UltraGene Assay

SARS-CoV-2 Multi Variants Deletions

V1

50
1000

Instructions for Use

Version 1.0

Qualitative SARS-CoV-2 assay - For use with qPCR Instruments

IVD **R_x only**

CE

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Intended Use

The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended for the qualitative detection of SARS-CoV-2 deletions HV 69/70 (Δ69), Y144 (Δ144) and 242-244 (Δ242) on the Spike (S) gene and the deletion SGF 3675-3677 (Δ3675) on the ORF1ab gene in upper respiratory specimens from patients already diagnosed positive to SARS-CoV-2 using an authorized assay for SARS-CoV-2 detection according to local competent authorities.

Positive results are used to detect a SARS-CoV-2 variant from a wild-type strain and to distinguish main circulating SARS-CoV-2 variants:

- the Spike Δ69 and Spike Δ144 and ORF1ab Δ3675 indicate an infection with SARS-CoV-2 lineage B.1.1.7 (i.e. United Kingdom VOC 202012/01, VOC 202102/02; "UK")
- the Spike Δ242 and ORF1ab Δ3675 indicate an infection with SARS-CoV-2 lineage B.1.351 (i.e. South Africa VOC 202012/02; "SA")
- the ORF1ab Δ3675 alone indicates an infection with SARS-CoV-2 lineage P.1 (i.e. Brazil VOC 202101/02; "BR").

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 are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection with a different variant 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 ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** is NOT intended for the qualitative detection of RNA from the SARS-CoV-2 in upper respiratory specimens from individuals suspected of COVID-19 by their healthcare provider.

Principles of the assay

The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test which includes five primer and probe sets designed to detect RNA from the SARS-CoV-2 nucleocapsid (N), Spike (S) and ORF1ab genes in extracted RNA from individuals' upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate and bronchoalveolar lavage (BAL) fluid specimens) already tested SARS-CoV-2 positive.

As the specimen is already tested positive, the N gene is used as a reverse transcription and PCR amplification positive control for each well. RNA priorily isolated from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the real-time PCR instrument.

During the amplification 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 bound probe, causing the reporter dye (FAM, HEX, ROX and Cy5) to separate from the quencher

dye (BHQ2), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the thermocycler.

Note: It is recommended to use with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** the same qPCR instrument used for the first SARS-CoV-2 detection assay in order to compare cycle threshold (Ct) values for the positive SARS-CoV-2 targets.

Assay components

The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** is provided in two formats: 50 reactions (REF 160A50, GTIN: **05407007960170**) or 1000 reactions (160A1000, GTIN: **05407007960163**). The specified number of reactions is the number of reactions for each target.

Label	Reagent	Description
CoV Reaction Solution	RT-PCR Premix	RT-PCR 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 dNTPs and buffer components.
Primer and Probe Mix	Primer/Probe Mix (N gene, S gene, ORF1ab gene)	N, S and ORF1ab specific primers and probes
Positive Control	Positive Control	Positive control RNA (N, S and ORF1ab genes)
Negative Control	Negative Control	PCR grade nuclease-free water
H_2O	Water	PCR grade nuclease-free water

Table 1 : Labeling

Label	Volume		Color Cap	Storage
	50 Rn.	**1000 Rn.**		
CoV Reaction Solution	1 * 688 µL	10 * 1375 µL	Green	-25°C to - 15 °C
Primer and Probe Mix	1 * 345 µL	5 * 1375 µL	Pink	-25°C to - 15 °C
Positive Control	1 * 20 µL	1 * 400 µL	White	-25°C to - 15 °C
Negative Control	1 * 20 µL	1 * 400 µL	Black	Can be stored at -25°C to - 15 °C with other reagents
H_2O	1 * 70 µL	1* 1375 µL	Blue	Can be stored at -25°C to - 15 °C with other reagents

Table 2 : Volumes and storage conditions of the UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1

Reagent storage and handling

The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** kit should be stored at - 25°C to - 15 °C and is stable until the expiration date stated on the label. Note the production date and expiration date listed on the label. Reagents from different lot numbers should not be mixed.

Note: Multiple thaw-freeze cycles should be avoided. Aliquoting should be considered.

Materials required but not provided

RNA extraction and purification of clinical specimen

The following nucleic acid extraction and purification kit is validated for use with this test:

Supplier	Equipment, Materials and Reagents
Roche Diagnostics	MagNa Pure 24 instrument Software version v1.1 Catalog #07290519001B
Roche Diagnostics	MagNA Pure 24 Total NA Isolation Kit Catalog #07658036001
Roche Diagnostics	MagNA Pure Tube 2.0mL Catalog #07857551001
Roche Diagnostics	MagNA Pure 24 ProcessingTip Park/Piercing Tool Catalog #07345585001
Roche Diagnostics	MagNA Pure Sealing Foil Catalog #06241638001

Table 3 : Validated Equipment, Materials and Reagents for RNA extraction and purification

Instruments

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

- UltraGene qPCR 48 (Advanced Biological Laboratories (ABL), Catalog #149A48, software version 1).
- QuantStudio 5 Real-Time PCR Instrument (96-Well 0.2mL Block) (Applied Biosystems, Catalog #A28139, Design & Analysis Software 2.4.3 / Firmware Version 1.3.3).

Materials

- For the QuantStudio 5 Real-Time PCR Instrument
  - Applied Biosystems MicroAmp™ Optical 96-Well Reaction Plate with Barcode, Catalog # 4306737
  - Applied Biosystems MicroAmp™ Optical Adhesive Film, Catalog # 4311971
- For the UltraGene qPCR 48 instrument:
  - Applied Biosystems MicroAmp 8-Tubes Strip (0.2 mL), Catalog #N8010580
  - Thermo Fisher Scientific Optically clear flat 8 Cap Strips, Catalog #AB-0866
- o be used for master mix preparation (applicable for both instruments):
  - Eppendorf Safe-Lock Tubes 1,5mL Catalog #0030123328
  - Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL)
  - Adjustable pipettes & fitting filtered pipette tips
  - Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
  - Benchtop vortex mixer.

- 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

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.

Warnings and precautions

- **The assay is for *in vitro* diagnostic (IVD) use.**
- For prescription use only.
- This product has been validated only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Handle all specimens as of infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafetyguidelines.html.
- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- The Positive Control should be handled in an area separate from sample receiving, accessioning and processing areas to avoid contamination of the samples with amplifiable material.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) Dilution of positive control material, 3) sample preparation area- isolation of the RNA/ DNA from sample and control, and 4) Amplification area- amplification and detection of nucleic acid target.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

Workflow

1. Batches of clinical samples tested for SARS-CoV-2 qualitative detection.
    - Negative and positive results are given to healthcare provider
    - PCR positive results are kept for second testing

2. Extracted RNA from positive results of previous PCR runs are used with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions:***
    a) PCR reaction setup
    b) PCR run
    c) Analysis of the presence of the deletions on the SARS-CoV-2 Spike and ORF1ab genes.

Storing specimens

- Original upper respiratory 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.

RNA isolation

Nucleic acids are isolated and purified from upper respiratory specimens via automated extraction using the Roche Diagnostics MagNA Pure 24 Total NA Isolation Kit (Catalog #07658036001) and Roche Diagnostics MagNa Pure 24 instrument (Catalog #07290519001B) with software version 1.1 and with the Pathogen 1000 protocol (sample input volume: 700 µL; elution volume: 50 µL).

- Poor RNA quality might result in failure to amplify the targets.
- If the downstream PCR of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** are not carried out immediately after the SARS-CoV-2 detection PCR run, extracted nucleic acid should be stored at -70°C or lower.
- It is recommended to make few aliquots of RNA extracts to prevent thawing and unthawing.

Do not vortex specimens as this will fragment the RNA and lead to failure of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** tests.

Quality Controls

Controls that are provided with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** are listed below:

a) A “no template” (negative) control (NTC) consisting of Water (molecular grade) is used and is needed to detect cross-contamination during all reaction steps. The NTC is used during the PCR and is used to determine validity of the test run.
b) A positive template control [(ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (Catalog# 0810587CFHI)], diluted into nuclease-free water is needed to ensure the rRT-PCR reaction setup and reagent integrity and is used to validate the rRT-PCR plate results. The positive control is used during PCR.
c) An additional primer/probe set labeled with a distinct fluorophore (FAM) is included in the Primer/Probe Mix that targets the SARS-CoV-2 Nucleocapsid gene (N) that is present in positive specimens. The internal control verifies that nucleic acid is present in the sample and adequately stored between the initial run and the new assay. It is used for every sample processed.

PCR reaction setup

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

Vortexing the mix of "CoV Reaction Solution" and "Primer and Probe Mix" reagents with or without extracted RNA solution, could lead to failures. As specificied in steps "4." and "5.", please don't vortex.

1. Thaw the following reagents on ice (protected from light) : CoV Reaction Solution (RT-PCR Master Mix), Primer and Probe Mix (for N, S and ORF1ab genes) and controls (Positive and Negative).
2. Briefly centrifuge (2000 rpm, 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 (RT-PCR Master Mix)	12.50 µL
Primer and Probe Mix	6.25 µL
H_2O	1.25 µL
Total volume	**20.00 µL**

Table 4: Premix reagents volume for 1 sample

Example: 40 samples and 2 controls to be analyzed in one run:

- 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.
- Volume of the premix required for all sample prep(s) to be tested = (40 + 2) * (20 µL)
- Volume of the premix required for all sample prep(s) to be tested = 840 µL

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**). **DON'T VORTEX.**
5. Add **5 µL** of extracted RNA solution to a single qPCR tube. **DON'T VORTEX.**

Note:

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

PCR cycling conditions

Thermal cycling program

Set up the cycling program for Real-Time PCR Instrument as indicated in the table below.

Stage	Cycle	Ramping rate (°C/s)		Temperature (°C)	Time (min:sec)
		QS5	UG48		
1	1	1.6	8.0	70.0	5:00
2	1	1.6	8.0	93.0	0:03
3	1	1.6	8.0	67.5	2:30
4	1	1.6	8.0	93.0	0:03
5	1	1.6	8.0	66.0	2:30
6	40	1.6	4.0	95.0	00:10
		1.6	4.0	58.0	00:15 (measurement in channels)
		1.6	4.0	72.0	00:15
7 (cooling)	1	1.6	4.0	40.0	00:01

Table 5: PCR Cycling program for QuantStudio 5 Real-Time PCR Instrument (Applied Biosystems, Catalog #A28139, Design & Analysis Software 2.4.3 / Firmware Version 1.3.3) and UltraGene qPCR 48 (Advanced Biological Laboratories (ABL), Catalog #149A48, software version 1)

Selection of fluorescence channels

- QuantStudio 5 Real-Time PCR Instrument:
  - FAM (Excitation: 470 nm; Emission: 520 nm) for the SARS-CoV-2 N target
  - HEX (Excitation: 520 nm; Emission: 555 nm) for the SARS-CoV-2 Spike Δ242
  - ROX (Excitation: 580 nm; Emission: 623 nm) for the SARS-CoV-2 Spike Δ69 and Δ144
  - Cy5 (Excitation: 640 nm; Emission: 682 nm) for the SARS-CoV-2 ORF1ab Δ3675

Note: Should be calibrated according to the Applied Biosystems QuantStudio 3 and 5 Real-Time PCR Systems Installation, Use and Maintenance Guide (Pub. No. MAN0010407).

- UltraGene qPCR 48 Instrument:
  - FAM (Excitation: 465 nm; Emission: 510 nm) for the SARS-CoV-2 N target
  - HEX (Excitation: 527 nm; Emission: 563 nm) for the SARS-CoV-2 Spike Δ242
  - ROX (Excitation: 580 nm; Emission: 616 nm) for the SARS-CoV-2 Spike Δ69 and Δ144
  - Cy5 (Excitation: 632 nm; Emission: 664 nm) for the SARS-CoV-2 ORF1ab Δ3675

Detection channels

The assay signal threshold should be set above the maximum noise level of the no template control curve. The threshold should be either set by auto threshold or manually adjusted after every PCR run based on no template control results for each instrument and software version. Manual threshold adjustment and subsequent interpretation of results should take place only when signal introduced from contamination can be ruled out. Deviations in the assay threshold setup could lead to false results.

Note:

- Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.
- Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Examination and Analysis of Results

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 analysis of results. If any of the controls are not valid, the results cannot be interpreted.

Ct cutoff values for controls

Please use the following Ct cutoff values for assay controls during run validity interpretation.

	SARS-CoV-2 N (FAM)	SARS-CoV-2 S Δ242 (HEX)	SARS-CoV-2 S Δ69 S Δ144 (ROX)	SARS-CoV-2 ORF1ab Δ3675 (Cy5)	Interpretation
Negative Control (NTC)	Ct ≥ 33 or No signal	Ct ≥ 33 or No signal	Ct ≥ 33 or No signal	Ct ≥ 33 or No signal	Valid
Positive Control	Detected (Ct ≤ 30)	Detected (Ct ≤ 30)	Detected (Ct ≤ 30)	Detected (Ct ≤ 30)	Valid

Table 6 : Controls validation

Results analysis

If the run is valid, then you analyse the PCR run results which will be reported as a cycle threshold (Ct) unit.

- Any sample displaying an exponential trace shall be considered as positive for the specific SARS-CoV-2 target.
- An absence of an exponential trace indicates an absence or undetectable load of nucleic acid, despite late Ct values (i.e. Ct ≥ 30) being reported. Please consider too possibly damaged extracted RNA.

Important!

- For each positive target, N (FAM), S (HEX, ROX) and ORF1ab (Cy5), with a Ct value ≥ 28, it is recommended to inspect manually the growth curve appearance. It must show a positive (i.e., exponential) amplification trace for SARS-CoV-2. If the trace is a flat curve, then you may consider the related target as negative for the analysis. See examples of SARS-CoV-2 target growth curves in the "Troubleshooting" section.
- If a positive target, N (FAM), S (HEX, ROX) and ORF1ab (Cy5), using the same qPCR instrument, has a Ct value greater than 10 cycles than the initial screening assay respective Ct values, then it is recommended to inspect manually the growth curve appearance. If the run is valid, then you analyse the PCR run results. The extracted RNA integrity is possibly damaged. Consider repeating the test after a new RNA extraction from the initial upper respiratory specimen.

Patient Sample Interpretation					
N (FAM)	**Δ 242 (HEX)**	**Δ 69 Δ 144 (ROX)**	**Δ 3675 (Cy5)**	**Result Interpretation**	**Action**
+	+	+	+	**Not detected (No deletion)**	• SARS-CoV-2 positive without known deletions. • Report results to sender and appropriate public health authorities.
+	+	-	-	**Detected (Deletions Δ69, Δ144 and Δ3675)**	• SARS-CoV-2 variant • Possible infection with SARS-CoV-2 lineage B.1.1.7 (i.e.United Kingdom VOC 202012/01, VOC 202102/02). • Report results to sender and appropriate public health authorities.
+	-	+	-	**Detected (Deletions Δ242 and Δ3675)**	• SARS-CoV-2 variant • Possible infection with SARS-CoV-2 lineage B.1.351 (i.e. South Africa VOC 202012/02). • Report results to sender and appropriate public health authorities.
+	+	+	-	**Detected (Deletion Δ3675)**	• SARS-CoV-2 variant • Possible infection with SARS-CoV-2 lineage P.1 (i.e. Brazil VOC 202101/02). • Report results to sender and appropriate public health authorities.
-	-	-	-	**Invalid**	• Extracted RNA integrity possibly damaged. • Consider repeating the test#.
+	-	-	-	**Invalid**	• SARS-CoV-2 positive with deletions associated with several variants • Possible infection with other SARS-CoV-2 lineage than identified Variants of Concern • Considering genotyping to detect all SARS-CoV-2 genomic variations
-	+	+	+	**Not Detected (Presumptive No deletion)**	• Extracted RNA integrity possibly damaged. • SARS-CoV-2 positive without known deletions. • Consider repeating the test#. • Report results to sender and appropriate public health authorities.
-	At least 1 negative (-) target (deletion)			**Invalid**	• Extracted RNA integrity possibly damaged. • Consider repeating the test#.

Table 7: Patient sample interpretation

(#) Consider repeating the test after a new RNA extraction from the initial upper respiratory specimen. If the repeated result remains the same, consider collecting a new specimen.

Note: For all results reported to sender and appropriate public health authorities, it is worth considering genotyping to detect all SARS-CoV-2 genomic variations (mutations, insertions and deletions) according to local competent authorities guidelines and policies.

Limitations

- The performance of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** was established using nasopharyngeal swab samples. Oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions***.
- The ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** can be used with the specimens listed in the Intended Use statement. Other specimen types should not be tested with this assay.
- Negative results do not preclude infection with other circulating variants strains of SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report all test results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the test performance.
- A false negative result may occur if a specimen is improperly stored or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the test target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- False-positive results may arise from cross contamination during specimen handling, preparation, assay set-up or product handling.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.

Troubleshooting

Baseline setting

The amplification curve baseline is one of the parameters that can affect PCR results. In case the baseline is incorrectly set, a Ct value can be displayed even if no real amplification occurred. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Real amplification curve

During the manual inspection of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** results, please ensure that the growth curve appearance shows exponential amplification traces such as :

- The SARS-CoV-2 strain not identified as a UK, BR or SA variant strain (Figure 1.)
- The SARS-CoV-2 Brazilian variant (Figure 2.)
- The SARS-CoV-2 UK variant (Figure 3.)
- The SARS-CoV-2 SA variant (Figure 4.).

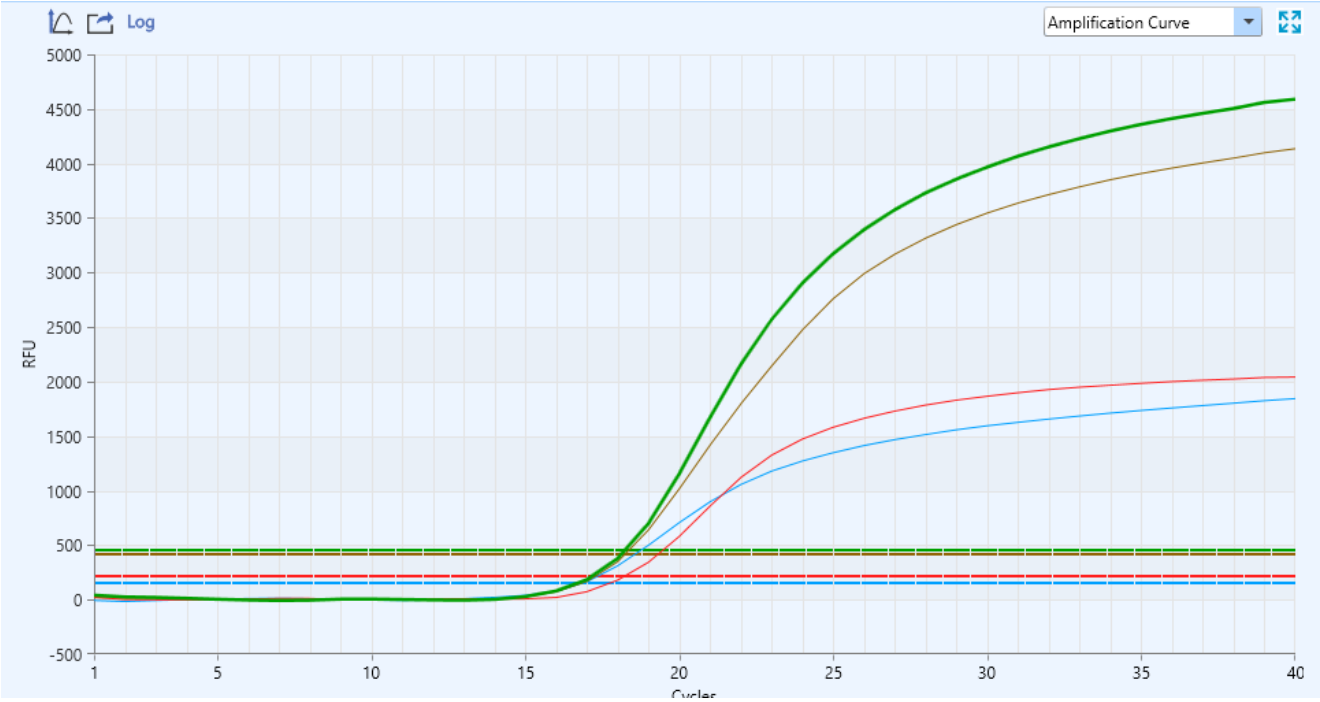

*Figure 1 : Exponential traces for all **UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1** targets which indicate a SARS-CoV-2 strain with no deletions and positive N target.*

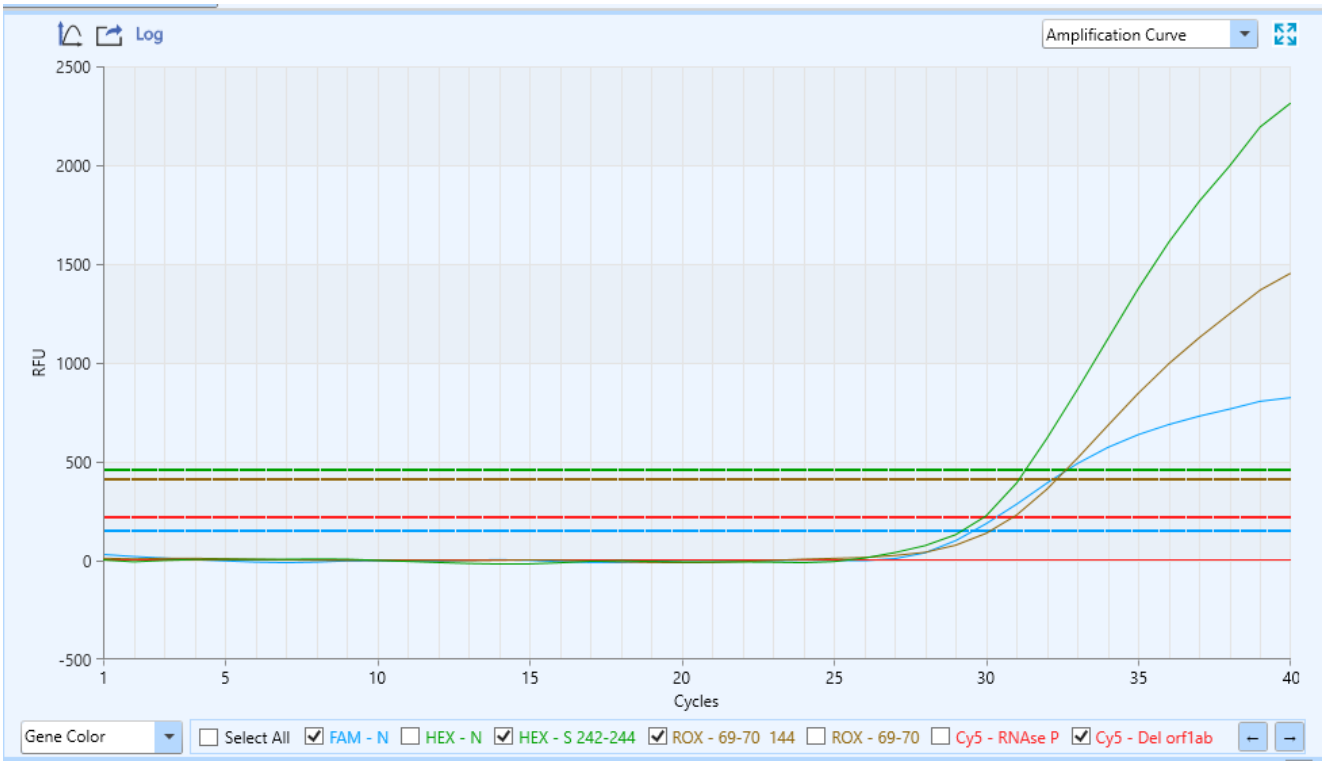

*Figure 2 : Exponential traces for three **UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1** targets which indicate a SARS-CoV-2 Brazilian variant strain : deletion Δ 3675 (absence of signal for Cy5 channel) and positive N target.*

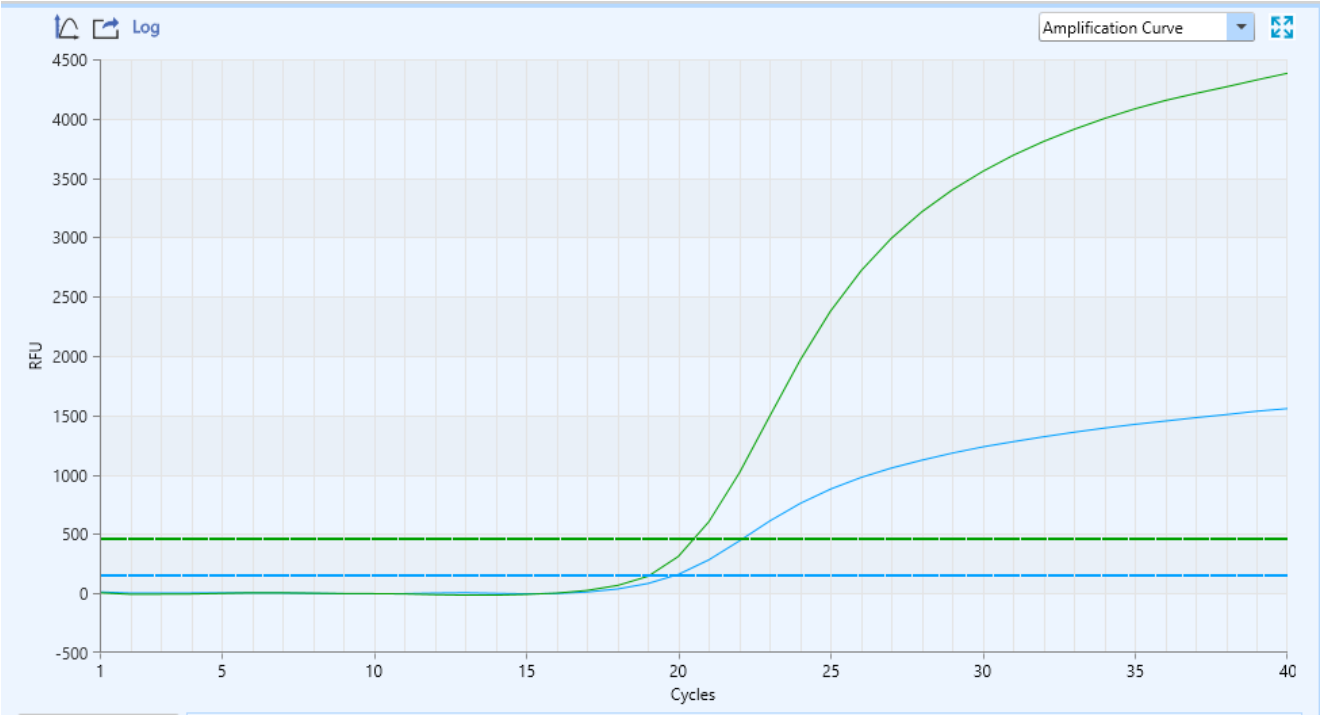

*Figure 3 : Exponential traces for two **UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1** targets which indicate a SARS-CoV-2 UK variant strain : deletion Δ 3675 (absence of signal for Cy5 channel), deletions Δ 69 and Δ 144 (absence of signal for ROX channel) and positive N target.*

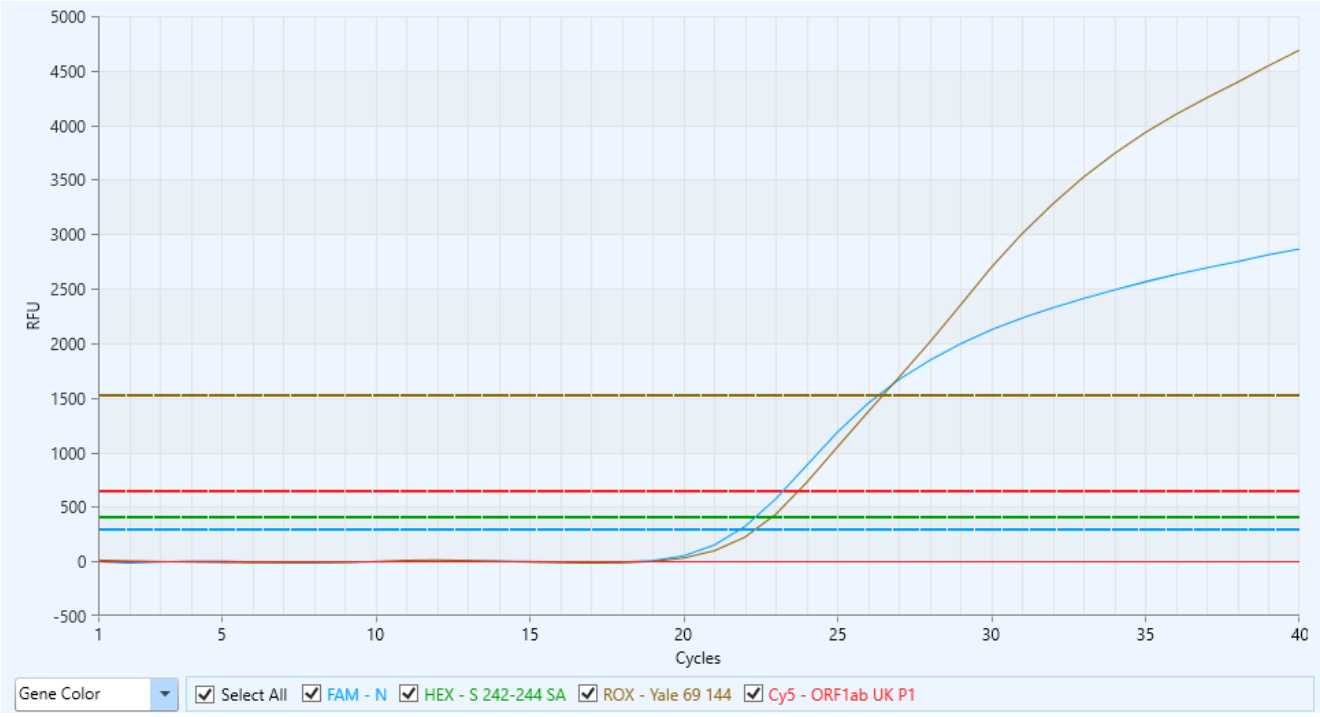

*Figure 4 : Exponential traces for two **UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1** targets which indicate a SARS-CoV-2 South Africa variant strain : deletion Δ 3675 (absence of signal for Cy5 channel), deletions Δ 242 (absence of signal for HEX channel) and positive N target.*

Comparison of initial screening assay Ct values and Assay results

Case #	*UltraGene Combo2Screen SARS-CoV-2 Assay V2.X* *Ct values*		*UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1* *Ct values*	**Difference > 10 cycles for N**	**Comment**
	N	E	N (FAM) target		
1	20.20	22.12	19.99	No (-0.21 Ct)	• Ct values are in the expected range for the Assay targets • Analyse the results
2	22.38	23.15	33.30	Yes (+10.92 Ct)	• Difference between Ct > 10 • Inspect manually the growth curve appearance • The extracted RNA integrity is possibly damaged. Consider repeating the test after a new RNA extraction from the initial upper respiratory specimen.
3	21.79	22.87	32.05	Yes (+10.26 Ct)	• Difference between Ct > 10 • Inspect manually the growth curve appearance • The extracted RNA integrity is possibly damaged. Consider repeating the test after a new RNA extraction from the initial upper respiratory specimen.
4	24.57	25.18	31.56	No (+6.99 Ct)	• With a Ct value ≥ 30, it is recommended to inspect manually the growth curve appearance. • It must show a positive (i.e., exponential) amplification trace for SARS-CoV-2. • If the trace is a flat curve, then you may consider the related target as negative for the analysis. • If positive amplification, analyse the results

Table 8 : Comparison of Ct values between initial detection and UltraGene Assay SARS-CoV-2 Multi Variants Deletions V1 PCR runs

Note:

- The examples are only indicative.
- The comparisons are done using the ***UltraGene Combo2Screen SARS-CoV-2 Assay V2.X*** (ABL, Catalog #139BX) Ct values to illustrate the example of differences between an initial SARS-CoV-2 detection run and a run to detect the deletions with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*****.** You shall use your own initial detection assay Ct values.

Performance characteristics

Non-clinical performance evaluation

Limit of detection (LoD) (analytical sensitivity)

Analytical sensitivity (LoD) is defined as the lowest concentration at which at least 95% of all replicates tested positive. The LoD of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** was determined using serial dilutions of a quantified heat inactivated virus (ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (0.5 mL), Catalog #0810587CFHI, lot# 324887, 1.15 * 10^7 $TCID_{50}$/mL)). Each dilution was prepared by spiking a known concentration of this viral stock solution into negative nasopharyngeal swab samples.

The contrived samples were extracted using the MagNa Pure 24 instrument (Roche; Catalog #07290519001B) with the MagNA Pure 24 Total NA Isolation Kit (Roche, Catalog #07658036001) and analyzed with the QuantStudio 5 Real-Time PCR Instrument (Applied Biosystems, Catalog #A28139) and the UltraGene qPCR 48 instrument (ABL, Catalog #149A48) according to this instructions for use document.

A total of 10 concentration levels, with 10-fold serial dilutions between the levels, were tested with a total of 3 replicates per concentration for the tentative LoD. The tentative LoD was established, between 1150 and 115 $TCID_{50}$/mL for the QuantStudio 5 and for UltraGene 48 qPCR. To confirm the tentative LoD, 20 replicates were tested at 1150 $TCID_{50}$/mL and 115 $TCID_{50}$/mL on both instruments, and at 11.5 $TCID_{50}$/mL for UltraGene 48 qPCR.

Real-Time PCR Instrument	Ccn. SARS-CoV-2 $TCID_{50}$ / mL	Result Interpretation	SARS-CoV-2 N gene (FAM)		SARS-CoV-2 S gene Δ242 (HEX)		SARS-CoV-2 S gene Δ69 Δ144 (HEX)		SARS-CoV-2 ORF1ab gene Δ3675 (Cy5)	
			% detection (# detected /# tested)	Mean Ct	% detection (# detected /# tested)	Mean Ct	% detection (# detected /# tested)	Mean Ct	% detection (# detected /# tested)	Mean Ct
QuantStudio 5	115.0	0% (0/20) positive	80% (16/20)	28.58	5% (1/20)	31.86	100% (20/20)	23.90	25% (5/20)	39.79
	1150.0	95% (19/20) positive	100% (20/20)	24.56	95% (19/20)	25.08	100% (20/20)	22.49	100% (20/20)	38.07
UltraGene qPCR 48	11.5	5% (1/20) positive	15% (3/20)	30.73	75% (15/20)	29.07	65% (13/20)	29.55	15% (3/20)	30.14
	115.0	100% (20/20) positive	100% (20/20)	27.89	100% (20/20)	28.04	100% (20/20)	27.91	100% (20/20)	29.44
	1150.0	100% (20/20) positive	100% (20/20)	26.02	100% (20/20)	26.35	100% (20/20)	25.97	100% (20/20)	27.66

Table 9 : Confirmatory LoD for UltraGene Assay SARS-CoV-2 Multi Variants Deletions with QuantStudio 5 and UltraGene 48 qPCR

The concentration level for the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** with observed rates greater than or equal to 95%, was 1150 and 115 $TCID_{50}$/mL for SARS-CoV-2, with the QuantStudio 5 Real-Time PCR instrument and the UltraGene qPCR 48 instrument, respectively.

Analytical specificity

We used 38 clinical samples characterized either as SARS-CoV-2 positive and mutant, per sequencing (n=4) or confirmed by specific qPCR (n=26), or as SARS-CoV-2 negative (n=8). All were provided as leftover samples from a French private pathology laboratory.

We ran the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** in reference for 38 samples on the RNA extracted (SARS-CoV-2) used for the initial screening with a CE-IVD rRT-PCR test (***UltraGene Combo2Screen SARS-CoV-2 Assay (V2.x)***, ABL, catalog #139BX). The initial RNA extractions were kept at +4°C for more than 24 hours.

The sequenced samples were reprocessed by ABL from the nasopharyngeal swabs in VTM used for the initial SARS-CoV-2 detection test: new RNA isolation (Roche MagNa Pure 24 instrument (Catalog #07290519001B) with the MagNA Pure 24 Total NA Isolation Kit (Catalog #07658036001)) followed by ***DeepChek Assay 8-plex CoV-2 Genotyping V1 (RUO)*** (ABL, catalog #142AX) or ***ABL DeepChek Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** (ABL, catalog #159A48) (PCR amplification with ThermoFisher Scientific Proflex PCR System) with next generation sequencing (Illumina iSeq100, SBS) and downstream analysis software (ABL DeepChek CoV2 software (RUO)).

The new RNA extractions were stored at -20°C and used subsequently with the assay in reference.

Procedure used (n=)	**SARS-CoV-2 characterization**	**Analysis using UltraGene SARS-CoV-2 Multi Variants Deletions**	**Comment**
Next Generation Sequencing (n=2)	Positive - Mutant – Brazil	100% Brazil	1 sample was with late Ct values due to low viral load of the sample
Next Generation Sequencing (n=2)	Positive - Mutant – South Africa	100% South Africa	
qPCR (n=26)	Positive - Mutant – UK	100% UK	8 samples were not analyzed: The N target used as the control was not determined due to damaged RNA.
qPCR (n=8)	Negative	100% negative	-

Table 10 : Analytical specificity of SARS-CoV-2 positive patients using UltraGene Multi Variants Deletions on the UltraGene qPCR 48 instrument

100% of the tested samples were adequately characterized as noted.

Inclusivity (analytical reactivity)

The NCBI (National Center for Biotechnology Information) Standard Nucleotide BLAST database was used for an *in-silico* inclusivity analysis and was composed of public full-length SARS-CoV-2 genomes for Human hosts as of the 15th of March 2021. There were 58,803 sequences (including the NC_045512.2 reference sequence) at that date matching those criteria, for a total of 47,393 unique sequences available after removal of 8,180 identical sequences, 8 sequences with less than 29,000 bp and 3,222 sequences with three (3) or more consecutive Ns in critical regions for the assay. Nucleotide BLAST+ (locally installed version 2.10.1+) was used to find the location of all primers and probes on each of the 47,393 sequences in the study database. Ambiguity characters in the complete genomes of the database compatible with the corresponding nucleotide in the prime/probe are considered as matches.

Targets	Percent Homology		
	Fw Primer	**Probe**	**Rv Primer**
N gene	99.5% (248 sequences with 1 mismatch each and 1 sequence with 2 mismatches)	99.7% (161 sequences with 1 mismatch each)	99.2% (357 sequences with 1 mismatch each and 2 sequences with 2 mismatches)
S gene Δ 242	96.8% (1530 sequences with 1 mismatch each and 1 sequence with 2 mismatches)	99.7% (161 sequences with 1 mismatch each and 3 sequence with 2 mismatches. 21 sequences with a deletion)	99.3% (325 sequences with 1 mismatch each, 4 sequences with 2 mismatches and 1 sequence without a good match)
S gene Δ 69	98.8% (545 sequences with 1 mismatch each and 1 sequence with 2 mismatches)	98.7% (586 sequences with 1 mismatch each, 10 sequences with 2 mismatches, 1 sequence with 3 mismatches and 5 sequences without a good match. 1053 sequences with a deletion)	98.2% (844 sequences with 1 mismatch each and 5 sequences with 2 mismatches)
S gene Δ 144	99.8% (81 sequences with 1 mismatch each and 1 sequence with 3 mismatches)	99.4% (271 sequences with 1 mismatch each, 2 sequences with 2 mismatches, 7 sequences with 4 mismatches and 8 sequences without a good match. 840 sequences with a deletion)	96.5% (1670 sequences with 1 mismatch each and 5 sequences with 2 mismatches)
ORF1ab gene Δ 3675	99.8% (96 sequences with 1 mismatch each and 1 sequence with 2 mismatches)	99.8% (90 sequences with 1 mismatch each. 781 sequences with a deletion)	99.5% (221 sequences with 1 mismatch each and 4 sequences with 2 mismatches)

Table 11 : In-silico inclusivity percent homology for primers and probes of the UltraGene Assay SARS-CoV-2 Multi Variants Deletions with public full-length SARS-CoV-2 genomes

The assay is expected to amplify and detect all the assay targets on N, S and ORF1ab genes in all current variants of the virus. The forward/reverse primers and probes for the N, the deletions HV 69/70 (Δ69), Y144 (Δ144) and 242-244 (Δ242) on the Spike (S) gene and the deletion SGF 3675-3677 (Δ3675) on the ORF1ab gene of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** aligned without mismatches on >96.5% of the 47,393 sequences in the Inclusivity dataset**.**

Cross-reactivity (*In Silico*)

NCBI Nucleotide BLAST (blastn) was used to query all primers and probe sequences in the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** against the complete genome sequences in the NCBI database for the microorganisms listed in the following table.

Microorganism (GenBank Accession #)	N gene			S gene Δ 242			S gene Δ 69			S gene Δ 144			ORF1ab gene Δ 3675		
	F	R	P	F	R	P	F	R	P	F	R	P	F	R	P
Human coronavirus 229E **(NC_002645.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human coronavirus OC43 **(NC_006213.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human coronavirus HKU1 **(NC_006577)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human coronavirus NL63 **(NC_005831.2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SARS-coronavirus **(NC_004718.3)**	94%	-	-	-	-	-	-	-	-	-	-	-	95%	-	-
MERS-coronavirus **(KJ556336.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenovirus type 1 **(MH183293.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenovirus type 2 (**J01917.1**)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenovirus type 3 (**AY599836.1**)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Human Metapneumovirus (hMPV) **(KJ627437.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parainfluenza virus 1 ***(*KX639498.1*)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parainfluenza virus 2 (**KM190939.1**)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parainfluenza virus 3 (**NC_001796.2**)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parainfluenza virus 4 (**JQ241176.1**)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Influenza A **(NC_026438.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Influenza B **(NC_002204.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Microorganism (GenBank Accession #)	N gene			S gene Δ 242			S gene Δ 69			S gene Δ 144			ORF1ab gene Δ 3675		
	F	R	P	F	R	P	F	R	P	F	R	P	F	R	P
Enterovirus (e.g. EV68) **(NC_001472.1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Respiratory syncytial virus ***(NC_001803.1)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhinovirus ***(NC_009996.1)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlamydia pneumoniae ***(NC_005043.1)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haemophilus influenzae ***(NZ_LN831035.1)***	-	-	-	-	-	95%	-	-	-	-	-	-	-	-	-
Legionella pneumophila ***(NZ_LR134380.1)***	-	-	-	90%	-	-	-	-	-	95%	-	-	-	-	-
Mycobacterium tuberculosis ***(NC_000962.3)***	94%	-	-	-	94%	-	-	-	-	-	-	-	-	-	-
Streptococcus pneumoniae ***(NZ_LN831051.1)***	-	-	-	-	94%	-	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes ***(NZ_LN831034.1)***	94%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bordetella pertussis ***(NC_018518.1)***	-	-	-	-	94%	-	-	-	-	-	-	-	-	-	-
Mycoplasma pneumoniae ***(NZ_CP010546.1)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pneumocystis jirovecii (PJP) ***(CAKM01000281.1)***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Candida albicans ***(SC5314)***	-	-	94%[c]	-	-	-	-	-		-	-	-	-	-	-
Pseudomonas aeruginosa ***(NC_002516.2)***	-	94%	-	-	94%	-	-	-	-	-	-	-	-	-	-
Staphylococcus epidermis ***(NZ_CP035288.1)***	-	-	-	-	94%	-	-	-	-	-	-	-	-	-	-
Streptococcus salivarius ***(NZ_CP009913.1)***	94%	-	-	-	94%	91%	-	-	-	-	-	-	-	-	-

Table 12 : Cross-reactivity with the UltraGene Assay SARS-CoV-2 Multi Variants Deletions with QuantStudio 5 and UltraGEne 48 qPCR

All assay query sequences with matches above the 80% threshold are identified together with the corresponding percent (%) of identical matches.

- (-): No match found (results with < 80% identical matches)
- Amplification and detection of any of the assay targets are not expected as it requires three matches to the same template (two matches at an adequate distance in opposite orientation to produce amplification and a third match of one of the probes between the other two).

All pathogens were determined to have no cross-reactivity with the primers used.

Cross-reactivity (Wet testing)

The possible cross-reactivity with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** for each microorganism listed in the table below was determined using the isolate (reference and concentration indicated in following table). The specimens were extracted using the MagNa Pure 24 instrument (Roche; Catalog #07290519001B) with the MagNA Pure 24 Total NA Isolation Kit (Roche, Catalog #07658036001).

Microorganism	ID	Test Ccn.	Replicates detected / total	SARS-CoV-2 Test Result QS5	SARS-CoV-2 Test Result UG48
Human Metapneumovirus	ZeptoMetrix #0810161CF	3.80 x 10^6 $TCID_{50}$/mL	(0/3)	Not detected	Not detected
Parainfluenza virus 1	ZeptoMetrix # 0810014CF	3.39 x 10^7 $TCID_{50}$/mL	(0/3)	Not detected	Not detected
Parainfluenza virus 2	ZeptoMetrix # 0810015CF	4.17 x 10^5 $TCID_{50}$/mL	(0/3)	Not detected	Not detected
Parainfluenza virus 3	ZeptoMetrix # 0810016CF	8.51 x 10^7 $TCID_{50}$/mL	(0/3)	Not detected	Not detected
Parainfluenza virus 4	ZeptoMetrix # 0810017CF	1.51 x 10^6 $TCID_{50}$/mL	(0/3)	Not detected	Not detected
Bordetella pertussis	ZeptoMetrix # 0801459	1.13 x 10^{10} CFU/mL	(0/3)	Not detected	Not detected
Mycoplasma pneumoniae	ZeptoMetrix # 0801579	3.16 x 10^8 CFU/mL	(0/3)	Not detected	Not detected
Haemophilus influenzae	ZeptoMetrix # 0801679	2.27 x 10^9 CFU/mL	(0/3)	Not detected	Not detected
Streptococcus pneumoniae	ZeptoMetrix # 0801439	2.26 x 10^9 CFU/mL	(0/3)	Not detected	Not detected
Streptococcus pyogenes	ZeptoMetrix # 0801512	1.64 x 10^9 CFU/mL	(0/3)	Not detected	Not detected
***Pneumocystis jirovecii* (PJP)**	ZeptoMetrix # 0801698	1.56 x 10^8 CFU/mL	(0/3)	Not detected	Not detected
Candida albicans	ZeptoMetrix # 0801504	6.24 x 10^8 CFU/mL	(0/3)	Not detected	Not detected
Pseudomonas aeruginosa	ZeptoMetrix # 0801519	8.4 x 10^9 CFU/mL	(0/3)	Not detected	Not detected
Staphylococcus epidermis	ZeptoMetrix # 0801651	1.21 x 10^{10} CFU/mL	(0/3)	Not detected	Not detected
Streptococcus salivarius	ZeptoMetrix # 0801896	8.17 x 10^8 CFU/mL	(0/3)	Not detected	Not detected
Human coronavirus NL63	ZeptoMetrix #0810228CFHI	1.41 x 10^5 $TCID_{50}$/mL	(0/3)	Not detected	Not done

Table 13 : Cross-reactivity results with the UltraGene Assay SARS-CoV-2 Multi Variants Deletions with QuantStudio 5 and UltraGEne 48 qPCR

Clinical performance evaluation

Clinical sensitivity

Clinical evaluation of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** was performed using sixty (60) leftovers of nasopharyngeal swab samples from patients suspected of COVID-19 by their health care provider. The testing was done at a French private pathology laboratory that characterized the samples for SARS-CoV-2 using the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** (ABL, Catalog #139BX) (CE-IVD).

The specimens were collected by qualified personnel from patients with signs and symptoms of an upper respiratory infection. Samples were extracted with the ThermoFisher CheMagic and tested using the Roche LightCycler 480 II instrument with the UltraGene Combo2Screen SARS-CoV-2 Assay (V2) (routine PCR runs for Covid-19 testing). Positive samples were then processed for SARS-CoV-2 variant detection by the ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) (EUROFINS GENOMICS EUROPE) using the RNA already extracted.

Because the extracted RNAs were not kept at -70°C after the first day, a new RNA isolation was done from the leftovers of nasopharyngeal swab samples in VTM using the MagNa Pure 24 instrument (Roche; Catalog #07290519001B) using the Roche MagNA Pure 24 Total NA Isolation Kit (Catalog # 07658036001). The samples were processed with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** using the QuantStudio 5 Real-Time PCR Instrument (Applied Biosystems, Catalog #A28139) and the UltraGene qPCR 48 instrument (ABL, Catalog #149A48) and according to the Instructions for Use.

The clinical performance of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** in nasopharyngeal swab samples is reported in the tables below.

Nasopharyngeal swab specimens			***UltraGene Combo2Screen SARS-CoV-2 Assay (V2.x)***		
			Positive	Negative	Total
QuantStudio 5	***UltraGene Assay SARS-CoV-2 Multi Variants Deletions***	Positive	30	0	30
		Negative	0	30	30
		Total	**30**	**30**	**60**
UltraGene qPCR 48	***UltraGene Assay SARS-CoV-2 Multi Variants Deletions***	Positive	30	0	30
		Negative	0	30	30
		Total	**38**	**30**	**60**

Table 14 : Clinical performance of the UltraGene UltraGene Assay SARS-CoV-2 Multi Variants Deletions in nasopharyngeal swab specimens using the QuantStudio 5 and UltraGene qPCR 48

The positive (PPA) and negative (NPA) percent agreements between the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** and the CE-IVD test are, both for the QuantStudio 5 and UltraGene 48 qPCR:

- PPA = 30/30 = 100% (95% C.I. = 88.65% - 100%)
- NPA = 30/30 = 100% (95% C.I. = 88.65% - 100%)

The N gene target Ct values of each assay were calculated for positive samples.

N gene target	*UltraGene Combo2Screen SARS-CoV-2 Assay (V2.x) (Roche LightCycler 480 II)*	*ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) (Roche LightCycler 480 II)*	*UltraGene Assay SARS-CoV-2 Multi Variants Deletions*	
			UltraGene qPCR 48	QuantStudio 5
Mean Ct values	21.71	24.02	22.47	21.67
St. Dev. Ct values	3.51	4.70	3.72	3.60
Mean coefficient of variation with the UltraGene Combo2Screen SARS-CoV-2 Assay (V2.x) initial testing		13%	10%	8%

Table 15 : Comparison of the N gene target Ct values between the various assays

The N gene target Ct values of the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** were comparable with the initial testing for the SARS-CoV-2 detection.

We compared then the variant detection results obtained with the ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** and the CE-IVD predicate ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2). ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** results obtained with the QuantStudio 5 and UltraGene 48 qPCR instruments are the same.

ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2)	**UltraGene SARS-CoV-2 Multi Variants Deletions**	
	Wild-Type	Variant
Wild-Type	1	1
Variant	1	27

Table 16 : Agreement between the ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) and the UltraGene SARS-CoV-2 Multi Variants Deletions

The agreement between the two tests (93.3%) was moderate (Cohen's kappa = 0.46).

We compared the variant typing between ***UltraGene Assay SARS-CoV-2 Multi Variants Deletions*** and the CE-IVD predicate ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2).

ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2)	**UltraGene SARS-CoV-2 Multi Variants Deletions**			
	Wild-Type	Lineage B.1.1.7 (UK)	Lineage B.1.351 (South Africa)	Lineage P.1 (Brazil)
Wild-Type	1	**1**	0	0
Lineage B.1.1.7 (UK)	0	23	**1**	0
Lineage B.1.351 (South Africa) / Lineage P.1 (Brazil)	**1**	0	3	0

Table 17 : Comparison of variant typing between ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) and the UltraGene SARS-CoV-2 Multi Variants Deletions

The ***UltraGene SARS-CoV-2 Multi Variants Deletions*** was able to detect the variant strains at least as well as the CE-IVD comparator ViroBOAR Assay. In addition, it was able also to discriminate between the South Africa and the Brazil variants for 3 samples where the ViroBOAR Assay only categorized them as either SA or Brazil variant.

On the 3 discordant samples (**bold underlined samples** in the previous table), we did a new RNA isolation to perform a genotyping analysis using ***DeepChek Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** (ABL, catalog #159A48) (PCR amplification with ThermoFisher Scientific Proflex PCR System) with next generation sequencing (Illumina iSeq100, SBS) and downstream analysis software ***DeepChek CoV2 software (RUO)*** (ABL).

ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2)	**UltraGene SARS-CoV-2 Multi Variants Deletions**	***DeepChek Whole Genome SARS-CoV-2 Genotyping V1***
Wild-Type	Lineage B.1.1.7 (UK)	Lineage B.1.1.7 (UK)
Lineage B.1.1.7 (UK)	Lineage B.1.351 (South Africa)	Lineage B.1.351 (South Africa)
Lineage B.1.351 (South Africa) / Lineage P.1 (Brazil)	WT	WT

Table 18 : Details about discordant results between ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) and UltraGene SARS-CoV-2 Multi Variants Deletions compared with Whole Genome Sequencing

The ***UltraGene SARS-CoV-2 Multi Variants Deletions*** results were 100% correct for the variant detection and variant typing as confirmed by Whole Genome sequencing. On the 30 positive samples, 3 of them (10%) were false results reported by the CE-IVD comparator ViroBOAR Assay.

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.

Symbols

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	SN	Serial Number
	Country of manufacturing	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Distributor		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: https://ablsa.odoo.com/fr_FR/issue; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up to date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer

Advanced Biological Laboratories (ABL) S.A.

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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. DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, ABL (S.A) AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Version 1.0

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