Multiplex

UltraGene Combo2Screen SARS-CoV-2 Assay (V2)

User Guide

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Version 0.4 - DRAFT

Qualitative in-vitro diagnostic - For use with qPCR Instruments

IVD

R_x only

Pending FDA review under the EUA program

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139B1000

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Introduction

This package insert must be read carefully prior to use. The package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Name

UltraGene Combo2Screen SARS-CoV-2 Assay (V2)

CAUTION : The UltraGene Combo2Screen SARS-CoV-2 Assay (V2) has been validated but FDA's independent review of this validation is pending.

Intended use

UltraGene Combo2Screen SARS-CoV-2 Assay (V2) is a real-time RT-PCR test intended for the qualitative detection of RNA from the SARS-CoV-2 in nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** 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 ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is only for use under the Food and Drug Administration's Emergency Use Authorization.

Pending FDA review under the EUA program.

Special conditions for use statements

For Emergency Use Authorization (EUA) only

For prescription use only

For in vitro diagnostic use only

Pending FDA review under the EUA program.

Indication of use

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 positive 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.

Pending FDA review under the EUA program.

Note: The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** should be used following the instructions given in this manual, in combination with validated reagents and instruments. Any off-label use of this product and/or modification of the components will void ABL's liability.

Principles of the assay

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens from patients suspected of COVID-19 by their healthcare provider.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is a real-time PCR-based assay directed against the E and N genes of SARS-CoV-2 virus.

During the amplification reaction, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable DNA polymerase. First, the SARS-CoV-2 reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences.

The Human ribonuclease P (RNase P) is used as an internal control with clinical specimens to confirm that nucleic acid was adequately extracted.

Amplification of the two targets (SARS-CoV-2 E and SARS-CoV-2 N) and the internal control (Rnase P) takes place simultaneously in the same thermal cycling program.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** uses two distinct fluorescent probes for the detection of PCR products. During each RT-PCR cycle, the fluorescent signals increase in a logarithmic manner, resulting in two amplification curves. As soon as the amplification curve of a target surpasses its threshold, the sample is considered positive for that target. The probe does not generate a signal unless it is specifically bound to the amplified product.

The two SARS-CoV-2-specific probes and internal control probe are labeled with three distinct fluorophores thus allowing for simultaneous detection of SARS-CoV-2 and internal control amplified products in the same reaction cycle.

A positive control and a negative control are processed after the extraction from the start of sample preparation to evaluate run validity.

The positive control in the test contains the SARS-CoV-2 RNA targets and confirms that the RT-PCR worked. Thus, the monitoring process in the closed reaction system can be realized and the occurrence of false negatives can be monitored effectively.

The primers and probe for:

- The E gene which is a highly conserved gene are specific to Sarbecovirus (SARS-CoV and SARS-CoV-2). Because SARS-CoV is currently not circulating, a positive result for the E gene is regarded as presumptive positive for the presence of SARS-CoV-2 RNA.
- The N gene are specific to SARS-CoV-2.

Pending FDA review under the EUA program.

Assay components

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is provided in two formats: 1000 reactions (REF 139B1000; GTIN: **05407007960095**) or 50 reactions (REF 139B50, GTIN: **05407007960088**) or.

The specified number of reactions is the number of reactions for each target.

Reagent	Label	Volume	Color Cap	Description	Storage
RT-PCR Premix	CoV Reaction Solution_VLC3	10 * 1375 µL	Green	RT-PCR Probe 2x Master Mix: Hotstart-formulated enzyme blend of thermostable DNA polymerase with reverse transcriptase activity and a Taq DNA polymerase. The blend is pre-mixed with ultrapure dNTPs and buffer components.	-25°C - 15 °C Cannot undergo more than 8 freeze/ thaw cycles.
Primer/Probe Mix **(E gene, N gene and RNAse P)**	CoV E-N-RNAseP Primer and Probe Mix	6 * 1375 µL	Pink	E, N and RNAse P specific primers and probe (each at a concentration of 10 µM)	-25°C - 15 °C
Positive Control	Positive Control	1 * 400 µL	White	Positive control RNA (E and N genes) (5 * 10^3 copies/ mL)	-25°C - 15 °C Several repetitive freeze-thaw cycles should be avoided.
Negative Control	Negative Control	1 * 400 µL	Black	PCR grade nuclease-free water	Can be stored at -25°C - 15 °C with other reagents

Table 1 : Assay components for 1000 reactions of each target

Reagent	Label	Volume	Color Cap	Description	Storage
RT-PCR Premix	CoV Reaction Solution_VLC3	1 * 688 µL	Green	RT-PCR Probe 2x Master Mix: Hotstart-formulated enzyme blend of thermostable DNA polymerase with reverse transcriptase activity and a Taq DNA polymerase. The blend is pre-mixed with ultrapure dNTPs and buffer components.	-25°C - 15 °C Cannot undergo more than 8 freeze/ thaw cycles.
Primer/Probe Mix **(E gene, N gene and RNAse P)**	CoV E-N-RNAseP Primer and Probe Mix	1 * 413 µL	Pink	E, N and RNAse P specific primers and probe (each at a concentration of 10 µM)	-25°C - 15 °C
Positive Control	Positive Control	1 * 20 µL	White	Positive control RNA (E and N genes) (5 * 10^3 copies/ mL)	-25°C - 15 °C Several repetitive freeze-thaw cycles should be avoided.
Negative Control	Negative Control	1 * 20 µL	Black	PCR grade nuclease-free water	Can be stored at -25°C - 15 °C with other reagents

Table 2 : Assay components for 50 reactions of each target

Note:

- All volumes include 10% overage for pipette error.
- Store all reagents between -25°C to -15°C in a non-frost-free freezer.
- Do not mix the reagents from different batches.
- The negative control can be referred as a “No Target Control” (NTC).
- The positive control is formulated with purified, intact viral particles, chemically modified to render them non-infectious. The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** targets regions within the positive control, including the internal control.
- The internal control is a RNase P which cleaves RNA. Its function is to normalize non-PCR related fluorescence fluctuations for accurate results when multiple probes (with different reporter dyes) are combined in a single tube. Clinical specimens tested for RNAse P gene are better controled for specimen quality and extraction.
- Controls should be validated by the end-user if used for the interpretation of test results.

Materials Required but Not Provided

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** test is to be used with qPCR instrument with FAM, HEX and Cy5 channels and related qPCR instrument software. As the test is opened, any RNA extraction and purification method validated at the laboratory for SARS-CoV-2 can be used.

Note: Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Note: Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

RNA extraction kits

The following nucleic acid extraction kits are validated for use with this test:

- MagNA Pure 24 Total NA Isolation Kit (Roche, #07658036001) with MagNa Pure 24 instrument (Roche, Software version v1.1)
- NUCLISENS easyMAG reagents (bioMérieux, #280130, 280131, 280132, 280133, 280134) with Emag (bioMérieux, Software version v1.1).

Note: the bioMérieux EMAG® instrument (software version 1.1), related reagents and consumables were used during external clinical validation.

Instruments

The following Realtime PCR instruments are validated for use with this test:

- Real-Time PCR System (Tianlong Science & Technology, Cat #Gentier 48E, software version 1)
- QuantStudio™ 5 Real-Time PCR System, 96-well, 0.2mL with Desktop (Applied BioSystems, Cat #A29221, QuantStudio Design and Analysis Software v1.4.3)

Note: the TianLong Real-Time PCR System Gentier 48E is not available in the USA.

Materials

- Applied Biosystems MicroAmp 8-Tubes Strip (0.2 mL), Cat #N8010580
- Thermo Fisher Scientific Optically clear flat 8 Cap Strips, Cat #AB-0866
- Eppendorf Safe-Lock Tubes 1,5mL Cat #0030123328
- Adjustable pipettes & fitting filtered pipette tips
- Appropriate PPE & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach
- Nuclease-free dH2O

Sample requirements (collection, handling and storage)

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-A may be referenced as an appropriate resource.

Collecting the Specimen

Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html or other collection specimen protocol validated at your facilities.

- Follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon and 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 media (Universal Transport Medium).

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to your laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to your laboratory on dry ice.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

Reagent Storage and Handling

- All reagents must be stored at –25°C to –15°C for long term storage.
- The thawing and freezing should not be more than eight times for the RT-PCR enzyme mix.
- Multiple thaw-freeze cycles should be avoided for other reagents. Aliquoting shall be considered.
- The opened reagents should be placed no more than 24 hours at room temperature.
- The products should be shipped on dry-ice or icepacks in a polystyrene box and via Express shipment. If the shipping time exceeds 5-6 days, we would furthermore recommend to quality test the reagents to be sure.

Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

Starting material for the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is extracted RNA.

Pending FDA review under the EUA program.

RNA isolation

Nucleic acids are isolated and purified from nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens using automated RNA extraction kits (magnetic beads type) from:

- MagNA Pure 24 Total NA Isolation Kit (Roche®, #07658036001)
  - Software version 1.1
  - Pathogen 1000 protocol
  - Sample input volume: 700 µL; Elution volume: 50 µL
- NUCLISENS easyMAG reagents (bioMérieux, #280130, 280131, 280132, 280133, 280134)
  - Software version 1.1
  - Standard manufacturer protocol
  - Sample input volume: 200 µL; Elution volume: 50 µL.

Workflow

The purified nucleic acid is reverse transcribed into cDNA which is then subsequently amplified in a qPCR instrument having at least 3 channels (FAM, HEX and Cy5) using:

1) **5µ** of extracted RNA solution added to
2) **20 µL** of the ABL ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** Premix reagents CoV (**12.5 µL** of ***CoV Reaction Solution_VLC3*** (RT-PCR Probe 2x Master Mix) and **7.5 µL** of ***CoV E-N-RNAseP Primer and Probe Mix***).

Pending FDA review under the EUA program.

Quantitative PCR (qPCR) reaction setup

Note: Plate set-up configuration can vary with the number of specimens and workday organization.

1. Thaw the following reagents on ice (protected from light) : CoV *Reaction Solution_VLC3*, CoV *E-N-RNAseP Primer and Probe Mix (CoV E, CoV N, IC) and controls (Positive and Negative)*.
2. Briefly centrifuge (2000rpm, 10sec) the reagents to collect the contents.
3. Set up a premix solution based on the number of sample preps to be tested. The volume of the premix required for all sample prep(s) to be tested = (number of sample preps + 2 controls) * the total volume of premix reagents (as listed in the table below).

Premix reagents CoV	Volume for 1 sample
CoV Reaction Solution_VLC3	12.5 µL
CoV E-N-RNAseP Primer and Probe Mix	7.5 µL
Total volume	**20 µL**

Table 3: Premix reagents volume for 1 sample

4. Evenly aliquot **20 µL** of the **Premix reagents CoV** into each qPCR tube (**one qPCR tube per sample to be tested + 2 for the controls**).Add **5 µL** of extracted RNA solution to a single qPCR tube.

Do not add more than one sample of extracted RNA into a single qPCR tube. Each qPCR tube shall have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a qPCR machine for the amplification.

Controls

Controls that will be provided with the test kit include:

a) A “no template” (negative) control (NTC) consisting of Water (molecular grade) is used and is needed to detect cross-contamination during reaction setup. The NTC is used to validate the rRT-PCR plate results. The NTC must be run on each assay plate. The NTC is not included during extraction.

b) A positive template control (purified, intact viral particles, chemically modified to render them non-infectious, isolate (USA-WA1/2020), (5 * 10^3 cp/mL)) is needed to insure the rRT-PCR reaction setup and reagent integrity and is used to validate the rRT-PCR plate results. The positive control must be run on each assay plate. The positive control is not included during extraction.

c) An internal control (IC) Human ribonuclease P (RNase P) (additional primer/probe set labeled with a distinct fluorophore from the two SARS-CoV-2 targets of the test) is needed to detect the human RNase P gene (RP) in control samples and clinical specimens and confirm that nucleic acid was adequately extracted. The IC is used to validate the rRT-PCR plate results. The IC is part of each sample amplification. The IC is not included during extraction.

Controls that are required but not provided with the test kit include:

An extraction control (EC) (i.e. MS2 Bacteriophage; any manufacturer with controlled GMP) is needed to demonstrate successful recovery of RNA as well as extraction reagent integrity. EC should be run using the same protocols as those used to amplify extracted clinical specimens. Purified nucleic acid from the EC should yield a positive result with the IC primer and probe set and negative results with all SARS-CoV-2 markers, here the E and N targeted genes. The EC is included in the extraction.

qPCR Cycling Condition

In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal.

With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the qPCR instruments and software, either from TianLong Gentier 48E or Applied BioSystems QuantStudio™ 5.

The operator sets up the following thermal cycling program.

Stage	Cycle	Temperature (°C)	Time (min:sec)
1	1	70	5:00
2	1	93	0:03
3	1	67.5	2:30
4	1	93	0:03
5	1	66	2:30
6	40	95	00:10
		58	00:15 (measurement in FAM, HEX, Cy5 channels)
		72	00:15
7 (cooling)	1	40	00:01

Table 4: qPCR Cycling program

With the TianLong Gentier 48E:

- FAM (Excitation: 465 nm; Emission: 510 nm) for the SARS-CoV-2 E target
- HEX (Excitation: 527 nm; Emission: 563 nm) for the SARS-CoV-2 N target
- Cy5 (Excitation: 632 nm; Emission: 664 nm) for the RNAse P (IC).

With the Applied BioSystems QuantStudio™ 5:

- FAM (Excitation: 470 +/- 15 nm; Emission: 520+/- 15 nm) for the SARS-CoV-2 E target
- HEX (Excitation: 520 +/- 10 nm; Emission: 558+/- 12 nm) for the SARS-CoV-2 N target
- Cy5 (Excitation: 640 +/- 10 nm; Emission: 682 +/- 14 nm) for the RNAse P (IC).

Detection Channels

It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform this calibration.

Threshold value setting principle:

- Manual setting: set the threshold value a little bit greater than the max fluorescence value of the normal negative control amplification curve.
- Auto setting: the instrument automatically set the threshold value.

Result Analysis

> ⚠ Sample results are only valid when negative controls yield no amplification. If the NTC is invalid in an assay, all sample results in the same assay are therefore also invalid and the operator shall repeat testing of the entire batch.

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Validation of Controls

For both, controls and patient specimens, the threshold for RNase P as well as for the SARS-CoV-2 targets (E and N) to be called positive is a Ct of ≤38.

- The cycle threshold (Ct) reported by the qPCR instrument for the NTC must be equal to 0 or no signal.
- The cycle threshold (Ct) reported by the qPCR instrument for the positive control should be less than 30.
- The cycle threshold (Ct) reported by the qPCR instrument for the internal control should be less than 30.
- If the (Ct) for the IC is equal to 0 or no signal for a patient specimen and at least one (Ct) of the SARS-CoV-2 targets is positive, the result should be considered valid.
- If the (Ct) of the two SARS-CoV-2 targets are equal to 0 or no signal and the (Ct) for the IC is equal to 0 or no signal for a clinical specimen, the result should be considered invalid.

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Note: If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact ABL for consultation.

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable.

Each patient specimen must be analyzed individually.

- For a patient specimen to be considered “Positive for SARS-CoV-2”, one of the SARS-CoV-2 targets (FAM/HEX channels) must give a positive Ct value.
    - Amplification of the IC in the Cy5 channel is expected around Ct 21-30. The IC may fail to amplify in the presence of high virus titers. Therefore, a sample with positive amplification of SARS-CoV-2 is positive even in the absence of RNase P amplification (IC).
- For a patient specimen to be considered negative for SARS-COV-2, “SARS-CoV-2 RNA NOT detected”, both SARS-CoV-2 targets (FAM/HEX channels) must not give a positive Ct value.
    - The IC must give a positive Ct value in the Cy5 channel (Ct 21-30) for these samples to ensure that sample material of suitable quality was present.
- For a patient specimen with a negative Target N result (HEX channel) and a positive Target E result (FAM channel), the interpretation shall be “Presumptive POSITIVE for SARS-COV-2 RNA” because the SARS-CoV-2 E gene allows for detection of Sarbecovirus and SARS-CoV is currently not circulating.

Note:

- A Positive Target N result (HEX channel) and a negative Target E result (FAM channel) is suggestive of:
  - A sample at low concentrations
  - A mutation in the Target E region, or
  - Other factors.
- A negative Target N result (HEX channel) and a positive Target E result (FAM channel) is suggestive of:
  - A sample at low concentrations
  - A mutation in the Target N region in the oligo binding sites
  - Infection with some other Sarbecovirus (i.e., SARS-CoV-1 or some other Sarbecovirus previously unknown to infect humans)
  - Other factors.

Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

A valid batch may include both valid and invalid sample results.

The table below lists the expected results for the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)***.

Case #	E (FAM)	N (HEX)	IC (Cy5)	PC	NTC	Interpretation
1	Neg (-)	Neg (-)	Neg (-)	Neg (-)	Neg (-)	Invalid result (inhibition/poor extraction)
2	Neg (-)	Neg (-)	Neg (-)	Pos (+)	Neg (-)	Invalid result (inhibition/poor extraction)
3	Neg (-)	Neg (-)	Pos (+)	Pos (+)	Neg (-)	**SARS-CoV-2 RNA NOT detected**
4	Pos (+)	Pos (+)	Pos (+) or Neg (-)	Pos (+)	Neg (-)	**POSITIVE for SARS-COV-2 RNA**
5	Neg (-)	Pos (+)	Pos (+) or Neg (-)	Pos (+)	Neg (-)	**POSITIVE for SARS-COV-2 RNA**
6	Pos (+)	Neg (-)	Pos (+) or Neg (-)	Pos (+)	Neg (-)	**Presumptive Positive SARS-CoV-2**

Table 5 : Examination and Interpretation of Patient Specimen Results

Note: For positive patient specimens, for low viral concentrations, a Ct of >38 and ≤40 can be reported for one or the two SARS-CoV-2 targets (E and N). The end user is required to review first fluorescent curves for these weakly positive patient specimens before final interpretation. The results shall be interpreted as follows.

Case #	Ct E (FAM)	Ct N (HEX)	Action
A	>38 and ≤40	≤38	**POSITIVE for SARS-COV-2 RNA**
B	≤38	>38 and ≤40	**Presumptive Positive SARS-CoV-2**
C	>38 and ≤40	>38 and ≤40	Recommended to re-test* after looking the Ct curves for E and N.

Table 6 : Examination and Interpretation of Weakly Positive Patient Specimen Results

(*): Sample retesting can be attempted from the RT-PCR only, however, re-extraction of the sample is needed if the RT-PCR is repeat invalid. Re-extraction can be attempted if left over sample material if adequately stored. If no additional material is available or if the re-extracted sample is still invalid a new sample needs to be requested.

Limitations

UltraGene Combo2Screen SARS-CoV-2 Assay (V2) is **Pending FDA review under the EUA program.** Laboratories must include in test reports to healthcare providers the following statement "**The test has been validated but FDA's independent review of this validation is pending**".

General

- For in vitro diagnostic use
- For Prescription Use Only (Rx only)

- For use under an Emergency Use Authorization (EUA) only
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Biosaftey, Contamination prevention

- ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** has been evaluated only for use in combination with the Roche® MagNA Pure 24 System[1] or bioMérieux EMAG®[2] and respective related reagents for RNA extraction (protocol Pathogen 1000, EMAG IFU standard protocol) and TianLong Gentier48R[3] or Applied BioSystems QuantStudio™ 5[4] qPCR instruments and respective related reagents for RT-PCR.
- The kit is to be used by personnel specially instructed and trained in in RT-PCR and the in vitro diagnostics procedures only.
- Strict compliance with the IFU is required for optimal results.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Perfect concordance between the results should not be expected due to differences between technologies. Users should follow their own specific policies/procedures.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Test results cannot be directly used as the basis for clinical diagnosis or exclusion, only for reference by clinicians. At the same time, sample collection, preservation and improper transportation conditions, variation in the virus target sequence or the sequence change caused by other causes could lead to a false negative result.
- A negative result does not exclude the possibility of infection because very low levels of infection or sampling error may cause a false-negative result.
- The test has been validated for use only with RNA extracted from nasopharyngeal swab samples. Testing of other sample types with ***UltraGene Combo2Screen SARS-CoV-2 Assay*** may result in inaccurate results.
- The performance of the ***UltraGene Combo2Screen SARS-CoV-2 Assay*** could be affected by future Covid-19 vaccinated individuals.
- Prevalence of SARS-CoV-2 infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

[1] https://diagnostics.roche.com/global/en/products/systems/magna-pure-24-system.html
[2] https://www.biomerieux-usa.com/clinical/emag
[3] http://e.medtl.com/?page_id=227
[4] https://www.thermofisher.com/search/browse/category/us/fr/602559/Real-Time+PCR+Instruments

Warnings and Precautions

Laboratory precautions

- DNase contamination which might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following:

- **Frequent cleaning of the wells of the qPCR instrument plate.**
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories shall be required to report all positive results to the appropriate public health authorities.
- The samples to be tested involved in this test shall be regarded as infectious substances, and the operation and treatment shall comply with the relevant requirements of the General Guidelines for Biosafety of Microbial Biomedical Laboratories and the Medical Waste Management Regulations issued by the Public Health Authorities[5], and using good laboratory procedures as outlined in the CLSI Document M29-A4[6].
- To make sure an accurate and reliable result, always use DNase/RNase-free disposable pipette tips, tubes, and calibration pipettes.
- Use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) sample preparation area- isolation of the RNA/ DNA from sample and control, and 3) Amplification area- amplification and detection of nucleic acid target.
- To avoid contamination, all the objects should be used in certain areas. All apparatus must be cleaned after each experiment.
- To avoid the contamination of fluorescent materials, disposable gloves, tubes, pipette and filter tips should not do contain fluorescent material.
- Nucleic acid samples stored at -70 ° C should be thawed, mixed, and centrifuged at low temperature for a short time before use.
- Try to avoid the generation of air bubbles when the reaction solution is dispensed.
- When adding the sample, the sample should be completely added to the reaction solution, and no sample should adhere to the tube wall. The tube cap should be closed as soon as possible after the sample is added.
- The reaction tube containing the reaction solution should be capped or packed in a sealed bag and then transferred to the sample processing area.
- Check whether the reaction tubes are tightly closed before loading on the machine to avoid the leakage contaminating the instrument.
- After the amplification, the reaction tube was taken out, sealed in a special plastic bag, and discarded at the designated place.
- The used tips should be thrown into disposal bottle which have 10% sodium hypochlorite solution and discarded with other waste.
- Use 10% sodium hypochlorite, 75% alcohol and ultraviolet light to disinfect the workbench and experimental items regularly.

[5] https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
[6] Protection of Laboratory Workers From Occupationally Acquired Infections, 4th Edition (M29-A24)(CLSI, M29A4E), https://clsi.org/standards/products/microbiology/documents/m29/

- The real-time PCR instrument requires frequent calibration.
- Material Safety Data Sheets are available upon request.

Product Quality Control

- In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality.
- Certificates of Analysis are available upon request.

Performance Characteristics

Non-clinical performance evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates are tested positive.

The LoD was determined using a reference control (ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (0.5 mL), Cat #0810587CFHI-0.5ML, lot# 324047, 3.16 * 10^6 $TCID_{50}$/mL)) which was serially diluted in negative patient (nasopharyngeal swab) samples.

Patient material was screened negative using New Coronavirus Nucleic Acid Detection Kit (PerkinElmer®, USA, # 2019-nCoV-PCR-AUS, CE-IVD). The samples were extracted using MagNA Pure 24 Total NA Isolation Kit (Roche®, #07658036001) and analyzed with Real-Time PCR System (Tianlong Science & Technology, Cat #Gentier 48E). The tentative LoD and confirmatory LoD was determined. Every sample was run through the extraction process. Replicas were generated by spiking multiple patient samples with RNA and full extraction of all replicas. The results are summarized below.

As real patient material was used for the spiking human RNA can be found in all target levels and RNase P gives a positive result in all samples. A total of 7 concentration levels, with 10-fold serial dilutions between the levels, were tested with a total of 3 replicates per concentration.

SARS-CoV-2 $TCID_{50}$ / mL	Concentration (Level)	Mean Ct Gene N	Mean Ct Gene E	Mean Ct IC
316000	1:10 (L1)	17,28	17,41	26,00
31600	1:100 (L2)	21,00	21,45	22,71
3160	1:1000 (L3)	24,06	24,79	22,86
316	1:10000 (L4)	28,17	28,96	23,02
31	1:100000 (L5)	34,09	36,43	22,85
3	1:1000000 (L6)	No results	No results	No results
0,3	1:10000000 (L7)	No results	No results	No results

Table 7 : Tentative LoD with Roche MagNA Pure

Once the preliminary LoD was established (L5 concentration (0,000001 $TCID_{50}$/mL)), it was confirmed in phase II by testing 20 replicates at level L5 by 3 different operators using 3 different qPCR instruments.

The concentration level for the assay with observed rates greater than or equal to 95%, was 0,000001 $TCID_{50}$/mL or 1*10^{-6} $TCID_{50}$/mL for SARS-CoV-2.

We defined the mean Ct value for each target and the internal control, and their respective upper and lower 95% confidence limit.

SARS-CoV-2 $TCID_{50}$ / mL	% Positives	Mean Ct Gene N [95% CL]	Mean Ct Gene E [95% CL]	Mean Ct IC [95% CL]
31 1:100000 (L5)	98,3% (59/60)	34,32 [33,57--35,06]	35,13 [34,34--35,92]	21,28 [21,12--21,43]

Table 8 : LoD with Roche MagNA Pure

Inclusivity (analytical sensitivity)

The inclusivity study database was composed of public full-length SARS-CoV-2 genomes for Human hosts as of the 21st of May 2020. The sequences were downloaded through the NCBI labs virus interface.

There were 2415 unique sequences available after removal of 243 identical sequences.

The Multi Sequence Alignment (MSA) was done using the online MAFFT tool (version 7) for better performances than standard desktop machines. We kept the default parameters to perform the alignment. We then downloaded the resulting alignment in a fasta format.

Using the graphic tool Ugene, we opened the MSA file. We selected the NCBI Reference Sequence: NC_045512.2 (SARS-CoV-2 isolate Wuhan-Hu-1, complete genome) and set it as the reference. We searched all primers and probes within this reference sequence (replacing the ambiguous characters with the corresponding ones from the reference). Because the MSA is 5'-3', we had to reverse complement the reverse primers when doing the look up.

We reported below the summary of the alignment.

Target gene	Percent Homology		
	Fw Primer	Probe	Rv Primer
E	100%	99.9% (2 sequences with 1 mismatch each)	100%
N	99.6% (10 sequences with 1 mismatch each)	100%	99.8% (3 sequences with 1 mismatch each and 1 sequence with 2 mismatches)

Table 9 : Summary table of the inclusivity study

The mismatches for the N reverse and forward primers are not expected to have any impact on the amplification and detection of the N gene. In silico analysis concluded that the product would detect all analyzed SARS-CoV-2.

Cross-reactivity (In Silico Specificity)

We did a database of complete genomes of all organisms listed in the "Recommended List of Organisms to be Analyzed in silico and by Wet Testing" (46 unique accession numbers). We launch a BLAST analysis of the 46 full-genome sequences. Nucleotide BLAST (blastn) was used to query all primers and probe sequences against the complete genome sequences in the database. Results with < 80% identical matches were discarded leaving 6 blast results.

Microorganism	Accession number	Assay sequence matches
Human coronavirus 229E	NC_002645.1	No match found
Human coronavirus OC43	NC_006213.1	No match found
Human coronavirus HKU1	NC_006577	No match found
Human coronavirus NL63	NC_005831.2	No match found
SARS-coronavirus	NC_004718.3	E primers and probe (100%) N forward primer (85%)
MERS-coronavirus	KJ556336.1	No match found
Adenovirus type 1	MH183293.1	No match found
Adenovirus type 2	J01917.1	No match found
Adenovirus type 3	AY599836.1	No match found
Human Metapneumovirus	KJ627437.1	No match found
Parainfluenza virus 1	KX639498.1	No match found
Parainfluenza virus 2	KM190939.1	No match found
Parainfluenza virus 3	NC_001796.2	No match found
Parainfluenza virus 4	JQ241176.1	No match found
Influenza A	GCF_000928555.1	No match found
Influenza B	NC_000907.1	No match found
Enterovirus	NC_001472.1	No match found
Respiratory syncytial virus	NC_001803.1	No match found
Rhinovirus	NC_009996.1	No match found
Chlamydia pneumoniae	NC_005043.1	No match found
Haemophilus influenzae	NZ_LN831035.1	No match found
Legionella pneumophila	NZ_LR134380.1	No match found
Mycobacterium tuberculosis	NC_000962.3	No match found
Streptococcus pneumoniae	NZ_LN831051.1	No match found
Streptococcus pyogenes	NZ_LN831034.1	No match found
Bordetella pertussis	NC_018518.1	No match found

Microorganism	Accession number	Assay sequence matches
Mycoplasma pneumoniae	NZ_CP010546.1	No match found
***Pneumocystis jirovecii* (PJP)**	CAKM01000281.1	No match found
Candida albicans	SC5314	N probe (94%)[c]
Pseudomonas aeruginosa	NC_002516.2	No match found
Staphylococcus epidermis	NZ_CP035288.1	E reverse primer (86%)
Streptococcus salivarius	NZ_CP009913.1	No match found

Table 10 : In silico analysis for detection of SARS-CoV-2 sequences

Using the 80% identity threshold the E set of sequences are not specific to SARS-CoV-2 as they would be able to amplify and detect the SARS coronavirus. But it presents no risk today as the SARS-CoV is not circulating in the US.

The primers and probe for the N gene seem to be specific to SARS-CoV-2.

Cross-reactivity (Wet testing)

The possible cross-reactivity with the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** for each microorganism described in the table below was determined using the isolate (reference and concentration indicated in the table) which was contrived in negative patient (nasopharyngeal swab) samples.

Patient material was screened negative using New Coronavirus Nucleic Acid Detection Kit (PerkinElmer®, USA, # 2019-nCoV-PCR-AUS, CE-IVD). The samples were extracted using MagNA Pure 24 Total NA Isolation Kit (Roche®, #07658036001) and analyzed with Real-Time PCR System (Tianlong Science & Technology, Cat #Gentier 48E).

Microorganism	ID	Test Concentration	Replicates detected / total	SARS-CoV-2 Test Result
Human coronavirus OC43	EQA	4.03 dPCR Log10 cp/ml	(0/1)	Not detected
Human coronavirus NL63	EQA	4.64 dPCR Log10 cp/ml	(0/1)	Not detected
Adenovirus type 3	ZeptoMetrix #NATPPA-BIO		(0/3)	Not detected
Human Metapneumovirus	ZeptoMetrix #0810161CF	3.80×10^6 $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 1	ZeptoMetrix # 0810014CF	3.39×10^7 $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 2	ZeptoMetrix # 0810015CF	4.17×10^5 $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 3	ZeptoMetrix # 0810016CF	8.51×10^7 $TCID_{50}$/mL	(0/3)	Not detected
Parainfluenza virus 4	ZeptoMetrix # 0810017CF	1.51×10^6 $TCID_{50}$/mL	(0/3)	Not detected
Influenza A	ZeptoMetrix # 0810036CF	$1 \times 10^{5.15}$ U/mL	(0/3)	Not detected
Influenza B	ZeptoMetrix # 0810255CF	$1 \times 10^{6.10}$ U/mL	(0/3)	Not detected
Respiratory syncytial virus	ZeptoMetrix # 0810040ACF	5.01×10^5 $TCID_{50}$/mL	(0/3)	Not detected
Rhinovirus	ZeptoMetrix #NATPPA-BIO		(0/3)	Not detected
Chlamydia pneumoniae	ZeptoMetrix #NATPPA-BIO		(0/3)	Not detected
Haemophilus influenzae	ZeptoMetrix # 0801679	2.27×10^9 CFU/mL	(0/3)	Not detected
Streptococcus pneumoniae	ZeptoMetrix # 0801439	2.26×10^9 CFU/mL	(0/3)	Not detected
Streptococcus pyogenes	ZeptoMetrix # 0801512	1.64×10^9 CFU/mL	(0/3)	Not detected
Bordetella pertussis	ZeptoMetrix # 0801459	1.13×10^{10} CFU/mL	(0/3)	Not detected
Mycoplasma pneumoniae	ZeptoMetrix # 0801579	3.16×10^8 CFU/mL	(0/3)	Not detected

Microorganism	ID	Test Concentration	Replicates detected / total	SARS-CoV-2 Test Result
***Pneumocystis jirovecii* (PJP)**	ZeptoMetrix # 0801698	1.56 x 10^8 CFU/mL	(0/3)	Not detected
Candida albicans	ZeptoMetrix # 0801504	6.24 x 10^8 CFU/mL	(0/3)	Not detected
Pseudomonas aeruginosa	ZeptoMetrix # 0801519	8.4 x 10^9 CFU/mL	(0/3)	Not detected
Staphylococcus epidermis	ZeptoMetrix # 0801651	1.21 x 10^{10} CFU/mL	(0/3)	Not detected
Streptococcus salivarius	ZeptoMetrix # 0801896	8.17 x 10^8 CFU/mL	(0/3)	Not detected
HIV-1	Clinical isoolate	> 1000 cp/mL	(0/3)	Not detected

Table 11 : Cross-reactivity test results

None of the microorganisms tested interfered with the product performance by generating false positive results.

Clinical performance evaluation

Clinical sensitivity

Clinical specimens were obtained from a French private pathology laboratory (ISO 15189 accredited laboratory) that characterized the samples for SARS-CoV-2 using CE-IVD and EUA authorized SARS-CoV-2 kits. The specimens were collected from patients with signs and symptoms of an upper respiratory infection and by qualified personnel according laboratory validated procedures for SARS-CoV-2 collection.

Clinical specimens (nasopharyngeal swab (NP)) were retrospectively selected from the private pathology laboratory RNA bank where enough leftover extracted RNA (≥30 µL) from patients tested recently for Covid-19 symptoms (May 2020) was available and it must have been tested with an authorized FDA test in the context of the Emergency Use Authorization.

Two EUA FDA tests were used for the clinical sensitivity study:

- New Coronavirus Nucleic Acid Detection Kit (PerkinElmer®, USA, # 2019-nCoV-PCR-AUS, CE-IVD), n=57 clinical specimens;
- SARS-COV-2 R-GENE® (bioMérieux, France, # 423735, CE-IVD); n=3 clinical specimens.

The RNA extraction method used at the private pathology laboratory was MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Cat #03730964001).

A total of 60 clinical specimens were retrieved from the RNA bank: 30 negative and 30 positive.

Each RNA was contrived in negative patient (nasopharyngeal swab) samples. Patient material was screened negative using New Coronavirus Nucleic Acid Detection Kit (PerkinElmer®, USA, # 2019-nCoV-PCR-AUS, CE-IVD) or SARS-COV-2 R-GENE® (bioMérieux, France, # 423735).

The contrived samples (30µL RNA + 500µL negative patient swab solution) were then extracted using MagNA Pure 24 Total NA Isolation Kit (Roche, #07658036001) (Pathogen 1000 protocol; sample input volume: 530 µL; Elution volume: 50 µL) and tested with Real-Time PCR System (Tianlong Science & Technology, Cat #Gentier 48E) in a blinded manner and according to the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** Instructions for Use.

The clinical performance of the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is reported in the table below.

Nasopharyngeal swab in VTM		**EUA Authorized Comparator**		**Total**
		Positive	Negative	
UltraGene Combo2Screen SARS-CoV-2 Assay (V2)	Positive	30	0	30
	Negative	0	30	30
	Total (NP/WTM)	**30**	**30**	**60**

The negative percent agreement was calculated based on the result obtained from the prior testing at the French laboratory using the EUA authorized SARS-CoV-2 RT-PCR tests. None of the SARS-CoV-2 negative clinical specimens gave positive test results for SARS-CoV-2. Diagnostic specificity of ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is 100 %.

The positive percent agreement was calculated based on the agreement of the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** result with the positive tested samples in NP swabs are shown below.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** performance against the expected results are:

- Positive Percent Agreement 30/30 = 100% (95% CI: 88.4%-100.00%)
- Negative Percent Agreement 30/30 = 100% (95% CI: 88.4%-100.00%)

The results interpretation agreement between the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** and the EUA comparator was perfect (Cohen's Kappa score=1).

Clinical reproducibility – External Quality Assurance (EQA)

We registered for the 2020 Coronavirus Outbreak Preparedness (CVOP) EQA Pilot Study (Cat# QAV204214_1) from QCMD which is composed of 8 tests.

The intention of this panel is to determine whether accurate results have been generated to ensure that the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** is performing to expectation and therefore providing the best possible service to all front-line clinicians during the outbreak.

The eight samples were spiked into negative specimen before extraction and blindly distributed by an operator, different than the technician who handled the specimens and processed them.

After unblinding the results, we obtained 100% clinical accuracy and the results were reproducible for the external quality assurance program.

References

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4. Processing of Sputum Specimens for Nucleic Acid Extraction - https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf
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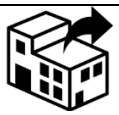
6. Publicly available report from French National Reference Centre for Covid-19 about the ***UltraGene Combo2Screen SARS-CoV-2 Assay*** https://www.sfm-microbiologie.org/wp-content/uploads/2020/05/RESULTATS-CNR-Combo2Screen-SARS-CoV-2-ABL.pdf

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Negative control
	Catalog number		Positive control
	Use by		Temperature limitation
	Manufacturer	**R_x only**	For provision and use only at a licensed physician's direction and under medical supervision
	Distributor	***Rn***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Serial Number		

Contact Information

- For technical assistance and more information, please see our Technical Support Center at support-diag@ablsa.com or contact your ABL Field-Application Specialist.
- For up to date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide.

Manufacturer and distributors

Advanced Biological Laboratories (ABL) S.A.

17 rue des Jardiniers, L-1835 Luxembourg, Luxembourg

USA and US territories

AdvancedDx Biological Laboratories USA Inc.

5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

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