UltraGene

Combo2Screen SARS-CoV-2 Assay

V1

User Guide

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Version 1

Qualitative in-vitro diagnostic - For use with qPCR Instruments

IVD **R_x only**

For use under an Emergency Use Authorization (EUA) Only

REF **139A100**
139A1000

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Introduction

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

Name

UltraGene Combo2Screen SARS-CoV-2 Assay

Contents

Intended use

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is a real-time RT-PCR test (nucleic acid technique (NAT)) intended to be used for the qualitative detection of nucleic acids from severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2) in a clinical specimen to aid the diagnosis of coronavirus disease (Covid-19) infection.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is for use only under Emergency Use Authorization (EUA) in U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and in U.S. laboratories certified under CLIA to perform high complexity tests.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

The current laboratory biosafety guidance for the 2019 novel coronavirus (SARS-CoV-2) shall be followed[1].

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

Indication of use

The amplified DNA result and its interpretation are used to aid the diagnosis of coronavirus disease (Covid-19) infection. Results are for the specific detection of SARS-CoV-2 RNA that are detectable during infection in nasopharyngeal swab samples from patients who meet Covid-19 clinical and/or epidemiological criteria.

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. Additional laboratory testing (i.e. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

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. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by the test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.

Note: The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** 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.

[1] https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

Summary and explanation of the test

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is a qualitative test for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in nasopharyngeal swab samples collected in Universal Transport Medium from patients with signs and symptoms of infection who are suspected of Covid-19.

In addition of the PCR amplification reagents, the test utilizes external controls (low titer positive control and a negative control).

Principles of the assay

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** 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. Amplification of the two targets (SARS-CoV-2 E and SARS-CoV-2 N) takes place simultaneously in the same thermal cycling program.

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** uses a fluorescent probe for the detection of PCR products. During each RT-PCR cycle, the fluorescent signal increases in a logarithmic manner, resulting in an amplification curve. As soon as the amplification curve of the 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 are labeled with the same fluorophore thus allowing for simultaneous detection of both SARS-CoV-2 amplified products in the same reaction cycle. The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is performed on a qPCR instrument having at least the FAM channel (Excitation: 465 / Emission: 510).

A positive control and a negative control are processed from the start of sample preparation to evaluate run validity. The positive control in the kit contains the test 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 product is compliant with EN ISO 13485:2016, EN ISO 14971:2012, EN 62366:2008, EN 13612:2002, EN 13975:2003, EN 14136:2004, EN ISO 15223-1:2016, EN ISO 18113-1:2011, EN ISO 18113-2:2011 and EN ISO 23640:2015.

Assay components

The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is provided in two formats: 100 reactions (REF 139A100) or 1000 reactions (139A1000). The specified number of reactions is the number of reactions for each target.

Ref no.		**139A100**	
Number of reactions		**100**	
RT-PCR reagents	**Per tube**	**Nb of tubes (color cap)**	**Total**
CoV Reaction Solution_VLC3	1375 µL	2 (green)	2750 µL
CoV E Primer and Probe Mix	248 µL	1 (yellow)	248 µL
CoV N Primer and Probe Mix	248 µL	1 (orange)	248 µL
Water	1000 µL	1 (blue)	1000 µL
Controls			
Positive Control	80 µL	1 (white)	80 µL
Negative Control	80 µL	1 (black)	80 µL
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Table 1 : Assay components for 100 reactions of each target

Ref no.		**139A1000**	
Number of reactions		**1000**	
RT-PCR reagents	**Per tube**	**Nb of tubes (color cap)**	**Total**
CoV Reaction Solution_VLC3	1375 µL	20 (green)	27500 µL
CoV E Primer and Probe Mix	1238 µL	2 (yellow)	2476 µL
CoV N Primer and Probe Mix	1238 µL	2 (orange)	2476 µL
Water	1000 µL	1 (blue)	1000 µL
Controls			
Positive Control	800 µL	1 (white)	800 µL
Negative Control	800 µL	1 (black)	800 µL
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Table 2: Assay components for 1000 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 a noninfectious purified nucleic acid which contains the complete SARS-CoV-2. It does not contain any viable virus. The ***UltraGene Combo2Screen SARS-CoV-2 Assay*** targets regions within the positive control.
- Controls should be validated by the end-user if used for the interpretation of test results.

Materials Required but Not Provided

- Microliter pipets* dedicated for PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL)
- Benchtop centrifuge* with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm)

- Benchtop vortex mixer*
- qPCR instrument* with FAM, i.e. Gentier real time PCR systems (ABL).

(*): 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.

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.
- 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*** is extracted RNA.

RNA isolation

- Extract the RNA using i.e. Roche MagNA Pure Total Nucleic Acid Isolation Kit (magnetic bead method).
- The resulting supernatant is used directly as the template in the ensuing analyses and will be referred to as the "extracted RNA".

Note: To achieve optimal and sensitive RNA analysis, the best representation of the RNA, it is recommended to extract 700 µL of sample and elute in the minimum volume required for your validated RNA extraction kit (i.e. 50 µL).

Workflow

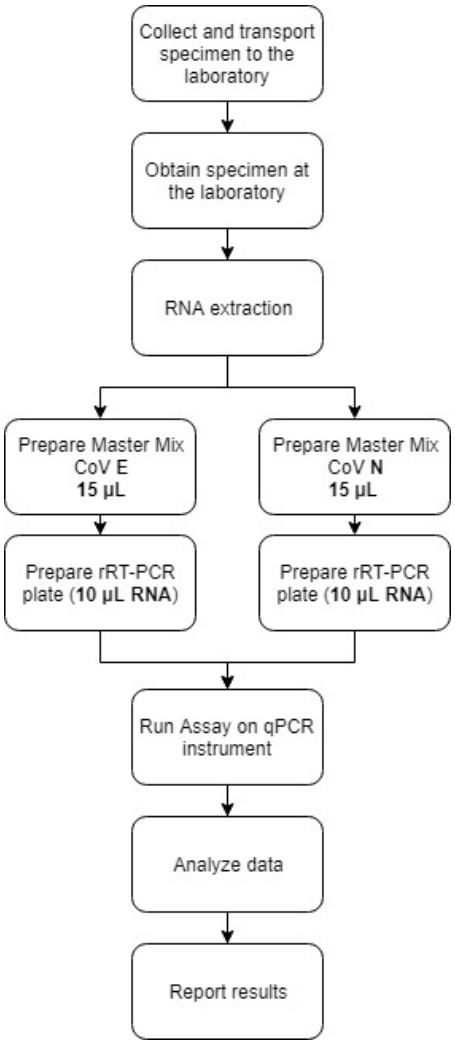

Figure 1 : Assay workflow for the 2 distinct CoV targets

Note: the workflow above is describing two distinct master mixes which are operated during the cycling reaction. For results consistency, the operator is recommended to perform reactions for both targets at the same time.

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: CoV *Reaction Solution_VLC3*, Water, *Primer and Probe Mixes (CoV E, CoV N) and controls (Positive and Negative)*.
2. Gently and evenly mix each individual reagent, then 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 2 tables below).

Premix reagents CoV E	Volume for 1 sample
CoV Reaction Solution_VLC3	12.5 µL
CoV E Primer and Probe Mix	2.25 µL
Water	0.25 µL
Total volume	**15 µL**

Table 3: Premix reagents E target

Premix reagents CoV N	Volume for 1 sample
CoV Reaction Solution_VLC3	12.5 µL
CoV N Primer and Probe Mix	2.25 µL
Water	0.25 µL
Total volume	**15 µL**

Table 4: Premix reagents N target

4. Evenly aliquot the premix(es) into qPCR tube(s) (**one qPCR tube per target and sample to be tested**).
5. Add 10 µL of extracted RNA solution to a single qPCR tube for each target.
6. 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.**

CONTROL − The NTC must be run on each assay plate and the cycle threshold (Ct) for the NTC must be equal to 0 for the sample(s) on the plate to be valid. If this criterion is not met, the sample(s) and controls on that plate are invalid and must be repeated.

CONTROL + The positive control must be run on each assay plate and the cycle threshold (Ct) should be less than 30.

qPCR Cycling Condition

- Set 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 channel)
		72	00:15
7 (cooling)	1	40	00:01

Table 5: qPCR Cycling program

- Selection of fluorescence channels: FAM (Excitation: 465 / Emission: 510).

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.

- If the above quality control conditions were met, the interpretation analysis is performed.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.
- The table below lists the expected results for the ***UltraGene Combo2Screen SARS-CoV-2 Assay***.
- 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.

Note:

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

- **For samples with a Ct value between 38 and 40, for the CoV E or CoV N target, additional confirmatory testing may be conducted.**

Case #	CoV E	CoV N	Positive control	NTC	Results interpretation (Report)	Decision
1	Neg (-)	Neg (-)	Neg (-)	Neg (-)	Invalid result (**Invalid**)	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.
2	Neg (-)	Neg (-)	Pos (+)	Neg (-)	SARS-CoV-2 not detected (**Not detected**)	Report results to sender. Consider testing for other respiratory viruses.
3	Neg (-)	Pos (+)	Pos (+) or Neg (-)	Neg (-)	SARS-CoV-2 (Target N specific RNA) detected[2] (**Positive SARS-CoV-2**)	Report results to sender and appropriate public health authorities.
4	Pos (+)	Neg (-)	Pos (+) or Neg (-)	Neg (-)	SARS-CoV-2 (Target E specific RNA) detected (**Presumptive Positive SARS-CoV-2**)	One positive target is enough, but you can consider doing again the negative target[3]. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
5	Pos (+)	Pos (+)	Pos (+) or Neg (-)	Neg (-)	SARS-CoV-2 (Target E and N specific RNA) detected (**Positive SARS-CoV-2**)	Report results to sender and appropriate public health authorities.

Table 6: Results interpretation and report based on 2 targets results

[2] A Positive Target N result and a negative Target E result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target E region, or 3) other factors.

[3] A negative Target N result and a positive Target E result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target N region in the oligo binding sites, or 3) infection with some other Sarbecovirus (i.e., SARS-CoV-1 or some other Sarbecovirus previously unknown to infect humans), or 4) other factors.

Limitations

- ***UltraGene Combo2Screen SARS-CoV-2 Assay*** is for in vitro diagnostic use under the FDA Emergency Use Authorization Only.
- **No internal control (RNA sequence that is unrelated to the SARS-CoV-2 target sequence) is provided with the assay to be introduced into each specimen at the beginning of sample preparation. There is no mean to demonstrate that the process has proceeded correctly for each negative sample.**
- ***UltraGene Combo2Screen SARS-CoV-2 Assay*** has been evaluated only for use in combination with the Roche® MagNA Pure 24 System[4] and related reagents for RNA extraction (protocol Pathogen 1000) and TianLong Gentier48R qPCR instrument[5] and related reagents for RT-PCR.
- The kit is to be used by personnel specially instructed and trained in 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.

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.

[4] https://diagnostics.roche.com/global/en/products/systems/magna-pure-24-system.html
[5] http://e.medtl.com/?page_id=227

- Laboratories within the United States and its territories are 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[6], and using good laboratory procedures as outlined in the CLSI Document M29-A4[7].
- 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.
- Avoid the bubbles when separate the reaction solution into tubes. Check the tubes before amplification to avoid contamination induced by leak of fluorescent materials.
- Nucleic acid samples stored at -70 ° C should be thawed, mixed, and centrifuged at low temperature for a short time before use.
- The reaction tube containing the reaction solution should be capped or packed in a sealed bag and then transferred to the sample processing area.
- 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.
- Try to avoid the generation of air bubbles when the reaction solution is dispensed, and 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.
- 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.

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

Performance Characteristics

Specimen Collection and Handling

Our performance evaluation approach was different to the routine procedure in terms of storing as we used leftover frozen clinical samples (patient's consent for research use) from a private pathology laboratory (ISO 15189 accredited) performing SARS-CoV-2 testing.

We used control reference materials from the industry (ISO 13485 certified production) and an external quality assessment panel (accredited ISO 17043:2010).

We also performed in-silico analyses to verify the inclusivity of our assay and simulate potential interferences of other DNA templates (viruses, microbes or human).

Nonclinical studies

Nonclinical studies were conducted to establish the analytical performance of the ***UltraGene Combo2Screen SARS-CoV-2 Assay***.

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.

An initial LoD was determined using a reference control (ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (0.5 mL), catalog# 0810587CFHI-0.5ML, lot# 324047, 3,16 * 10^6 $TCID_{50}$/mL)) which was serially diluted in simulated clinical matrix.

A total of 7 concentration levels, with 10-fold serial dilutions between the levels, were tested with a total of 3 replicates per concentration for each target, as shown in table 7.

SARS-CoV-2 $TCID_{50}$ / mL	Concentration (Level)	Mean Ct Gene N	Mean Ct Gene E
316000	1:10 (L1)	17,22	16,62
31600	1:100 (L2)	21,01	19,60
3160	1:1000 (L3)	24,57	22,95
316	1:10000 (L4)	28,17	26,81
31	1:100000 (L5)	31,54	41,27
3	1:1000000 (L6)	34,77	
0,3	1:10000000 (L7)	38,26	

Table 7 : Initial LoD determination using USA-WA1/2020 strain

Once the preliminary LoD was established, it was confirmed in phase II by testing 20 replicates for each Target regions at three levels.

As shown in table 8, the concentration level, for both Targets (E and N), 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.

SARS-CoV-2 $TCID_{50}$ / mL	Concentration (Level)	% Positives Gene N	Mean Ct Gene N	% Positives Gene E	Mean Ct Gene E
316	1:10000 (L4)	Not done		100% (20/20)	26,40
31	**1:100000 (L5)**	**100% (20/20)**	**31,66**	**100% (20/20)**	**32,32**
3	1:1000000 (L6)	80% (16/20)	36,26	0% (0/20)	
0,3	1:10000000 (L7)	40% (8/20)	37,45	Not done	

Table 8 : LoD determination in Nasopharyngeal specimens

The Target N may have a greater sensitivity but did not reach the 95% hit rate with the lower concentration (L6).

Note: Even if we were able amplify adequately the SARS-CoV-2 N region at lower concentrations (L6 and L7), a laboratory shall proceed to its own evaluation of the UltraGene Assay performance for concentrations below the LoD.

Inclusivity (in-silico)

The study database was composed of public full-length SARS-CoV-2 genomes for Human hosts as of the 7th of April 2020. The sequences were downloaded through the NCBI labs virus interface. There were 414 sequences available. 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 NC_045512.2 sequence 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. For each primer and probe, we then searched for mismatches in any of the other 413 sequences.

- The studied forward/reverse primers and probes for the E and N genes aligned without mismatches on >99% of the 414 sequences in the Inclusivity dataset.
- When allowing for 1 mismatch, we aligned all primers and probes of the ***UltraGene Combo2Screen SARS-CoV-2 Assay*** to 100% of the inclusivity study sequences.
- One sequence had a mismatch at position 10 of the N reverse primer and another one at position 1 on the N reverse primer. Those mismatches are expected to not have an impact on the amplification and detection. One sequence had a mismatch at position 23 of the E probe which is not expected to have an impact on the amplification and detection of the E gene according to the original peer-reviewed publication describing the E primers and probe.

Cross-reactivity (in-silico)

We conducted a two steps in-silico cross-reactivity analysis.

First, a primer-BLAST analysis where the designated SARS-CoV-2 template sequence for our study was NC_045512.2. We also set the amplicon size to be between 30 and 1500 bp. The selected organisms/database to search for unintended targets were Coronaviridae (taxid:11118) and Homo sapiens (taxid:9606) from the RefSeq representative genomes database.

Second, we did an in-silico cross-reactivity manual analysis with the Human genome reference (GRCh38) and six families of viruses (only RefSeq complete genomes filtered by human host): Adenoviridae (9 viruses),

Coronaviridae (11 viruses where SARS-CoV-2 virus sequences were removed), Orthomyxoviridae (10 viruses), Paramyxoviridae (13 viruses), Parvoviridae (18 viruses), Picornaviridae (37 viruses).

Our study database had 166 RefSeq virus sequences and 639 human sequences. We defined conditions for potential off targets to be identified for each organism available in the study database. We used Blast 2.10.0 and Python 3.6.8 (Pandas) for the analysis.

- The two intended targets (from the assay PCR reaction products) in our reference sequence (NC_045512.2) were confirmed by Primer-BLAST.
- Primer-BLAST identified two potentially unintended targets for the E gene primers and probe : the Bat coronavirus (BM48-31/BGR/2008) with 1 mismatch in the forward primer, 1 in the reverse primer and 1 in the probe; and the SARS coronavirus but no mismatches found in either primers or probe.
- For the N gene primers and probe, we identified no potentially unintended targets. Even though the forward and reverse primers may amplify a target region in SARS coronavirus, the probe would not bind to the amplicon.
- The primers and probe of the E gene may amplify and detect the SARS and Bat coronaviruses and were not specific with an 80% identity threshold. Few other nonspecific bindings were found but would not result in either an amplification or detection.
- The primers and probe for the N gene were specific to SARS-CoV-2.

Cross-reactivity (wet testing)

The Analytical Specificity of the assay was established by direct testing of organisms in the assay ("wet" testing). The wet testing used 12 micro-organisms identified as high priority for evaluation due to the reasonable likelihood they may be present in upper respiratory samples. We used a reference panel (ZeptoMetrix NATtrol™ Pneumonia Panel – Atypical Bacteria & Viruses (NATPPA-BIO), Lot# 322617) with high titer stocks of the potentially cross-reacting microorganisms which were spiked into negative simulated clinical matrix.

As shown in table 9, all micro-organisms were undetectable with the ***UltraGene Combo2Screen SARS-CoV-2 Assay***.

Panel Member (strain)	Result	Panel Member (strain)	Result
Adenovirus Type 3 (N/A)	Negative	**L. pneumophila (Philadelphia)**	Negative
Adenovirus Type 31 (N/A)	Negative	**M. pneumoniae (M129)**	Negative
C. pneumoniae (CWL-029)	Negative	**Metapneumovirus 8 (Peru6-2003)**	Negative
Coronavirus NL63 (N/A)	Negative	**Parainfluenza virus Type 1 (N/A)**	Negative
Influenza A H3 (A/Brisbane/10/07)	Negative	**Rhinovirus 1A (N/A)**	Negative
Influenza B (B/Florida/02/06)	Negative	**RSV A2 (N/A)**	Negative

Table 9 : Cross-reactivity testing analysis on 12 upper respiratory micro-organisms

Clinical studies

Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the ***UltraGene Combo2Screen SARS-CoV-2 Assay*** using sixty (60) nasopharyngeal swab specimens.

Samples were contrived by spiking known concentrations of diluted reference material SARS-CoV-2 relative to the product LoD into extracted RNA from previously reported negative clinical samples.

The leftover extracted RNA was aliquoted and frozen at -20°C. Negative results were reported using a laboratory-developed test which was validated against a CE-IVD marked test.

For each target, E and N genes of the SARS-CoV-2, the sixty samples were randomly and blindly distributed over 3 instruments runs by a person, different than the technician who handled the specimens and processed them.

All negative samples yielded negative results (100%). Results for positive samples[8] are shown in the table 10.

Final RNA concentration in sample	Number of positives	Mean Ct E gene	Mean Ct N gene
~ 1.5 LOD	100% (20/20)	31,58	31,55
~4 LOD	100% (10/10)	27,20	29,95

Table 10 : nasopharyngeal swab (NP) specimens Clinical Evaluation Study

Clinical sensitivity

We did a comparison of detection of SARS-CoV-2 on clinical samples using 3 different rRT-PCR methods.

Clinical samples (nasopharyngeal swab (NPS)) were retrospectively selected from the RNA bank at an ISO 15189 accredited laboratory where enough leftover extracted RNA (≥30 µL)[9] from patients tested recently for Covid-19 symptoms (March to April 2020) was available.

The specimens used for the clinical sensitivity study were frozen leftover extracted RNA after the process of the lab developed test (LDT) method. This LDT was validated against the VIASURE SARS-CoV-2 Real-Time PCR Detection Kit adapted for the BD MAX™ System (CE-IVD marked, targeting the SARS-CoV-2 S region)).

Forty-four (44) extracted RNA from NPS samples were processed in parallel with the ***UltraGene Combo2Screen SARS-CoV-2 Assay*** and a comparator test (CE-IVD marked, ORF1a/b and N SARS-CoV-2 targets with a claimed LoD of 200 copies/mL or 5 GEC/reaction) : 22 clinical samples were initially reported positive and 22 negative by the validated LDT results.

[8] The interpretation was determined following instructions of the Results Analysis section.

[9] A blinded selection of samples was not possible due to lack of human resources in the emergency context of the Covid-19 outbreak and also a lack of enough leftover extracted RNA needed for our study.

Method of qualitative detection of SARS-CoV-2	**Lab developed test (initial results)**		**UltraGene Combo2Screen SARS-CoV-2 Assay**		**CE-IVD Assay**	
	N	RdRp	N	E	ORF1a/b	N
Mean Ct for positive clinical samples	22,67	23,40	26,23	24,41	18,85	17,64
Number of positive samples	22	22	22	22	22	22

Table 11 : Comparison of 3 qualitative detection of SARS-CoV-2 for 22 clinical samples (NSP)

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

- Positive Percent Agreement 22/22 = 100% (95% CI: 84.56%-100.00%)
- Negative Percent Agreement 22/22 = 100% (95% CI: 84.56%-100.00%)

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

Clinical reproducibility

We registered for the 2020 Coronavirus Outbreak Preparedness (CVOP) EQA Pilot Study (Ref# QAV204214_1) from QCMD which is composed of 8 tests. We ran the panel according the instructions and submitted our results online. A rapid Expected Results report was made available detailing the composition of the EQA panel.

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

We obtained ***100%*** clinical reproducibility.

References

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Symbols

The following symbols may appear on the packaging and labeling:

Σ <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution	CONTROL -	Negative control
REF	Catalog number	CONTROL +	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
SN	Serial Number		

Contact Information

- For technical assistance and more information, please see our Technical Support Center at support-diag@ablsa.com, call +1 (857) 330-7474, or contact your ABL Field-Application Specialist.
- For up to date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide.

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

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