WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: Primerdesign Ltd COVID-19 genesig Real-Time PCR assay
EUL Number: EUL-0489-185-00
Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

The Primerdesign Ltd COVID-19 (genesig Real-Time PCR assay) with product code Z-Path COVID-19-CE, CE-mark regulatory version manufactured by Primerdesign Ltd, York House, School Lane, Chandlers Ford, SO53 4DG, United Kingdom and Northen Ireland**,** was listed as eligible for WHO procurement on 7 April 2020.

Intended use:
According to the claim of intended use from Primerdesign Ltd, "*Primerdesign Ltd COVID-19 genesig Real-Time PCR assay is intended to be used to achieve qualitative detection of COVID-19 viral RNA extracted from nasopharyngeal swabs, oropharyngeal swabs and sputum from patients in association with a CE IVD extraction system. The kit is intended for use by laboratory trained personnel".*

Specimen type(s) that were validated:

Nasopharyngeal swabs, oropharyngeal swabs and sputum.

Test kit contents:

Component	96 tests (product code Z-Path COVID-19-CE)
oasig Lyophilised qPCR OneStep Master Mix	2 x 525 µL vial
COVID-19 Primer & Probe Mix	2 x 110 µL vial
Master Mix Resuspension buffer	2 x 600 µL vial
Kit Resuspension buffer	2 x 1 500 µL vial
Water RNase/DNase Free	1 x 500 µL vial
COVID-19 Positive control template	1 x 600 µL vial
RNA Internal extraction control	2 x 1 000 µL vial

Items required but not provided:

Extraction reagent

- HAIN Lifescience GmbH GXT DNA/RNA Extraction Kit VER 2.0 (IFU-120102-10)

Equipment

- PCR hood
- Benchtop centrifuge
- Vortex mixer
- Depending on the Real-Time PCR instrument to be used: White Roche LightCycler 480 Multiwell plate 96, White Bio-Rad CFX96 Multiwell plate 96, or Transparent Applied Biosystems 7500 Real-Time PCR System Multiwell Plate 96
- Adjustable pipettes
- Pipette tips with filters
- Disposable gloves
- 1.5mL microcentrifuge tubes for extraction

Validated Real-Time PCR platforms:

- Applied Biosystem® 7500 Real-Time PCR System (software version 2.3) product code: 4351104)
- Bio-Rad CFX96 Maestro (software version 1.1) product code: 1855201,1855195
- Roche® LightCycler 480 II (software version 1.5) product code: 05015278001

Storage:

-20°C.

Shelf-life upon manufacture:
Claimed shelf-life is 18 months, real time stability studies are ongoing.

Warnings/limitations:
Please refer to the instructions for use.

Product dossier assessment

Primerdesign Ltd submitted a product dossier for Primerdesign Ltd COVID-19 genesig Real-Time PCR assay as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347 version 2)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external product evaluating committee (PEC) assessor appointed by WHO.

Post listing Commitments for EUL:

1. As a requirement to listing, the manufacturer is required to participate in the WHO collaborative study for the assessment of the suitability of an interim standard for SARS-CoV-2 virus nucleic acid amplification tests.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Primerdesign Ltd was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff and external technical experts (assessors), it was established that sufficient information was provided by Primerdesign Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_ 347 version 2"*.

Quality management system assessment conclusion: acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL status:

1. Notification to WHO of any planned changes to an EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and

2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3).*[1]

Primerdesign Ltd is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

Scope and duration of procurement eligibility

The Primerdesign Ltd COVID-19 genesig Real-Time PCR assay with product code Z-Path COVID-19-CE manufactured by Primerdesign Ltd is considered to be eligible for WHO procurement for 12 months from the day of listing. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Primerdesign Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Primerdesign Ltd is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

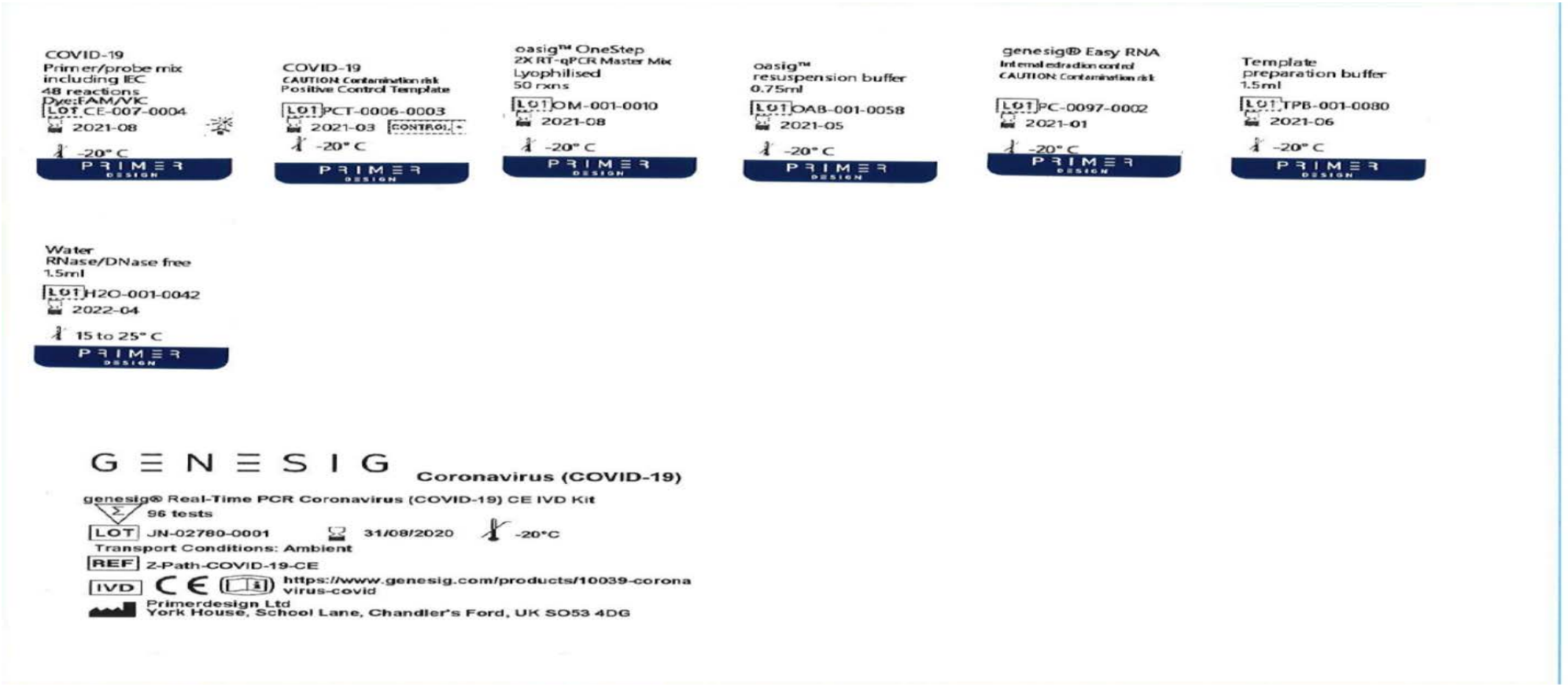
WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

[1] Available on the web page https://www.who.int/diagnostics_laboratory/postmarket/en/

Labelling

1. **Labels**
2. **Instructions for use**

1.0 Labels


COVID-19
Primer/probe mix
including IEC
48 reactions
Dye:FAM/VIC
LOT CE-007-0004
2021-08
-20° C
PRIMER DESIGN
COVID-19
CAUTION Contamination risk
Positive Control Template
LOT PCT-0006-0003
2021-03
CONTROL +
-20° C
PRIMER DESIGN
oasig™ OneStep
2X RT-qPCR Master Mix
Lyophilised
50 rxns
LOT OM-001-0010
2021-08
-20° C
PRIMER DESIGN
oasig™
resuspension buffer
0.75ml
LOT OAB-001-0058
2021-05
-20° C
PRIMER DESIGN
genesig® Easy RNA
Internal extraction control
CAUTION: Contamination risk
LOT PC-0097-0002
2021-01
-20° C
PRIMER DESIGN
Template
preparation buffer
1.5ml
LOT TPB-001-0080
2021-06
-20° C
PRIMER DESIGN
Water
RNase/DNase free
1.5ml
LOT H2O-001-0042
2022-04
15 to 25° C
PRIMER DESIGN
GENESIG
Coronavirus (COVID-19)
genesig® Real-Time PCR Coronavirus (COVID-19) CE IVD Kit
96 tests
LOT JN-02780-0001
31/08/2020
-20°C
Transport Conditions: Ambient
REF Z-Path-COVID-19-CE
IVD
https://www.genesig.com/products/10039-coronavirus-covid
Primerdesign Ltd
York House, School Lane, Chandler's Ford, UK SO53 4DG

Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Primerdesign™ Ltd

Coronavirus (COVID-19)

genesig® Real-Time PCR assay

Instructions for Use (IFU)

Issue 2.0

GENESIG

Kits by Primerdesign

genesig®

Coronavirus (COVID-19)

Real-Time PCR Assay

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

For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	CE IVD Extraction System, suitable for the directed sample types	Applied Biosystem® 7500 Real-Time PCR System Bio-Rad CFX96 Roche® LightCycler 480 II
Oropharyngeal Swabs		
Sputum		

96 tests

Z-Path-COVID-19-CE

Primerdesign Ltd, School Lane, Chandler's Ford, UK, SO53 4DG
Freephone: +44 (0) 800 0156 494

Contents

1. Intended Use

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD is intended to be used to achieve qualitative detection of COVID-19 viral RNA extracted from nasopharyngeal swabs, oropharyngeal swabs and sputum from patients in association with a CE IVD extraction system and the designated PCR platforms listed above. The kit is intended for use by laboratory trained personnel.

2. Kit Components

Kit Component	Number of Vials	Volume (µL per vial)	Lid colour	Resuspended with?
oasig® Lyophilised qPCR OneStep Master Mix	2	525*	Red	Master Mix Resuspension buffer
COVID-19 Primer & Probe Mix	2	110*	Amber	Kit Resuspension buffer
Master Mix Resuspension buffer	2	600	Blue	n/a
Kit Resuspension buffer	2	1500	Yellow	
Water RNase/DNase Free	1	500	White	
COVID-19 Positive control template	1	600*	Red, vial stored in silver foil	Kit Resuspension buffer
RNA Internal extraction control	2	1000*	Blue, vial stored in gold foil	

* The projected volume once resuspended

3. Storage & Handling Conditions

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD is shipped at ambient temperatures but must be stored at -20°C upon arrival.

If the protective kit packaging is damaged upon receipt, please contact Primerdesign for instructions. Attention should be paid to the “use by” date specified on the pack label and individual tube labels. On this date, the kit should be discarded following the disposal instructions in **Section 8**.

3.1. In Use Stability

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD should be stored in the original packaging and is stable for up to 1 month once resuspended, if stored at -20°C, but should not be used past the “use by” date as indicated on the pack label and individual tube labels. When in use the kit components should be returned to the freezer promptly after use to minimise the time at room temperature. Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles. Components may be aliquoted into smaller volumes after resuspension, if necessary.

4. Identification of Material & Devices Required but Not Provided

4.1. Reagents

- Appropriate nucleic extraction system and/or kit (please refer to **Section 9.1** 'sample preparation procedure').

4.2. Equipment

- PCR hood
- Benchtop centrifuge
- Vortex mixer
- White Roche® LightCycler 480 Multiwell plate 96
- White Bio-Rad® CFX96 Multiwell plate 96
- Transparent Applied Biosystems® 7500 Real-Time PCR System Multiwell Plate 96
- Adjustable pipettes
- Pipette tips with filters
- Disposable gloves
- 1.5ml microcentrifuge tubes for extraction

4.3. Real-Time PCR Machine

- Appropriate Real-Time PCR instrument (please refer to **Section 6.3**).

4.4. Facilities/Training Requirements

- Samples should be handled in a Biosafety Level 2 facility, World Health Organization Interim guidance on laboratory biosafety from 12 Feb 2020 should be followed. Testing for the presence of COVID-19 should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures.

5. Background Information

The target is important to be screened for patient displaying relevant symptoms of Coronavirus. Reliable and regular diagnosis of individuals for this target will help to ensure a reduction in the spread of infections but also help rapidly treat infected patients.

COVID-19 is a contagious, novel strain of Coronavirus that emerged from Wuhan, China in December 2019. The virus is thought to be of zoonotic origin and likely to have spread from large seafood and animal markets by human-animal contact in the city of Wuhan. The virus causes respiratory infection, with symptoms including fatigue, fever, shortness of breath, respiratory failure, renal failure and death. As of the time of writing, February 2020, globally confirmed cases exceed 60,000.

Patients can become infected with COVID-19 by person-person contact (through contact with a contaminated environment or person). The young and old (and other immunocompromised individuals) are most at risk of serious complications however, approximately 20% of those infected can develop a critical condition.

6. Product Description

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD assay is an in vitro diagnostic test based on Real-Time PCR technology, developed for specific detection of SARS-CoV-2 viral RNA. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology. The COVID-19 specific probe is labelled with the FAM fluorophore and the internal control is labelled with the HEX fluorophore.

The assay includes an Internal Control to identify possible PCR inhibition, to measure extraction purity and to confirm the integrity of the PCR run.

Real-Time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

6.1. Positive Control

The Positive Control Template (PCT) contain standardised concentrations of SARS-CoV-2 RNA (Coronavirus COVID-19) specific sequence in concentration 1.67 x 10^5 copies per µl. To ensure PCR run validity, the PCT should produce Cq value ≤22 in the FAM channel.

6.2. Extraction Kits / Instruments

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD was developed to be used with an extraction system validated as a CE IVD device for use in the extraction of RNA (either exclusively or with DNA) from clinical samples, including nasopharyngeal swabs, oropharyngeal swabs and/or sputum.

Validation of the assay was performed on the automated extraction system GenoXtract® from HAIN Lifescience GmbH (Brucker) using GXT DNA/RNA Extraction kit (CE IVD).

6.3. Real-Time PCR instruments

The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD was developed and validated to be used with the following Real-Time PCR instruments.

- **Applied Biosystem® 7500 Real-Time PCR System (software version 2.3)**
- **Roche® LightCycler 480 II (software version 1.5)**
- **Bio-Rad CFX Maestro (software version 1.1**

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

7. Warning and Precautions

7.1. Safety Information

7.1.1. Samples

Testing for the presence of COVID-19 should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances:

7.1.2. genesig Real-Time PCR Coronavirus (COVID-19) CE IVD

Please consult the material safety data sheet (MSDS) before using this kit. This is available on request.

- The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD component “Kit Resuspension Buffer” contains EGTA. This component should be handled according to the MSDS. In the event of damage to protective packaging, contact Primerdesign for instructions.

7.1.3. RNA Extraction Kit (CE IVD) Warnings

Please consult the relevant MSDS, available from the supplier, before using your chosen CE IVD extraction kit.

7.2. Handling and Procedural Requirements

7.2.1. General

- Always wear disposable gloves when handling kit components.
- Use separated working areas for specimen preparation, reaction set up and amplification.
- Supplies and equipment should be separated in each work area and not moved between them.
- When mixing reagents by pipetting up and down this should be done with a volume roughly equal to 50% of the total component volume
- Please ensure that water is not used to resuspend the kit components.

7.2.2. Preventing Template Contamination

- 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.
- 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. For further instruction for disposal see **Section 8** Disposal Instructions.

7.2.3. Prevention of DNase 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.

8. Limitations of Use

- The genesig Real-Time PCR Coronavirus (COVID-19) CE IVD has been validated for use with sputum, oropharyngeal swab and nasopharyngeal swab samples run on the Roche® LightCycler 480 II Real-Time PCR System, Bio-Rad CFX96 and Applied Biosystem® 7500 Real-Time PCR System.
- 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.
- All samples should be handled as if they are infectious following proper biosafety precautions.
- Interpretation of results must account for the possibility of false negative and false positive results.
- 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 COVID-19 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.

9. Procedure

9.1. Sample Preparation Procedure

Prepare at least 1 negative extraction control (NEC) each time an extraction is performed (i.e. an extraction with no sample added). This NEC will serve as the negative control for the entire testing system.

	Nasopharyngeal swabs	Oropharyngeal swabs	Sputum
Collection	Dacron or polyester flocked swabs in viral transport medium	Dacron or polyester flocked swabs in viral transport medium	Sterile container**
Transport temperature*	4°C	4°C	4°C
Short-term storage (pre-extraction)*	4°C for ≤ 5 days	4°C for ≤ 5 days	4°C for ≤ 48 hours
Long-term storage (pre-extraction)*	-70°C for longer periods	-70°C for longer periods	-70°C for longer periods
Extraction System	CE IVD extraction system intended for use in the isolation of RNA	CE IVD extraction system intended for use in the isolation of RNA	CE IVD extraction system intended for use in the isolation of RNA
Extraction sample volume	700µL***	700µL***	700µL
Extraction elution volume	85µL	85µL	85µL

*These are World Health Organisation (WHO) recommendations. Local regulations pertaining to sample handling must take priority.
**Sputum must be from the lower respiratory tract
***Sample refers to the viral transport medium provided in the sample container serving as the repository for the swab.

9.1.1. RNA Extraction

Please consult the IFU of the chosen CE IVD extraction system for full usage details.

The internal extraction control should be resuspended in 1000µl template preparation buffer. It should be incorporated in the extraction as directed by the extraction system IFU. Primerdesign recommends 20µl is added per sample. **The internal extraction control should not be added directly to the raw sample (i.e. before the sample is mixed with a lysis buffer). Doing so may compromise the testing.**

Where the IFU provides no specific guidance for the application of an internal extraction control or where an automated system does not support the addition of 20µl, please contact Primerdesign for guidance.

GXT DNA/RNA Extraction Kit VER 2.0 (IFU-120102-10)

During the validation studies, the GXT DNA/RNA Extraction Kit VER 2.0 for the GenoXtract® Automated Extraction System was used to conduct the extractions (according to IFU-120102-10). The internal extraction control (20µl) was applied directly to Well 12 (lysis buffer) of the GXT DNA/RNA Extraction Kit cartridge before proceeding with the protocol

9.2. Master Mix Setup

a) Resuspend the Coronavirus (COVID-19) CE IVD primer/probe tube in kit resuspension buffer, 110µl of buffer per each tube; vortex to mix.

b) Resuspend the oasig Lyophilised qPCR Master Mix in 525µl oasig resuspension buffer; gently swirl to mix.

9.3. Reaction Setup

a) Prepare a mix of the following reagents:

Component	1 x volume required (µl)*
oasig qPCR OneStep Master Mix	10*
Coronavirus (COVID-19) CE IVD Primer/Probe	2*

*Multiply all numbers according to experimental requirements

b) Add 12µl into the number of wells required for your testing into an appropriate 96 well plate for your chosen PCR platform, include 1 well for the PCT and 1 well for the NEC (an additional well for an NTC can be prepared if desired)

c) Add 8µl of the following into the appropriate wells according to your plate setup:
   i. Sample(s)
   ii. PCT; resuspended in 600µl kit resuspension buffer (vortex to mix)
   iii. NEC
   iv. NTC (optional)

d) Seal the plate with an appropriate seal and place in the instrument.

9.4. Programming the Real-Time PCR Instrument

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

- Applied Biosystem® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (© Copyright 2007, 2010 Applied Biosystem, all rights reserved).
- LightCycler 480 instrument Operator's manual (July 2016, Addendum 4, Software version 1.5)
- CFX96™ Touch Instruction Manual (© 2013, Bio-Rad Laboratories Inc.)

a) Enter the following amplification program:

Steps	Time	Temperature	Cycles	Detection Format
Reverse Transcription	10 min	55°C	1	COVID-19 = FAM (465-510) 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

b) Ensure the well loaded with PCT are designated as "Sample Type - Standard" and assigned the appropriate concentration (see **Section 6.1**)

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

9.5. Data Analysis

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
b) NEC produces a Cq < 30 in the VIC / HEX / Yellow555 (533-580) channel
c) PCT produces a Cq of 14-22 in the FAM (465-510) channel

For instrument specific guidance on correctly assigning Cq values please see below.

9.5.1. Roche® LightCycler 480 II

- Select the “Abs Quant/2nd Derivative Max” option
- Switch on the automated color compensation function; “Universal CC FAM (510) – VIC (580)”, this can be accessed from “Color Comp” drop down button on the analysis screen

9.5.2. Bio-Rad® CFX96

- Select the ‘Cq Determination Mode’ to Regression. This option can be accessed in the menu bar under ‘View’.

9.5.3. Applied Biosystem® 7500 Real-Time PCR System

- Select “Graph Type: Linear”
- Select “Target: FAM”
- Untick box for “Threshold: Auto”
- Tick box for “Show: Threshold”
- Manually set the threshold line at the 1/10th of the End point fluorescence value for the PCT
- Select the VIC/HEX channel
- Manually set the threshold line at the 1/10th of the average End point fluorescence of the VIC/HEX amplification curves.

9.6. Interpretation of Results

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

		FAM (465-510)	
		Cq Positive	**Cq Negative**
VIC / HEX / Yellow555 (533-580)	**Cq ≤ (NEC Cq + 6)**	**COVID-19 Detected***	**COVID-19 Not detected**
	Cq > (NEC Cq + 6)	**COVID-19 Detected***	**Result invalid**
	Cq Negative	**COVID-19 Detected***	**Result invalid**

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

10. Performance Evaluation

The results for the Coronavirus (COVID-19) CE IVD assay performance evaluation have been generated on the Applied Biosystem® 7500 Real-Time PCR system with additional testing on the Roche® LightCycler 480 II and Bio-Rad CFX 96 instruments for analytical sensitivity (LoD).

10.1. Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed via spiking 3 negative oropharyngeal samples with known copy number template. The eluates were then serially diluted to give the 11 contrivance levels that were tested over 3 days, producing at least 36 replicates for each concentration tested.

10.1.1. Analytical Sensitivity Results

This data demonstrates that the Coronavirus (COVID-19) CE IVD genesig® kit detects 0.58 copies/µl of COVID-19 viral RNA with a confidence ≥95%. This concentration therefore serves as the limit of detection of the kit.

	ABI 7500 Data			
Contrivance Level	**Overall Mean Concentration (copies/µl)**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
1	2.28×10^3	100	26.77	1.577
2	1.34×10^3	100	27.71	1.386
3	4.09×10^2	100	29.50	1.404
4	2.36×10^2	100	30.36	1.373
5	3.55×10^1	100	32.92	1.373
6	2.53×10^1	100	33.98	1.353
7	0.8	100	36.25	1.447
8	0.58	100	38.33	1.125
9	1.4	97.2	38.41	2.564
10	0.90	72.2	39.30	2.077
11	0.14	19.4	40.53	0.790

10.1.2. Alternative Instrument Testing

The CFX 96 (Bio-Rad®) and LightCycler 480 II (Roche®) qPCR machines were used to re-validate the analytical sensitivity to ensure that 0.58 copies/µl. 36 replicates of positive control template representing the analytical sensitivity concentration were tested on each instrument.

	Lightcycler 480 II			Bio-Rad CFX96		
Overall Mean Concentration (copies/µl)	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
1.25×10^5	100	15.49	0.061	100	14.87	0.030
1.25×10^3	100	22.54	0.061	100	21.93	0.071
1.25 x 10	100	29.56	0.078	100	28.75	0.119
6.25	100	30.44	0.266	100	29.71	0.015
1.25	100	33.88	0.304	100	33.07	0.366
0.58	100	33.92	0.329	100	32.84	0.251

10.2. Analytical Specificity

The genesig Real-Time Coronavirus (COVID-19) CE IVD has been designed to detect all publicly available COVID-19 viral RNA sequences. This was assessed with *in silico* sequence comparison analyses and in vitro specimen testing. Upon *in silico* analysis the genesig Real-Time PCR Coronavirus (COVID-19) CE IVD design was found to detect all COVID-19 virus strains and exhibited no cross reactivity with non-COVID-19 species.

For testing, in vitro two panels were sourced from Qnostics: the Respiratory Evaluation Panel and QCMD Past Panel from the Coronavirus EQA programme. The results of the in vitro specimen testing are presented below

Organism	Interpreted Result*
Influenza A H1N1	COVID-19 not detected
Influenza A H3N2	COVID-19 not detected
Influenza B Victoria	COVID-19 not detected
Influenza B Yamagata	COVID-19 not detected
RSV A	COVID-19 not detected
RSV B	COVID-19 not detected
Coronavirus NL63	COVID-19 not detected
Coronavirus 229E	COVID-19 not detected
Coronavirus HKU	COVID-19 not detected
Coronavirus OC43	COVID-19 not detected

* Results were interpreted according to **Section 9.6**.

10.3. Precision

Assay precision for the genesig Real-Time PCR Coronavirus (COVID-19) CE IVD Kit was determined by the repeated testing of clinical samples contrived to represent 3 viral load levels:

- 120 copies/reaction (15 copies/µl)
- 100 copies/reaction (12.5 copies/µl)
- 80 copies/reaction (10 copies/µl)

Precision was expressed in the form of the Cq standard deviation and coefficient of variation.

10.3.1. Repeatability

Repeatability was measured by analysing 10 replicates of each sample on a single plate:

	COVID-19 (FAM)			IEC (HEX/VIC/Yellow555)		
Sample Concentration (copies/µl)	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**
15	31.48	100	0.31	19.77	100	0.72
12.5	31.83	100	0.45	19.37	100	0.33
10	32.19	100	0.36	19.84	100	0.91

10.3.2. Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by running 10 replicates of each sample across 2 qPCR instruments.

	COVID-19 (FAM)			IEC (HEX/VIC/Yellow555)		
Sample Concentration (copies/µl)	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**
15	31.39	100	2.99	19.83	100	6.98
12.5	31.77	100	3.41	19.45	100	7.01
10	32.04	100	3.02	19.86	100	8.2

10.3.3. Operator Reproducibility

Three different operators tested 10 replicates of each sample with the genesig Real-Time PCR Coronavirus (COVID-19) CE IVD to assess operator reproducibility. For each test, the same batch and the same instrument was used.

	COVID-19 (FAM)			IEC (HEX/VIC/Yellow555)		
Sample Concentration (copies/µl)	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**
15	31.47	100	3.44	19.96	100	8.53
12.5	31.73	100	3.20	19.33	100	7.39
10	32.12	100	3.35	19.91	100	8.14

10.3.4. Daily Reproducibility

Daily reproducibility was assessed by analyzing 40 replicates of each sample across 4 days, 10 replicates of each concentration per day. For each test, the same batch and the same instrument was used.

	COVID-19 (FAM)			IEC (HEX/VIC/Yellow555)		
Sample Concentration (copies/µl)	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variation (%)**
15	31.50	100	3.51	20.02	100	7.86
12.5	31.77	100	3.32	19.45	100	6.99
10	32.311	100	3.28	19.94	100	7.57

10.4. Accuracy

Clinical evaluation of the genesig Real-Time PCR Coronavirus (COVID-19) CE IVD was conducted with contrived oropharyngeal swabs (50 positive and 50 negative) in Copan universal transport medium. 50 swabs were contrived with positive control template and tested blindly to generate the Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and overall percentage agreement (OPA) as a measurement of estimated Diagnostic Accuracy:

		Contrived Sample Status	
		Positive	Negative
genesig Real-Time PCR Coronavirus (COVID-19) CE IVD	Positive	49	0
	Negative	1	50
		Positive Percentage Agreement (PPA)	Negative Percentage Agreement (NPA)
		98%	100%
		Overall Percentage Agreement (OPA)	
		99%	

11. Quality Control

In accordance with Primerdesign Ltd ISO 13485 certified Quality Management System, each batch of genesig Real-Time PCR Coronavirus (COVID-19) CE IVD Kit is tested against predetermined specifications to ensure consistent product quality.

12. Technical Support

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

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

13. Trademarks and Disclaimers

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

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

14. 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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