Primerdesign™ Ltd

genesig®

SARS-CoV-2 Winterplex Real-Time PCR assay

CE IVD

Instructions for Use (IFU)

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GENESIG

Kits by Primerdesign

genesig®

SARS-COV-2 Winterplex

Real-Time PCR Assay

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

Validated For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	CE IVD Extraction System, suitable for the directed sample types QIAamp® Viral RNA Mini kit (Qiagen extraction system) exsig® Mag extraction kit	Applied Biosystem® 7500 Bio-Rad CFX Connect™ Roche® LightCycler 480 II genesig® q32 (Primerdesign, Novacyt)
Oropharyngeal Swabs		
Sputum		

96 tests

Catalogue number: D00020

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1. Intended Use

The genesig® Real Time PCR SARS-COV-2 Winterplex is a CE marked, *in vitro* diagnostic, real-time, reverse transcriptase PCR (RT-PCR) multiplex assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 (ORF1ab and S gene targets), Influenza A (Flu A), Influenza B (Flu B) and Respiratory Syncytial Virus (RSV A and B) in nasopharyngeal swabs, oropharyngeal swabs and sputum specimens. This multiplex assay provides rapid screening of individuals suspected of SARS-CoV-2, Flu A, Flu B and RSV infections and aids the diagnosis of suspected disease in patients.

The assay has been designed to be used with Real Time PCR instruments capable of simultaneously detecting FAM (Max Absorption 499nm, Maximum Emission 519nm), HEX (Max Absorption 538nm, Maximum Emission 559nm), ROX (Max Absorption 575nm, Maximum Emission 602nm) and Cy5 (Max Absorption 643nm, Maximum Emission 667nm) fluorophores.

The genesig® Real Time PCR SARS-COV-2 Winterplex assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The assay has been validated for use with the extraction systems and the designated PCR platforms listed in **Sections 7 and 8**.

Positive results are indicative of the presence of SARS-CoV-2, Flu A, Flu B or RSV RNA. Positive results do not rule out co-infection with other bacteria or other viruses. Negative results do not preclude SARS-CoV-2, Flu A, Flu B or RSV infections and should not be used as the sole basis for patient management decisions. Individual target confirmatory tests should be used to exclude co-infections. Positive and Negative results must be combined with clinical observations, patient history, and epidemiological information.

Specimen test results are available to interpret in under three hours using the genesig® Real Time PCR SARS-COV-2 Winterplex assay. This time includes the time to extract nucleic acid from a specimen, PCR set-up, PCR run time, and to availability of results.

2. Materials Provided

The genesig® Real-Time PCR SARS-COV-2 Winterplex assay contains:

Reagent label	Number of Vials 96 tests	Volume (µL per vial)	Lid colour	Resuspended with?
Onestep Lyophilised Master Mix	4	525*	Gold, vial stored in sealed foil pouch	Master Mix Resuspension Buffer
ORF1ab/S Primer & Probe Mix (including IEC primer/probe mix)	1	220*	Amber, vial stored in sealed foil pouch	Template preparation buffer
FluA/FluB/RSV Primer & Probe Mix (including IEC primer/probe mix)	1	220*	Yellow (Amber body), vial stored in sealed foil pouch	Template preparation buffer
Master Mix Resuspension Buffer	4	750	Blue	n/a
Template preparation buffer	3	1500	Yellow	
Water RNase/DNase Free	1	1500	White	
genesig® Winterplex Positive control template	1	800*	Red, vial stored in sealed foil pouch	Template preparation buffer
genesig® Winterplex Internal extraction control (IEC)	2	1000*	Blue, vial stored in sealed foil pouch	

*The projected volume once resuspended

The SARS-COV-2 Winterplex Primer & Probe Mix consists of two tubes. ORF1ab/S primer & probe tube contains the primers and FAM labeled probe specific to the ORF1ab region of SARS-CoV-2 and the primers and Cy5 labeled probe specific to the S gene of SARS-CoV-2, as well as the primers and HEX labelled probe for the genesig® Winterplex RNA Internal extraction control (IEC). FluA/FluB/RSV primer & probe tube contains the primers and FAM labeled probe for the detection of Influenza A, primers and Cy5 labelled probe specific to Influenza B, primers and ROX labeled probe for RSV and the primers and HEX labelled probe for the genesig® Winterplex RNA IEC.

The OneStep Lyophilised Master Mix, the two Primers & Probes mixes, genesig® Winterplex Positive control template and genesig® Winterplex RNA IEC are all provided lyophilised. The table above indicates which buffer to use, as well as the volume to add, to resuspend these reagents.

3. Warnings and Precautions

3.1 General

- For in vitro diagnostic use (IVD) only.
- Handle all specimens as if infectious using safe laboratory procedures. Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) biological safety cabinet (refer to the guidance detailed in **Section 9**).
- 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 genesig® Real-Time PCR SARS-COV-2 Winterplex assay component "Template preparation buffer" contains EDTA. This component should be handled according to the SDS. In the event of damage to protective packaging, contact Primerdesign for instructions.

3.2 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® SARS-COV-2 Winterplex positive control template is provided in a sealed foil envelope and contains a mixture of high copy number synthetic DNA templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
    - Maintain separate areas for handling of 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 minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Good aseptic technique should always be used when working with nucleic acids.
    - 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 2.**
    - Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g.10% bleach, ethanol, DNA/RNA remover) to minimize risk of nucleic acid contamination.
- RNA samples should be maintained on a cold block or on ice during preparation and used to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.
- Dispose of unused kit reagents and human specimens according to national regulations (refer to guidance detailed in **Section 9**).

3.3 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.

3.4 Specimen nucleic acid extraction kit/system

- Please consult the relevant Instruction For Use (IFU) and Safety Data Sheet (SDS), available from the manufacturer, before using your chosen extraction kit/ system.

4. Reagent Storage, Handling and Stability Conditions

4.1 Storage conditions

- The genesig® Real-Time PCR SARS-COV-2 Winterplex assay is shipped at ambient temperatures but must be stored at -20°C upon arrival.
- The genesig® Real-Time PCR SARS-COV-2 Winterplex assay should be stored in the original packaging and is stable for up 6 months once stored at -20°C.
- Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
- If the kit's protective packaging is damaged upon receipt or the tamper proof seal has been compromised, 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 11.**
- Always check the expiration date prior to use. Do not use expired reagents.
- Primer/probe mixes, 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 2.**
- Once resuspended, components may be aliquoted into smaller volumes, if required, and are stable for up to one month if stored at -20°C.
- It is important to protect the fluorogenic primer/probe mixes from light as this reagent is photosensitive.

4.2 In Use Stability

- The genesig® Real-Time PCR SARS-COV-2 Winterplex assay should be stored in the original packaging and is stable for up to one month once resuspended and stored at -20°C.
- The kit should not be used past the "use by" date as indicated on the pack label and individual tube labels.
- When *in use* the kit components should be returned to the freezer promptly after use to minimize 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 required.

5. Specimen Collection, Handling and Storage

5.1 Collecting the Specimen

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.

- Refer to the 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

- Refer to the 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

- Refer to 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

- 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. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport medium.

5.2 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.

5.3 Storing Specimens

- Extracted nucleic acid should be stored at -70ºC or lower.
- Refer to **Section 5.1** weblinks for guidance

6. Reagent and Controls Preparation

6.1 OneStep Lyophilised Master Mix preparation

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

6.2 SARS-COV-2 Winterplex and IEC Primer/Probe mix preparation

- Two primer/probe mixes are provided, ORF1ab/S primer probe mix and FluA/FluB/RSV primer probe mix. Both mixes also include primers and probe for the detection of the IEC.
- Upon receipt, the dried primers/probes mix can be stored at -20ºC. Do not use after the expiry date (see product label).
- Precautions: this reagent should only be handled in a clean area and not exposed to light.
- Using aseptic technique, resuspend each dried primer/probe mix in 220µl of Template preparation buffer and vortex to mix.
- Store at -20ºC. Resuspended primer/probe mix is stable for one month when stored at -20ºC.
- Freeze/ thaw cycles should be minimized 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.

6.3 genesig® SARS-COV-2 Winterplex Positive control template preparation

- The genesig® SARS-COV-2 Winterplex Positive control template (PCT) is provided in a sealed foil envelope and contains a mixture of high copy number synthetic DNA templates and should be opened and processed away from clinical specimens and kit components to avoid cross-

contamination.

- The PCT tube contains synthetic DNA representing the genomic regions of interest for the assay targets of: ORF1ab for SARS-CoV-2, S gene of SARS-CoV-2, Influenza A, Influenza B and RSV. Following resuspension, this will be at a concentration of 1.25 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. Do not use after the expiry date (see product label).
- Using aseptic technique, resuspend the dried PCT in 800µl of Template preparation buffer, mix gently. Resuspended PCT is stable for one month when stored at -20ºC.
- Freeze/ thaw cycles should be minimized 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 for the ORF1ab (SARS-CoV-2) and Influenza A targets, amplification in the Cy5 channel for the S gene (SARS-CoV-2) and Influenza B targets, and amplification on the ROX channel for RSV.

6.4 genesig® Winterplex RNA Internal extraction control (IEC) preparation

- The genesig® Winterplex RNA Internal extraction control (IEC) can be added to the nucleic acid extraction system (not provided) to provide an RNA template control, detect PCR inhibition and confirm the integrity of the PCR run.
- Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination.
- Upon receipt, the dried IEC can be stored at -20ºC. Do not use after the expiry date (see product label).
- Using aseptic technique, resuspend the dried IEC in 1000µl of Template preparation buffer, mix gently. Resuspended IEC is stable for one month when stored at -20ºC.
- Freeze/ thaw cycles should be minimized and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.

6.5 Negative Extraction Control (NEC) preparation

- Prepare at least 1 negative extraction control (NEC) every time RNA is extracted from a cohort of samples. In addition, each PCR run should include a minimum of 1 NEC, prepared in parallel with the clinical samples.
- The NEC is an extraction with no clinical specimen/sample added, it is prepared by extracting from RNase/DNase free water. The IEC is added to the NEC sample during extraction as directed by the manufacturer's IFU. This NEC will serve as the negative control for the entire testing system and to check for contamination during PCR plate set-up.

6.6 No Template Control

- DNase/RNase free water is a provided to use as a No Template control (NTC), if required, in addition to the NEC (refer to **Section 6.5)**
- The NTC is used to check for contamination during PCR plate set-up.

7. General Preparation

7.1 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 minimize the risk of nucleic acid contamination.
- Performance of the genesig® Real-Time PCR SARS-COV-2 Winterplex assay is dependent upon the amount and quality of RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been validated for recovery and purity of RNA for use with this assay:
    - Automated extraction system GenoXtract® from Bruker HAIN Lifescience GmbH using the GXT NA Extraction kit.
    - Qiagen extraction system with QIAamp® Viral RNA Mini kit (Qiagen, Germany)
    - exsig™ Mag extraction kit from Primerdesign Ltd.

Please consult the manufacturer's IFU of the chosen extraction system for full usage details.

8. Assay Set Up

8.1 Sample Preparation Procedure

	Nasopharyngeal swabs, oropharyngeal swabs, sputum*
Collection	Swabs: Dacron or polyester flocked swabs in viral transport medium Sputum: Viral transport medium in sterile container
Transport temperature**	2-8ºC ≤ 72hrs
Short-term storage (pre-extraction) **	2-8ºC ≤ 72hrs
Long-term storage (pre-extraction) **	≤ -70˚C for longer periods
Extraction sample volume	550µL***
Extraction elution volume	50µL

*Sputum must be from the lower respiratory tract
**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

***Sample refers to the viral transport medium provided in the sample container serving as the repository for the swab.
Extraction sample volume and extraction elution volume as recommended for the GXT NA (CE) on the HAIN GenoXtract® from Bruker.

8.2 RNA extraction

The results of the genesig® Real-Time PCR SARS-COV-2 Winterplex assay is dependent upon the amount and quality of template RNA purified from human specimens.

- Consult the IFU of the extraction system for full usage details.
- Prepare at least 1 negative extraction control (NEC) each time a cohort of samples are processed for RNA extraction (i.e. an extraction with no clinical specimen/sample added).
- The genesig® Winterplex RNA Internal extraction control (IEC) 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, directly into the lysis stage of the extraction.

The internal extraction control should not be added directly to the clinical specimen/ sample before RNA extraction (i.e. not before the clinical specimen/sample is mixed with a lysis buffer of the nucleic acid extraction kit/system). Doing so may compromise the testing.

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

8.3 Master Mix Setup

Each of the two primer/probe tubes (ORF1ab/S and FluA/FluB/RSV) in this assay must be treated separately for plate setup, i.e. each patient sample, PTC, NTC and NEC must be tested against each primer/ probe mix. Therefore, follow the below master mix setup instructions for each of the two tubes.

a) Resuspend each of the two primer/probe tubes in 220µl of template preparation buffer, vortex to mix.
b) Resuspend the OneStep Lyophilised Master Mix in 525µl Master Mix 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 6.5 and 8.2** on how to prepare NEC). NTCs should be included in each plate set-up. A PCT must be included in each plate set-up (note: one positive well is sufficient for all targets for simultaneous detection on multiple channels)
    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) 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:
    1. If number of samples (n) is ≤ 10, then N = n+1
    2. If number of samples (n) is > 10 and ≤ 20, then N = n+2
    3. If number of samples (n) is > 20, then N = n+ 10% of total number of samples

e) Prepare two reaction mixes, one for each primer/probe mix (ORF1ab/S and FluA/FluB/RSV). Label two 1.5ml DNase/RNase free tubes (for example, COVID-19 and Flu/RSV). Dispense the following resuspended components into the correct labelled tube:

Reaction mix Component	1 x volume required (µl) *
Onestep Lyophilised Master Mix	10*
Primer & Probe mix (either ORF1ab/S or FluA/FluB/RSV)	2*

*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.

f) Add 12µl of the ORF1ab/S reaction mix 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.
g) Add 12µl of the FluA/FluB/RSV reaction mix into empty wells of the PCR plate or a second PCR plate, according to your plate setup. Include 1 well for the PCT, 1 well for the NEC and 1 well for the NTC for each primer/probe reaction mix.
h) Add 8µl of the following into the appropriate wells according to your plate setup:

a. NEC (please refer to **Sections 6.5**)
b. NTC (please refer to **Sections 6.6**)

i) Cover the entire plate and move the plate to the specimen nucleic acid handling area.
j) Gently vortex nucleic acid sample tubes for approximately 5 seconds.
k) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
l) Change gloves often and when necessary to avoid contamination.
m) Add 8µl of the RNA/nucleic acid extracted from clinical specimen/sample(s) into the appropriate wells according to your plate setup.
n) Cover the entire plate and move the plate to the positive template control handling area.
o) Add 8µl of PCT (please refer to **Sections 6.3**) into the appropriate wells according to your plate set up. Seal the plate with an appropriate seal and place in the instrument.

8.4 Programming the Real-Time PCR Instrument

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

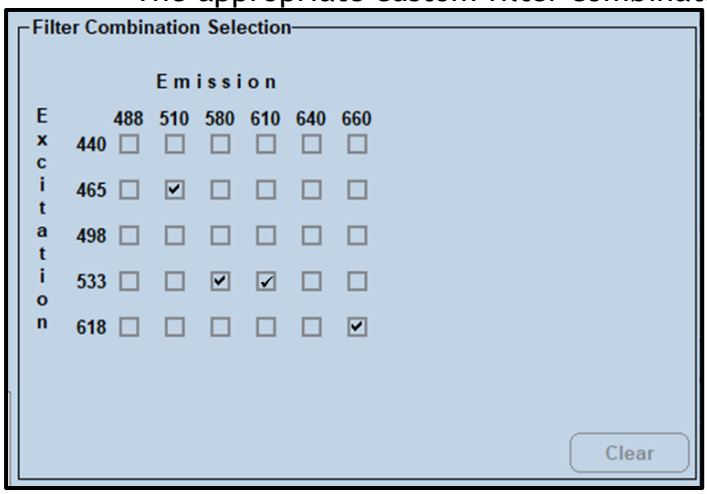
- Applied Biosystems® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (as per Applied Biosystems manual (2010)).
- LightCycler 480 instrument Operator's manual (July 2016, Addendum 4, Software version 1.5)
- Bio-Rad CFX Connect™ Real-Time PCR Detection System Instrument Guide (as per Bio-Rad Laboratories Inc. Manual (2017))
- genesig® q32 Instrument Guide (2020) software version 3.5.21

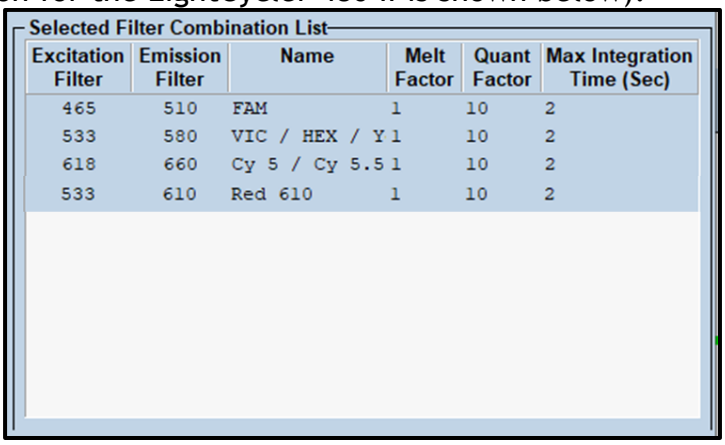
a) Enter the following amplification program:

Steps	Time	Temperature	Cycles	Detection Format
Reverse Transcription	10 min	55˚C	1	FAM (465-510) VIC / HEX / Yellow555 (533-580) Red 610 / ROX (533-610) Cy5 (618-660)
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 you will need to create a custom detection format as directed in the LightCycler 480 instrument Operator's manual (2.1.1 Setting Detection Formats)
- The appropriate custom filter combination for the LightCycler 480 II is shown below):





- 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"

9. Interpretation of Results

9.1 Acceptance criteria of controls included in the genesig® Real-Time PCR SARS-COV-2 Winterplex 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), Cy5 (618-660) and ROX (533-610) channel, and that NEC produces positive amplification in the VIC/HEX (533-580) channel (this is detection of the genesig® Winterplex RNA Internal Extraction control).
b) PCT produces a Cq of between 14-22 in the FAM (465-510), Cy5 (618-660) and ROX (533-610) channels.

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.

9.2 Interpretation of Patient Specimen Results

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

ORF1ab/S reaction mix:

SARS CoV-2 Targets		Internal Extraction Control	Result †
ORF1ab FAM (465-510)	S gene Cy5 (618-660)	VIC/HEX/Yellow555 (533-580)	
Cq Positive (+)	Cq Positive (+)	Cq Positive (+) / Negative (-)	SARS-CoV-2 Positive*
Cq Positive (+)	Cq Negative (-)	Cq Positive (+) / Negative (-)	SARS-CoV-2 Positive*
Cq Negative (-)	Cq Positive (+)	Cq Positive (+) / Negative (-)	SARS-CoV-2 Positive*
Cq Negative (-)	Cq Negative (-)	Cq Positive (+)	SARS-CoV-2 Negative**
Cq Negative (-)	Cq Negative (-)	Cq Negative (-)	Result invalid, repeat testing of sample

FluA/FluB/RSV reaction mix:

Flu A	Flu B	RSV	Internal Extraction Control	Result †
FAM (465-510)	Cy5 (618-660)	ROX (533-610)	VIC/HEX/Yellow555 (533-580)	
Cq Positive (+)	Cq Negative (-)	Cq Negative (-)	Cq Positive (+) / Negative (-)	Flu A Positive*
Cq Negative (-)	Cq Positive (+)	Cq Negative (-)	Cq Positive (+) / Negative (-)	Flu B Positive*
Cq Negative (-)	Cq Negative (-)	Cq Positive (+)	Cq Positive (+) / Negative (-)	RSV Positive*
Cq Negative (-)	Cq Negative (-)	Cq Negative (-)	Cq Positive (+)	Test Negative**
Cq Negative (-)	Cq Negative (-)	Cq Negative (-)	Cq Negative (-)	Result invalid, repea testing of sample

*All instances of FAM and/or Cy5 sample amplification in the ORF1ab/S testing indicates a SARS-CoV-2 positive sample. For the FluA/FluB/RSV test, all instances of FAM, Cy5, or ROX channel sample amplification indicates a positive result for the respective pathogen. Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

**If there is no target amplification in the FAM or Cy5 channels (plus ROX for the FluA/FluB/RSV test) for a test sample, before the result is confirmed as a true negative the Internal Extraction VIC/HEX channel should be analysed. Positive amplification in the VIC/HEX channel confirms the PCR run is valid and the genesig® Winterplex RNA IEC added to the test sample during the RNA extraction process has been detected. The following acceptance criteria should be applied to confirm the negative samples:

- The IEC Cq value produced by the patient sample should be < 36 and should not exceed the NEC IEC Cq value + 6 (i.e. sample RNA IEC Cq < NEC RNA IEC Cq + 6). Failure to satisfy this criterion indicates a compromised sample extraction and an invalid result; testing of the sample must be repeated.

† Please note: Please check all channels even after identifying a positive result. The above interpretation table does not include the test case of more than one pathogen producing positive amplification, however this is a possibility. In the event of co-infection with more than one pathogen, positive amplification will be detected in the relevant channels.

10. Performance Evaluation

The genesig® Real-Time PCR SARS-COV-2 Winterplex assay performance evaluation has been generated on the CFX384 (Bio-Rad®) Real-Time PCR system for analytical sensitivity (LoD). A set of additional testing at the LoD level has been performed for the additional qPCR instruments and extraction systems listed in **Sections 7 and 8**.

10.1 Analytical Sensitivity

The Limit of detection (LoD) is defined as the lowest concentration of analyte that could be reliably detected with > 95% confidence. First a tentative LoD was established by preparing negative oropharyngeal swab samples spiked with synthetic RNA (COVID-19, Flu A, Flu B) or cultured and extracted RNA (RSV) of each target. Four contrived samples were extracted for each target. Once the tentative LoD was established it was confirmed by analysing three contrived samples per target in quadruplicate on the CFX384 platform giving a total of 36 data points per target.

Samples were extracted with the GXT NA Extraction kit on the Automated GenoXtract® system (Bruker HAIN Lifescience). The LoD results are described in **Sections 10.1.1, 10.1.2 and 10.1.3 and 10.1.4.**

10.1.1 Analytical Sensitivity Results

LoD was established by testing negative oropharyngeal swabs contrived with either SARS-CoV-2 synthetic whole genome RNA (Twist Synthetic SARS-CoV-2 RNA Control (Twist BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (Twist BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (Twist BioScience ®) or Respiratory Syncytial Virus A (RSV) inactivated standard (Helix Elite, BioConnections®) at an known copy/ml concentration.

Samples were extracted with the GXT NA Extraction kit on the Automated GenoXtract® system (Bruker HAIN Lifescience). Extracted samples were diluted down to limiting concentrations to establish the tentative LoD for each target. Diluted extracted samples were tested on the CFX384 (Bio-Rad®) Real-Time PCR System.

Once the tentative LoD concentration for each target was established, it was confirmed by preparing 3 - 4 levels of dilutions around the tentative LoD and tested on the CFX384 (Bio-Rad®) Real-Time PCR System across three different days. Samples were tested with a five-point standard curve of synthetic DNA containing the relevant target amplicon sequence.

CFX384 Real-Time PCR System COVID-19 ORF 1ab assay						
Contrivance level	Positive calls/Total no. results included in analysis	Positive Calls	Overall Mean Conc (copies/µl)	Overall mean Conc (copies/rxn)	Overall Mean Cq	Cq Standard Deviation
Dilution 1	36/36	100	1.5	11.9	35.2	0.5
Dilution 2	35/36	97	0.8	6.3	36.1	0.7
Dilution 3	35/36	97	0.4	3.4	37.2	0.7
Dilution 4	29/36	81	3.5	28.3	38.1	0.8

CFX384 Real-Time PCR System COVID-19 S gene assay						
Contrivance level	Positive calls/Total no. results included in analysis	% Positive Calls	Overall Mean Conc (copies/µl)	Overall mean Conc (copies/rxn)	Overall Mean Cq	Cq Standard Deviation
Dilution 1	36/36	100	2.9	23.5	35.8	0.8
Dilution 2	36/36	100	1.4	11.2	37.6	1.1
Dilution 3	34/36	94	0.7	5.6	38.2	1.7
Dilution 4	24/36	69	0.6	4.4	38.4	0.8

CFX384 Real-Time PCR System Flu A assay						
Contrivance level	Positive calls/Total no. results included in analysis	% Positive Calls	Overall Mean Conc (copies/µl)	Overall mean Conc (copies/rxn)	Overall Mean Cq	Cq Standard Deviation
Dilution 1	36/36	100	1.4	11.0	36.4	0.5
Dilution 2	36/36	100	0.7	5.6	37.6	0.9
Dilution 3	33/36	92	0.4	3.2	38.6	1.1
Dilution 4	25/36	69	0.3	2.1	39.1	1.0

CFX384 Real-Time PCR System Flu B assay						
Contrivance level	Positive calls/Total no. results included in analysis	% Positive Calls	Overall Mean Conc (copies/µl)	Overall mean Conc (copies/rxn)	Overall Mean Cq	Cq Standard Deviation
Dilution 1	35/36	97	0.6	4.9	36.8	1.0
Dilution 2	30/36	83	0.6	5.0	37.3	1.7
Dilution 3	19/36	53	0.1	0.9	39.6	1.6
Dilution 4	N/A	N/A	N/A	N/A	N/A	N/A

CFX384 Real-Time PCR System RSV assay						
Contrivance level	Positive calls/Total no. results included in analysis	% Positive Calls	Overall Mean Conc (copies/µl)	Overall mean Conc (copies/rxn)	Overall Mean Cq	Cq Standard Deviation
Dilution 1	34/34	100	0.75	6.0	36.7	1.0
Dilution 2	29/36	81	0.3	2.3	38.0	0.9
Dilution 3	19/36	53	0.2	1.3	39.0	1.9
Dilution 4	1/36	3	0.2	1.9	37.7	0.0

This data demonstrates that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.4 copies/µl** of **SARS-CoV-2** whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

This data demonstrates that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.7 copies/µl** of **Flu A** whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

This data demonstrates that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.6 copies/µl** of **Flu B** whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

This data demonstrates that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.75 copies/µl** of **RSV** whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

10.1.2 Alternative Instrument Testing

The LoD was further confirmed by testing seven replicates per sample from three negative oropharyngeal swabs samples contrived with either SARS-CoV-2 RNA Control (Twist BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (Twist BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (Twist BioScience ®) or Respiratory Syncytial Virus A (RSV) inactivated standard (Helix Elite, BioConnections®).

Each extracted sample was further diluted down to three dilutions around the tentative LoD for each target.

Samples were extracted with the GXT NA Extraction kit on the Automated GenoXtract® system (Bruker HAIN Lifescience) and tested on four Real-time qPCR platforms: CFX96 Connect™ Real-Time PCR Detection System (Bio-Rad®), LightCycler 480 Instrument II (Roche®), Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®) and the genesig® q32 Real-Time PCR Instrument (Primerdesign ®).

COVID-19 ORF1ab assay						
PCR Instrument	Positive calls/Total no. results included in analysis	% Calls	Mean Cq	Overall Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Cq Standard Deviation
CFX96 Connect™ Real-Time PCR (Bio-Rad ®)	21/21	100	37.1	0.2	1.6	1.4
LightCycler 480 Instrument II (Roche®	21/21	100	36.8	0.2	1.6	0.8
Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®)	21/21	100	37.2	0.2	1.6	1.0
genesig q32 Real-Time PCR Instrument (Primerdesign ®)	20/20	100	35.6	0.4	3.2	0.7

COVID-19 S gene assay						
PCR Instrument	Positive calls/Total no. results included in analysis	% Calls	Mean Cq	Overall Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Cq Standard Deviation
CFX96 Connect™ Real-Time PCR (Bio-Rad ®)	21/21	100	36.9	1.0	8.0	0.8
LightCycler 480 Instrument II (Roche®)	21/21	100	37.2	0.7	5.9	1.2
Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®)	21/21	100	37.0	1.1	8.8	0.8
genesig q32 Real-Time PCR Instrument (Primerdesign ®)	20/20	100	35.6	1.2	9.6	0.8

Flu A assay						
PCR Instrument	Positive calls/Total no. results included in analysis	% Calls	Mean Cq	Overall Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Cq Standard Deviation
CFX96 Connect™ Real-Time PCR (Bio-Rad ®)	21/21	100	0.8	6.5	36.2	0.6
LightCycler 480 Instrument II (Roche®	20/21	95	38.3	0.2	1.6	1.1
Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®)	21/21	100	37.6	0.5	3.9	0.9
genesig q32 Real-Time PCR Instrument (Primerdesign ®)	20/20	100	36.4	0.4	3.2	0.8

Flu B assay						
PCR Instrument	Positive calls/Total no. results included in analysis	% Calls	Mean Cq	Overall Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Cq Standard Deviation
CFX96 Connect™ Real-Time PCR (Bio-Rad ®)	21/21	100	35.4	0.3	2.4	0.6
LightCycler 480 Instrument II (Roche®	20/21	95	36.1	0.5	4.0	1.6
Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®)	21/21	100	37.0	0.2	1.6	0.5
genesig q32 Real-Time PCR Instrument (Primerdesign ®)	20/20	100	33.7	1.0	8.0	0.3

RSV assay						
PCR Instrument	Positive calls/Total no. results included in analysis	% Calls	Mean Cq	Overall Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Cq Standard Deviation
CFX96 Connect™ Real-Time PCR (Bio-Rad ®)	20/21	95	37.2	0.1	1.1	0.9
LightCycler 480 Instrument II (Roche®	21/21	100	35.5	0.3	2.4	0.4
Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®)	21/21	100	36.2	0.8	6.4	0.5
genesig q32 Real-Time PCR Instrument (Primerdesign ®)	20/20	100	33.5	1.1	8.8	0.3

The results above confirm that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.40 copies/µl** of **SARS-CoV-2** whole viral genome RNA ≥95% of the time across the following Real-Time qPCR platforms: CXF96 Connect™ Real-Time PCR (Bio-Rad®), LightCycler 480 Instrument II (Roche®, Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®) and genesig q32 Real-Time PCR Instrument (Primerdesign®).

The results above confirm that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.70 copies/µl** of **Flu A** whole viral genome RNA ≥95% of the time across the following Real-Time qPCR platforms: CXF96 Connect™ Real-Time PCR (Bio-Rad®), LightCycler 480 Instrument II (Roche®, Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®) and genesig q32 Real-Time PCR Instrument (Primerdesign®).

The results above confirm that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **1 copies/µl** of **Flu B** whole viral genome RNA ≥95% of the time across the following Real-Time qPCR platforms: CXF96 Connect™ Real-Time PCR (Bio-Rad®), LightCycler 480 Instrument II (Roche®, Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®) and genesig q32 Real-Time PCR

Instrument (Primerdesign®).

The results above confirm that the genesig® Real-Time PCR SARS-COV-2 Winterplex assay detects ≤ **0.8 copies/µl** of **RSV** whole viral genome RNA ≥95% of the time across the following Real-Time qPCR platforms: CXF96 Connect™ Real-Time PCR (Bio-Rad®), LightCycler 480 Instrument II (Roche®, Applied Biosystems ABI 7500 Real-Time PCR System (Thermofisher ®) and genesig q32 Real-Time PCR Instrument (Primerdesign®).

10.1.3 exsig™ Mag extraction system

For this bridging study, seven replicates were tested per sample from three negative oropharyngeal swabs samples contrived with either SARS-CoV-2 RNA Control (Twist BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (Twist BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (Twist BioScience ®) or Respiratory Syncytial Virus A (RSV) inactivated standard (Helix Elite, BioConnections®).

Each extracted sample was further diluted down to three dilutions around the tentative LoD for each target.

Target	Dilution 1	Dilution 2	Dilution3
	% Positive calls	% Positive calls	% Positive calls
COVID-19 ORF1ab	100	100	100
S gene	100	100	100
FluA	100	100	100
FluB	100	100	57
RSV	100	100	80.9

In this study, 100% sensitivity was maintained for each of the targets in the genesig® Real-Time PCR SARS-COV-2 Winterplex assay when the same starting concentration of relevant whole genomic RNA control was used as determined in **Section 10.1.2.**

10.1.4 QIAamp® Viral RNA Mini Kit extraction

For this bridging study, seven replicates were tested per sample from three negative oropharyngeal swab samples contrived with either SARS-CoV-2 RNA Control (Twist BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (Twist BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (Twist BioScience ®) or Respiratory Syncytial Virus A (RSV) inactivated standard (Helix Elite, BioConnections®). Each extracted sample

was further diluted down to three dilutions around the tentative LoD for each target.

Target	Dilution 1	Dilution 2	Dilution 3
	% Positive calls	% Positive calls	% Positive calls
COVID-19 ORF1ab	100	100	100
S gene	100	100	100
FluA	100	100	100
FluB	100	95	76
RSV	100	100	100

This data demonstrates that analytical performance is maintained when samples are extracted with the QIAamp® Viral RNA Mini Kit.

10.2 Inclusivity

10.2.1 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 27th of August 2020, *in silico* analysis confirms the COVID-19 assay primers and probe still show 100% detection with the 59,917 full length, good quality SARS-CoV-2 sequences published on the GISAID EpiCoV database.

10.2.2 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 SARS-COV-2 Winterplex 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 SARS-COV-2 Winterplex assay.

In vitro testing:
For *in vitro* testing, 5 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)
- QCMD panel from the 2019 MERS Coronavirus EQA Programme

The samples from these panels are representative of true clinical human specimens and evaluated by the SARS-COV-2 Winterplex genesig® Real-Time PCR assay in single replicate. The results of the *in vitro* cross-reactivity testing are presented below:

ORF1ab/S gene

Virus	Strain	Source	Detected	Final result
INF A H1N1 positive	-	Isolate	N/A	Negative
INF A H3N2 positive	-	Isolate	N/A	Negative
INF B Victoria	-	Isolate	N/A	Negative
INF B Yamagata	-	isolate	N/A	Negative
RSV A	-	isolate	N/A	Negative
RSV B	-	isolate	N/A	Negative
Coronavirus	NL63	isolate	N/A	Negative
Coronavirus	229E	isolate	N/A	Negative
Coronavirus	HKU	isolate	N/A	Negative
Coronavirus	OC43	isolate	N/A	Negative
Influenza AH1	-	isolate	N/A	Negative
Influenza AH3	-	isolate	N/A	Negative
Influenza H1N1 2009	-	isolate	N/A	Negative
Influenza B		isolate	N/A	Negative
Metapneumovirus	-	isolate	N/A	Negative
Enterovirus	-	isolate	N/A	Negative
Adenovirus 3	-	isolate	N/A	Negative
Parainfluenza 3	-	isolate	N/A	Negative
Rhinovirus	-	isolate	N/A	Negative
S. pyogenes	Z018	isolate	N/A	Negative
Parainfluenza 2	-	isolate	N/A	Negative
S. pneumoniae	Z022	isolate	N/A	Negative
S. marcescens	Z053	isolate	N/A	Negative
S. aureus	MRSA, COL	isolate	N/A	Negative
S. agalactiae	Z019	isolate	N/A	Negative
K. pneumoniae	Z460; NDM-1	isolate	N/A	Negative
Coronavirus SARS	-	isolate	N/A	Negative
Parainfluenza	-	isolate	N/A	Negative
K. pneumoniae	Z138	isolate	N/A	Negative

Virus	Strain	Source	Detected	Final result
K. pneumoniae	Z460	isolate	N/A	Negative
P. aeruginosa	Z139, VIM1	isolate	N/A	Negative
P. mirabilis	Z050	isolate	N/A	Negative
K. aerogenes	Z052	isolate	N/A	Negative
K.oxytoca	-	isolate	N/A	Negative
M.catarrhalis	-	isolate	N/A	Negative
H. influenzae	MinnA	isolate	N/A	Negative
E. coli	Z297	isolate	N/A	Negative
E. cloacae	Z101	isolate	N/A	Negative
A. baumannii	307-0294	isolate	N/A	Negative
MERS Coronavirus		isolate	N/A	Negative

FluA/FluB/RSV

Virus	Strain	Source	Detected	Final result
INF A H1N1	-	Isolate	32.11 Cq	Positive
INF A H3N2	-	Isolate	31.04 Cq	Positive
INF B Victoria	-	Isolate	33.77 Cq	Positive
INF B Yamagata	-	isolate	33.9 Cq	Positive
RSV A	-	isolate	32.45 Cq	Positive
RSV B	-	isolate	29.24 Cq	Positive
Coronavirus	NL63	isolate	N/A	Negative
Coronavirus	229E	isolate	N/A	Negative
Coronavirus	HKU	isolate	N/A	Negative
Coronavirus	OC43	isolate	N/A	Negative
Influenza AH1	-	isolate	28.83 Cq	Positive
Influenza AH3	-	isolate	33.55 Cq	Positive
Influenza H1N1 2009	-	isolate	27.38 Cq	Positive
Influenza B	-	isolate	27.29 Cq	Positive
RSV A	-	isolate	24.06 Cq	Positive
RSV B	-	isolate	27.23 Cq	Positive
Metapneumovirus	-	isolate	N/A	Negative
Enterovirus	-	isolate	N/A	Negative

Adenovirus 3	-	isolate	N/A	Negative
Parainfluenza 3	-	isolate	N/A	Negative
Rhinovirus	-	isolate	N/A	Negative
S. pyogenes	Z018	isolate	N/A	Negative
Parainfluenza 2	-	isolate	N/A	Negative
S. pneumoniae	Z022	isolate	N/A	Negative
S. marcescens	Z053	isolate	N/A	Negative
S. aureus	MRSA, COL	isolate	N/A	Negative
S. agalactiae	Z019	isolate	N/A	Negative
K. pneumoniae	Z460; NDM-1	isolate	N/A	Negative
Coronavirus SARS	-	isolate	N/A	Negative
Parainfluenza	-	isolate	N/A	Negative
K. pneumoniae	Z138	isolate	N/A	Negative
K. pneumoniae	Z460	isolate	N/A	Negative
P. aeruginosa	Z139, VIM1	isolate	N/A	Negative
P. mirabilis	Z050	isolate	N/A	Negative
K. aerogenes	Z052	isolate	N/A	Negative
K.oxytoca	-	isolate	N/A	Negative
M.catarrhalis	-	isolate	N/A	Negative
H. influenzae	MinnA	isolate	N/A	Negative
E. coli	Z297	isolate	N/A	Negative
E. cloacae	Z101	isolate	N/A	Negative
A. baumannii	307-0294	isolate	N/A	Negative
MERS Coronavirus		isolate	N/A	Negative

10.3 Precision

Assessment of repeatability (intra-run) and reproducibility (inter-run) of the genesig® Real-Time PCR SARS-COV-2 Winterplex assay has been performed by contriving oropharyngeal swab samples negative for SARS-CoV-2 (for tube 1 targets) and FluA/FluB/RSV (for tube 2 targets) with a known copy number synthetic RNA template representing either: SARS-CoV-2 synthetic whole genome RNA (Twist Synthetic SARS-CoV-2 RNA Control (BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (BioScience ®) and RSV RNA synthetic RNA genomic region of interest (Primerdesign®) respectively at three contrivance levels* (reproducing high, medium and low viral load samples):

- High viral load sample: SARS-CoV-2 at 3.7 x 10^4 copies/ml, Flu A at 5.2 x 10^4 copies/ml, Flu B at 6.3 x 10^3 copies/ml and RSV at 3.6 x 10^3 copies/ml

- Medium viral load sample: SARS-CoV at 1.6 x 10^4 copies/ml, Flu A at 2.6 x 10^4 copies/ml, Flu B at 3.1 x 10^3 copies/ml and RSV at 1.8 x 10^3 copies/ml

- Low viral load sample: SARS-CoV-2 at 1.2 x 10^4 copies/ml, Flu A at 1.6 x 10^4 copies/ml, Flu B at 2 x 10^3 copies/ml and RSV at 1.1 x 10^3 copies/ml.

*Contrivance level concentrations were based on analytical sensitivity of the assay in **Sections 10.1.1 and 10.1.2.**

Samples were extracted with the GXT NA Extraction kit on the Automated GenoXtract® system (Bruker HAIN Lifescience) and tested on the CFX96 (Bio-Rad ®) Real Time PCR system.

A total of 7 replicates of each dilution were tested across four PCR plates. Two different operators performed the study over 2 days on two CFX96 (Bio-Rad ®) Real-time PCR instruments.

The precision was measured by reporting the Coefficient of Variance which were well below an accepted industrial standard of 9% for all studies:

	Coefficient of variance (%) Flu A assay			
Contrivance level	Repeatability	Inter-Instrument	Inter-operator	Inter-day
High viral load	0.70	0.72	0.68	0.20
Medium viral load	0.65	1.60	0.58	0.43
Low viral load	0.50	0.94	0.80	0.62

	Coefficient of variance (%) Flu B assay			
Sample concentration (copies /ml)	Repeatability	Inter-Instrument	Inter-operator	Inter-day
High viral load	1.71	1.53	1.92	2.01
Medium viral load	0.73	1.12	2.06	2.07
Low viral load	1.36	1.29	2.11	1.91

	Coefficient of variance (%) RSV assay			
Sample concentration (copies /ml)	Repeatability	Inter-Instrument	Inter-operator	Inter-day
High viral load	0.39	0.91	1.50	1.25
Medium viral load	0.37	1.23	1.82	1.02
Low viral load	0.40	1.27	0.87	0.86

Sample concentration (copies /ml)	Coefficient of variance (%) COVID-19 ORF1ab assay			
	Repeatability	Inter-Instrument	Inter-operator	Inter-day
High viral load	0.69	0.66	0.46	0.66
Medium viral load	0.84	1.14	0.70	0.66
Low viral load	0.91	1.03	0.70	0.75

Sample concentration (copies /ml)	Coefficient of variance (%) COVID-19 S gene assay			
	Repeatability	Inter-Instrument	Inter-operator	Inter-day
High viral load	0.44	0.59	0.84	0.69
Medium viral load	0.33	0.66	0.63	0.83
Low viral load	1.38	1.14	0.80	0.98

10.4 Clinical Performance Evaluation

Clinical evaluation of SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay was conducted with 60 oropharyngeal swabs (30 positive and 30 negative) for each target.

Both blinded clinical samples and contrived samples were assessed. Contrived samples were spiked with either SARS-CoV-2 RNA Control (Twist BioScience ®), Flu A H1N1 synthetic whole genome RNA (Twist Synthetic Flu A H1N1 RNA Control (Twist BioScience ®), Flu B synthetic whole genome RNA (Twist Synthetic Flu B RNA Control (Twist BioScience ®) or Respiratory Syncytial Virus A (RSV)inactivated standard (Helix Elite, BioConnections®) at x5 LoD of the relevant assay target.

Target	Clinical samples	Contrived samples
Flu A	7	23
Flu B	1	29
RSV	12	18
SARS-CoV-2	-	30

Samples were extracted with the GXT NA CE IVD kit on the HAIN Lifescience (Brucker®) CE extraction system and tested on the LightCycler 480 Instrument II (Roche®).

SARS-CoV-2 positive contrived samples were tested with the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay (test) and with the COVID-19 (CE - IVD) genesig® Real-Time PCR assay (control).

Flu A and Flu B positive contrived samples and positive clinical samples were tested with the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay (test) and with the Influenza A/B R-GENE™ (Biomérieux ®) (control).

RSV positive contrived samples and positive clinical samples were tested with the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay (test) and with the RSV/hMPV R-GENE™ (Biomérieux ®) (control).

Negative samples were tested with the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay (test) and with the COVID-19 (CE - IVD) genesig® Real-Time PCR assay, the Influenza A/B R-GENE™ (Biomérieux ®) and the RSV/hMPV R-GENE™ (Biomérieux ®) (controls).

Results between both test and control assays for each target to compare to generate Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA) and 95% Confidence Intervals (CIs). Original sample status was assessed to resolve discrepant results between control and test assays.

Target	Positive Percentage Agreement (PPA) (95% CIs)	Negative Percentage Agreement (NPA)	Overall Percentage Agreement (OPA)
Flu A	96% (83.3 - 99.4%)	100% (88.7 - 100%)	98% (88.8 - 102.1%)
Flu B	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (91.1 - 102.9%)
RSV	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (91.1 - 102.9%)
SARS-CoV-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (91.1 - 102.9%)

SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay demonstrated 100% of specificity and between 96% and 100% of sensitivity.

10.5 Interfering substances

Potential interfering substances present within target samples (oropharyngeal swab samples) were evaluated. Changes in performance of the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay were analysed by comparing Cq values of samples containing the potential interfering substances at relevant clinical concentration.

Interfering Substance	Tested concentration
Blood (Haemoglobin)	0.2g/ml
Nasacort Allergy Nasal Spray (Triamcinolone acetonide)	10% v/v
Dymista Allergy Nasal Spray (Corticosteroids - Azelastine hydrochloride & Fluticasone)	6.85 mg/ml Azelastine 2.5mg/ml Fluticasone
Corticosteroid - Dexamethasone	1.52µmol/L
Corticosteroids - Fluticasone	0.1mg/ml
Antiviral medication - Guaifenesin	3mg/ml

Antiviral medication – Oseltamivir	0.1mg/ml
Antibacterial medication – Mupirocin	5µg/ml
Antibacterial eye drops – Tobramycin	0.03mg/ml
Throat lozenge (Strepsils – 2,4-Dichlorobenzyl Alcohol, & Amylmetacresol)	1% w/v
Mucin	0.2mg/ml
α-Amylase	7.92mg/ml

From the 12 interfering substances screened, 3 were found to have a significant effect on the SARS-COV-2 Winterplex (CE- IVD) genesig® Real- Time PCR assay – Nasacort, Dymista and Mucin.

Nasacort was shown to impact the number of replicates amplified past 10% v/v for all targets (COVID-19 ORF1ab, COVID-19 S gene, Flu A, Flu B and RSV).

Dymista had a similar effect (impacting the number of replicates amplifying past 10% v/v), however only for Flu A target.

Although Mucin was shown to have a significant effect on COVID-19 S gene, Flu A and Flu B during the screen, the dose response suggested this drop in assay performance does not worsen with increased Mucin concentration.

11. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates as laboratory clinical waste according to local, state and federal regulations. Refer to **Section 5** for guidance weblinks

12. Technical Support

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

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