For use under Emergency Use Authorization Only

For Prescription Use Only.
For *in vitro* diagnostic (IVD) use

Consult instructions for use. Electronic Instruction for Use (IFU) is available via a linked URL on our website BillionToOne.com or directly here:
https://www.dropbox.com/s/tg5wa5mlfryc2h2/EUA201022.IFU.BillionToOne.pdf?dl=1

Intended use

The qSanger-COVID-19 Assay is a Sanger sequencing-based RNA extraction-free diagnostic test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory swab specimens (such as nasal swab, mid-turbinate swab, nasopharyngeal swab, and oropharyngeal swab specimens) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The qSanger-COVID-19 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, Sanger Sequencing and in vitro diagnostic procedures. The qSanger-COVID-19 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principle

The qSanger-COVID-19 Assay is a Sanger sequencing-based test for detection of SARS-CoV-2 RNA. The qSanger-COVID-19 Assay is designed to detect RNA from SARS-CoV-2 in nasopharyngeal and oropharyngeal swabs from patients who are suspected of COVID-19. Patient samples are tested directly without extraction. VTM from Nasopharyngeal (NP)/oropharyngeal (OP) swabs is added directly to a reverse transcription and an endpoint PCR reaction. The amplification product of this reaction is then sequenced using a modified Sanger sequencing approach. Detection of the correct sequencing involves use of a frame-shifted spike-in sequence that serves as an internal control for each sample and is described in detail in the Instructions For Use document.

Materials Provided and Storage

Table 1. BillionToOne, Inc. qSanger-COVID-19 Assay

Component (for 2000 reactions, with 20% overage)	Volume	Shipping	Storage
Reagent A1: Sars-CoV-2 Primer and Spike-In Reagent	12 mL	Dry ice	-20˚C (-15 to -25˚C)
Enzyme A2: Luna® WarmStart ® RT Enzyme Mix (20x)	3 mL		
Enzyme A3: Luna® Universal Probe One Step Reaction Mix No ROX (2x)	30 mL		
Reagent B1: Sanger Sequence Primer	5 mL		
Reagent C1: SARS-CoV-2 Primer Mix (No Spike-In)	0.2 mL		

Warnings and Precautions

- For *in vitro* diagnostic use
- For prescription use only
- For use under FDA emergency use authorization (EUA) only
- The qSanger-COVID-19 Assay has not been FDA cleared or approved;
- The qSanger-COVID-19 Assay has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests;
- The qSanger-COVID-19 Assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The qSanger-COVID-19 Assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Only personnel proficient in handling infectious materials and trained in the use of the qSanger-COVID-19 Assay should perform this procedure.
- RNA is highly unstable and extra precaution should be employed to ensure it does not degrade in samples.
- Maintain separate areas for sample accessioning, RT-PCR and the set-up of the sequencing reaction to minimize the risk of contamination with amplifiable material in the sample processing steps.
- The performance of the qSanger-COVID-19 Assay was established using nasal swab specimens. Performance with other specimen types has not been established.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

- Laboratory personnel must be familiar with the protocol and all instrumentation used.
- Maintain dedicated equipment (e.g., pipettes, microcentrifuges, vortexes) and supplies (e.g., 1.5mL tubes, pipette tips, gloves) for assay reagent set-up and handling of extracted nucleic acids.
- Workflow must always be in the direction of clean area to dirty area.
- Carefully clean pipettes, containers, and surfaces with RNaseAway, RNaseZap, or a similar product before and between sample batches. Change gloves frequently.
- Never bring extracted nucleic acid or amplification products into the assay set-up area.
- Use nuclease-free, aerosol barrier (filter) pipette tips only.

Symbols

Table 2. Symbols and Description

Symbol	Description
	"Batch code" or "Lot number"
	"Temperature limitation." The upper and lower temperature limits will be indicated on either side of the symbol.
	"Use By" This symbol is intended to indicate that the kit should not be used after the end of the year and month shown.
	"Reference Number" or Manufacturer's catalog number. This number can be used for reordering.

References

- BigDye™ Terminator v3.1 Cycle Sequencing Kit USER GUIDE (link)
- 3730xl DNA Analyzer USER GUIDE (link) [for "latest version", catalog number A41046]

Contact

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