution** tubes on ice (or on a cold rack).
l) Transfer 45µl of each **Sample Suspension** into the **Sample Dilution** tube designated for that sample.
m) Add 2ul of **PCR Optimiser** to each **Sample Dilution** tube (including the NEC).
n) Add 10µl of the resuspended **genesig® Easy RNA IEC** into each **Sample Dilution** tube (including the NEC).
o) Mix the **Sample Dilution** tubes by pipetting each solution up and down 3 times.
p) The samples (**Sample Dilutions**) are now ready for testing with the genesig® Real-Time PCR COVID-19 (CE IVD) assay. Proceed immediately to the PCR set up placing the tubes on ice (or on a cold rack) until you are ready to dispense into the PCR reaction plates.

Please note the following:

- The genesig® Easy RNA Internal extraction control (IEC) is supplied in the genesig® Real-Time PCR COVID-19 (CE IVD) assay pack and should be resuspended in 500µl template preparation buffer.
- **The internal extraction control should not be added directly to the clinical specimen/ sample before the clinical specimen/sample is mixed with exsig® Sample Preparation Buffer. Doing so may compromise the testing.**

14.3 genesig® Real Time PCR Coronavirus (COVID-19) CE IVD Reaction Mix Setup

a) Resuspend the COVID-19 probe tube in template preparation buffer, 110µl of buffer per tube, vortex to mix.
b) Resuspend the oasig™ OneStep 2X RT-qPCR Master Mix in 525µl oasig™ resuspension buffer, gently swirl to mix.
c) Plate set-up configuration can vary with the number of specimens. An NEC must be included in each plate set-up (refer to **Section 12.5** on how to prepare NEC). NTCs should be included in each plate set-up. A PCT must be included in each plate set-up.
    a. The PCT will be added after all other reagents and samples have been added to the plate.
    b. This will be in an area for handling nucleic acid and away from the NEC, NTC and any clinical specimen/ samples.
    c. This is to prevent plate set-up, reagent or specimen contamination with the PCT.
d) Prepare a reaction mix of the following reagents from resuspended components in a 1.5ml DNase/RNase free tube:
    a. 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 excess reaction mix to allow for pipetting error. Use the following guide to determine volume of reagents to add to the reaction mix:
        i. If number of samples (n) is ≤ 10, then **N = n+1**
        ii. If number of samples (n) is > 10 and ≤ 20, then **N = n+2**
        iii. If number of samples (n) is > 20, then **N = n+10% of total number of samples**

Reaction mix Component	Volume required for 1 sample (µl)	Volume required for n samples (µl)*
Oasig™ OneStep 2X RT-qPCR Master Mix	10	10 x N*
COVID-19 and IEC Primer & Probe	2	2 x N*

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

e) Add 12µl into the number of wells required for your testing, in an appropriate 96 well plate for your chosen PCR platform. Include 1 well for the PCT, 1 well for the NEC and 1 well for the NTC for each PCR plate.
f) Add 8µl of the following into the appropriate wells according to your plate setup:
    a. NEC (please refer to **Sections 12.5**)
    b. NTC
g) Temporarily cover the reaction plate and transfer to the specimen nucleic acid handling area.
h) Gently vortex the **Sample Dilution** from the processed clinical specimen/sample(s) tubes for approximately 1 second.
i) Briefly spin down (< 5 seconds) in a mini-centrifuge to collect contents at the bottom of the tubes, and then place tubes on ice (or on a cold rack).
j) Change gloves often and when necessary to avoid contamination.
k) Add 8µl of the **Sample Dilution** into the appropriate wells according to your plate setup.
l) Temporarily cover the entire reaction plate and move the reaction plate to the positive template control handling area.
m) Add 8µl of PCT (please refer to **Sections 12.3**) into the appropriate well according to your

plate set up. Seal the plate with an appropriate seal and place in the instrument.

14.4 Programming the Real-Time PCR Instrument

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

- Applied Biosystems® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (as per Applied Biosystems manual (2010)).
- LightCycler 480 instrument Operator's manual (July 2016, Addendum 4, Software version 1.5)
- Bio-Rad CFX Connect™ Real-Time PCR Detection System Instrument Guide (as per Bio-Rad Laboratories Inc. Manual (2017))
- FluoroCycler® XT (FC XT 101, Bruker Hain Lifescience)
- QuantStudio 5 (ThermoFisher Scientific, QuantStudio Design & Analysis Software v 1.4.3)
- genesig® q16 (Primerdesign, Novacyt, software version 2.10.1) (cycling conditions are preloaded in the software)
- genesig® q32 (Primerdesign, Novacyt, software version 1.2.2) (Cycling conditions are provided in template run files

a) Enter the following amplification program:

Steps	Time	Temperature	Cycles	Detection Format
Reverse Transcription	10 min	55°C	1	COVID-19 = FAM (465-510) RNA Internal Extraction Control (IEC) = VIC / HEX / Yellow555 (533-580)
Initial Denaturation (Taq Activation)	2 min	95°C	1	
Denaturation	10 sec.	95°C	45	
Annealing and Extension	60 sec.	60°C*		

*Acquisition must be performed at the end of this stage
When using Roche® LightCycler 480 II please select the following detection format: Dual Color Hydrolysis Probe / UPL Probe
When using the ABI 7500® please select 'none' for the dye to use as passive reference dye in the plate set up

b) Ensure wells loaded with clinical sample(s) are designated as "Sample Type – Unknown"
c) Ensure the well loaded with PCT is designated as "Sample Type – Positive Control"

15. Interpretation of Results

15.1 Acceptance Criteria of controls included in the exsig® COVID-19 Direct (CE IVD) assay

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

a) NEC is free from amplification in the FAM (465-510) channel and NEC produces positive amplification in the VIC/HEX/Yellow555 (533-580) channel (this is detection of the genesig® Easy RNA Internal Extraction control).
b) PCT produces a Cq of between 14-22 in the FAM (465-510) channel.

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

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

15.2 Interpretation of Patient Specimen Results

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

		SARS-CoV-2 Target (FAM (465-510))	
		Cq Positive	Cq Negative
IEC (VIC / HEX / Yellow555 (533-580))	Cq Positive	SARS-CoV-2 Positive*	SARS-CoV-2 Negative**
	Cq Negative	SARS-CoV-2 Positive*	Result invalid Repeat testing of sample

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

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

- The IEC Cq value produced by the patient sample should be < 27 and should not exceed the NEC IEC Cq value + 6 (i.e. sample RNA IEC Cq < NEC RNA IEC Cq + 6). Failure to satisfy this criterion indicates a compromised sample preparation and an invalid result; testing of the sample must be repeated. Please note that the NEC value is run specific variable.

16. Limitations of The Procedure

- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- As with any molecular test, mutations within the target sequence of SARS-CoV-2 could affect the genesig® COVID-19 primer and/or probe binding, resulting in failure to detect the presence of the virus.
- False negative results may be caused by:
    - Unsuitable collection, handling and/or storage of samples.
    - Sample outside of viraemic phase.
    - Failure to follow procedures in this handbook.
    - Use of unauthorised extraction kit or PCR platform.
- False positive results may be caused by:
    - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
    - Unsuitable handling of amplified product.
- All results should be interpreted by a 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.

17. Performance Evaluation

The exsig® COVID-19 Direct (CE IVD) assay performance evaluation has been generated on the Bio-Rad CFX Connect™ Real-Time PCR Detection System instruments for analytical sensitivity (LoD) with additional testing on the Applied Biosystems® 7500 Real-Time PCR system and Roche® LightCycler 480 II.

17.1 Analytical Sensitivity

17.1.1 Analytical Sensitivity Data genesig® q16 and q32

The genesgi® q16 and q32 instruments have faster cycling conditions and therefore run protocols (provided in software); as such the Limit of detection (LoD) is different for exsig® COVID-19 Direct on these two instruments. The LoD is defined as the lowest concentration of analyte that can be reliably detected with at least 95% confidence. The LoD of exsig® COVID-19 Direct (CE IVD) assay was validated by contriving nasal samples with whole SARS-CoV-2 viral genome RNA representing 6 isolates (MT007544.1, MN908947.3, LC528232.1, MT106054.1, MT188340 and MT118835). Two different donors were subjected to nasal swabbing at two different time periods in order to generate samples for the contrivance level at the below defined concentration, for determining the analytical sensitivity of PROmate™ COVID-19.

The analytical sensitivity for **dry swabs** is defined as **1.00** RNA copies/µl in the PCR reaction.

The LoD results are summarised in below:

Viral RNA Concentration in PCR reaction (copies/µl)	% Replicate Detection	Mean Cq	Run variability (Cq)
1.00	100%	35.80	0.61

17.1.2 Analytical Sensitivity Data from Applied Biosystem® 7500, Bio-Rad CFX Connect™, Roche® LightCycler 480 II, FluoroCyler®XT (Bruker Hain Lifescience) and Quanstudio 5 (Thermofisher Scientific)

The Limit of detection (LoD) is defined as the lowest concentration of analyte that can be reliably detected with at least 95% confidence. The LoD of exsig® COVID-19 Direct (CE IVD) assay was validated by contriving swab samples with whole SARS-CoV-2 viral genome RNA representing 6 isolates (MT007544.1, MN908947.3, LC528232.1, MT106054.1, MT188340 and MT118835).

The LoD was assessed over 3 days. Each day a fresh set of 3 contrived swab samples was used to produce 3 biological replicates of each contrivance level. The biological replicates of each contrivance level were tested in quadruplicate, producing 12 replicates in total per day. Across the 3 days, 36 technical replicates were produced for each contrivance level.

- The analytical sensitivity for **dry swabs** is defined as **0.48** RNA copies/µl in the PCR reaction

Viral RNA Concentration in PCR reaction (copies/µl)	% Replicate Detection	Mean Cq	Standard Deviation
1.52	100%	33.99	0.61
0.76	97.2%	36.09	1.48
0.48*	95.0%	n/a	n/a
0.38	94.4%	36.73	1.23
0.19	83.3%	37.14	0.94

***Determined via probit analysis of the empirical data**

17.2 Inclusivity

17.2.1 Latest in silico Specificity Analysis:

To ensure the COVID-19 primers and 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 16th of November 2020, *in silico* analysis confirms the COVID-19 assay primers and probe show 100% detection with the 137,221 full length, good quality SARS-CoV-2 sequences published on the GISAID EpiCoV database.

17.2.2 Analytical Specificity

Related Pathogens and pathogens that are likely to be present in the clinical specimen 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® Real-Time PCR COVID-19 (CE IVD) assay exhibited no cross-reactivity with non-SARS-CoV-2 species except for two sequences, Bat coronavirus (NCBI Accession No. MN996532.1) and Pangolin coronavirus (NCBI Accession No. MT084071.1) sequences. The primers/probe sequence has 5 mismatches and 7 mismatches respectively, with these viruses and therefore show limited possibility of being detected with the genesig® Real-Time PCR COVID-19 (CE IVD) assay.

In vitro testing:

For *in vitro* testing, 4 panels were sourced:

- Respiratory Evaluation Panel (Qnostics, Scotland, UK)
- QCMD panel from the 2019 Coronavirus EQA programme (Qnostics)
- Respiratory validation panel (ZeptoMetrix)
- Pneumonia Validation panel (ZeptoMetrix)

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

Virus	Strain	Source	Detected/Replicates	Final result
INF A H1N1 positive	-	Isolate	0/3	Negative
INF A H3N2 positive	-	Isolate	0/3	Negative
INF B Victoria	-	Isolate	0/3	Negative
INF B Yamagata	-	isolate	0/3	Negative
RSV A	-	isolate	0/3	Negative
RSV B	-	isolate	0/3	Negative
Coronavirus	NL63	isolate	0/3	Negative
Coronavirus	229E	isolate	0/3	Negative
Coronavirus	HKU	isolate	0/3	Negative
Coronavirus	OC43	isolate	0/3	Negative
Influenza AH1	-	isolate	0/3	Negative
Influenza AH3	-	isolate	0/3	Negative
Influenza B		isolate	0/3	Negative
Metapneumovirus	-	isolate	0/3	Negative
Enterovirus	-	isolate	0/3	Negative
Adenovirus 3	-	isolate	0/3	Negative
Parainfluenza 3	-	isolate	0/3	Negative
Rhinovirus	-	isolate	0/3	Negative
S. *pyogenes*	Z018	isolate	0/3	Negative
Parainfluenza 2	-	isolate	0/3	Negative

Virus	Strain	Source	Detected/Replicates	Final result
S. pneumoniae	Z022	isolate	0/3	Negative
S. marcescens	Z053	isolate	0/3	Negative
S. aureus	MRSA, COL	isolate	0/3	Negative
S. agalactiae	Z019	isolate	0/3	Negative
K. pneumoniae	Z460; NDM-1	isolate	0/3	Negative
Coronavirus SARS	-	isolate	0/3	Negative
Parainfluenza	-	isolate	0/3	Negative
K. pneumoniae	Z138	isolate	0/3	Negative
K. pneumoniae	Z460	isolate	0/3	Negative
P. aeruginosa	Z139, VIM1	isolate	0/3	Negative
P. mirabilis	Z050	isolate	0/3	Negative
K. aerogenes	Z052	isolate	0/3	Negative
H. influenzae	MinnA	isolate	0/3	Negative
E. coli	Z297	isolate	0/3	Negative
E. cloacae	Z101	isolate	0/3	Negative
A. baumannii	307-0294	isolate	0/3	Negative

17.3 Precision

Assessment of repeatability (intra-run) and reproducibility (inter-run) of the exsig® COVID-19 Direct (CE IVD) assay has been performed by contriving negative swab samples from healthy individuals with whole SARS-CoV-2 viral genome RNA representing 6 isolates (MT007544.1, MN908947.3, LC528232.1, MT106054.1, MT188340 and MT118835). Dry swab samples were agitated in exsig® Sample Preparation Buffer and contrived at 2 levels for this assessment:

- 0.76 copies/µl in the PCR reaction
- 0.38 copies/µl in the PCR reaction

*Contrivance level concentrations were based on analytical sensitivity of the assay in **Sections 18.1.1 and 18.1.2.**

17.3.1 Repeatability

Repeatability was measured by analysing within-run variation of replicates. A total of 12 replicates for each contrivance level was produced on the CFX96. The statistical analysis of imprecision (SD and %CV) is shown below.

	exsig® COVID-19 Direct FAM				exsig® COVID-19 Direct VIC (IEC)			
Viral RNA Concentration in PCR reaction (copies/µl)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)
0.76	100%	34.45	0.58	1.67	100%	23.09	0.09	0.39
0.38	100%	35.56	1.03	2.89	100%	23.18	0.11	0.46

17.3.2 Operator Reproducibility

Inter-operator reproducibility was measured by analysing the between-run variation of replicates from plates prepared by 2 different operators. A total of 24 replicates at each contrivance level was prepared, comprising two sets of 12 replicates per operator. The same machine was used by each operator, and the runs were done on consecutive days. The statistical analysis of imprecision (SD and %CV) is shown below.

	exsig® COVID-19 Direct FAM				exsig® COVID-19 Direct VIC (IEC)			
Viral RNA Concentration in PCR reaction (copies/µl)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)
0.76	95.8%	35.59	1.61	4.54	100%	23.64	0.78	3.30
0.38	95.8%	36.28	1.02	2.81	100%	23.67	0.69	2.92

17.3.3 Daily Reproducibility

Daily reproducibility was measured by analysing the between-run variation of replicates from plates prepared on 3 separate days. A total of 36 replicates at each contrivance level was prepared, comprising 3 sets of 12 replicates per day. The statistical analysis of imprecision (SD and %CV) is shown below.

	exsig® COVID-19 Direct FAM				exsig® COVID-19 Direct VIC (IEC)			
Viral RNA Concentration in PCR reaction (copies/µl)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)	% Replicate Detection	Mean Cq	Standard Deviation	Coefficient of variance (%)
0.76	97.2%	36.11	1.48	4.09	100%	23.94	0.66	2.75
0.38	94.4%	36.77	1.28	3.48	100%	24.06	0.71	2.97

17.4 Performance Evaluation

17.4.1 Internal Performance Evaluation

An internal Performance evaluation of the exsig® COVID-19 Direct (CE IVD) assay was conducted with contrived swab specimens (30 positive and 30 negative). The 60 specimens were contrived with whole SARS-CoV-2 viral genome RNA representing 6 isolates (MT007544.1, MN908947.3, LC528232.1, MT106054.1, MT188340 and MT118835) and tested blindly to generate the Positive Percentage Agreement (PPA) and Negative Percentage Agreement (NPA):

		Contrived Sample Status	
		Positive	Negative
exsig® COVID-19 Direct (CE IVD) assay	Positive	30	0
	Negative	0	30
		Positive Percentage Agreement (PPA)	Negative Percentage Agreement (NPA)
		100%	100%
		Overall Percentage Agreement (OPA)	
		100%	

17.4.2 External Performance Evaluation

As part of an external evaluation of the exsig™ COVID-19 direct technology a total of 453 anterior nasal swab samples have been collected and processed according to the published instructions for use. Samples were processed as part of the CICERO study, conducted at the Blizard Institute (QMUL).

Dry anterior nasal and nasopharyngeal swabs were collected in parallel from asymptomatic participants at the care homes. The dry anterior nasal swabs were tested using the exsig™ COVID-19 Direct (CE-IVD) on the q16 qPCR instrument. The dry nasopharyngeal swabs were tested using the reference method of the Roche COVID-19 testing system in the Royal London Hospital (Barts NHS Trust) Pathology laboratory. Patient's swabs were collected and stored in viral transport medium (VTM) or dry.

In addition to this, contrived positive dry swabs* were generated with Twist Synthetic SARS-CoV-2 RNA (Twist Bioscience). These contrived samples prepared and blinded from the investigator for

testing with exsig™ COVID-19 Direct (CE-IVD) on the q16 qPCR instrument.

To evaluate diagnostic accuracy, these results were compared to either the contrived sample status or the results derived from the reference method to generate the clinical sensitivity, clinical specificity, NPV and PPV.

***samples were contrived at of 0.38 copies/µL in the PCR reaction**

Please note: where swabs were provided in viral transport media, samples were treated adding viral inactivation buffer and RNase inhibitor, heated and cooled down as indicated by the protocol for exsig direct and then diluted 1:15 in exsig™ sample preparation buffer (84µl of buffer + 6µl of sample). All other steps proceeded as per the IFU.

PCR set up: NPT using the exsig™ COVID-19 Direct (CE-IVD) kit was carried out on the genesig q16 and q32 Real-Time PCR system according to the genesig® Covid-19 CE IVD qPCR assay IFU. Each plate contained negative extraction controls (NEC), a positive control and samples were run as single replicates.

		Known Sample Status	
		Positive	Negative
exsig® COVID-19 Direct (CE IVD) assay	Positive	154	2
	Negative	0	943

Clinical Sensitivity	100%
Clinical Specificity	99.7%
PPV	98.7%
NPV	100%

18. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates as laboratory clinical waste according to national regulations. Refer to **Section 8** for guidance weblinks.
The Viral Inactivation Buffer contains Triton X 100 reduced and is very toxic to aquatic life with long lasting effects. Do not let product enter drains and discharge into the environment must be avoided.

19. Primerdesign Ltd Quality Control

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

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

20. Technical Support

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

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

21. Trademarks and Disclaimers

Trademarks: exsig®, oasig™, genesig® and the Primerdesign logo.

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

22. Explanation of Symbols

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

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