DeepChek® Assay

Whole Genome SARS-CoV-2 Genotyping

V1.X

48

Instructions for Use

Version 1.0

Qualitative *in-vitro* diagnostics

For use with PCR Instruments and downstream next generation sequencing analyzers

IVD

CE

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Intended use

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is intended to be used for amplifying whole-genome of severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2) in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens, and nasopharyngeal wash/aspirate or nasal aspirate specimens) from individuals already tested positive to SARS-CoV-2.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** aims to identify SARS-CoV-2 infection by a variant (or lineage) which might aid in clinical applications.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of PCR, next generation sequencing workflow and in vitro diagnostic procedures. The current laboratory biosafety guidance for the 2019 novel coronavirus (SARS-CoV-2) shall be followed.

This nucleic amplification test is indicated for use on previously diagnosed COVID-19 patients ONLY. This test is NOT intended to be used as a screening or confirmation test for the detection, confirmation or quantification in upper respiratory specimens of SARS-CoV-2.

Indication of use

The amplified DNA and its interpretation are used:

- to support clinical applications for acute respiratory infections (ARI) in community medicine, serious cases hospitalized in intensive care, suspicions of reinfection, infections after vaccination (vaccine failures) and treatment failures with monoclonal antibodies
- to investigate specific situations (i.e.: clusters) or any other situation deemed to be abnormal (eg: abnormally high incidence, suspicion of transmission from the animal to humans or vice versa, etc.)
- to complement the epidemiological data collected to the orientation of the public health actions
- to identify the share of the different variants on a territory
- to identify new variants by analyzing databases and feed research projects from strengthened sequencing capacities and standardized, centralized and shared genomics data.

Results are for the specific detection of SARS-CoV-2 genomic variations that are detectable during infection in samples from patients already tested positive to SARS-CoV-2. The amplified DNA might be used as input material in validated laboratory procedures for genomic variability testing (genotyping) potentially affecting SARS-CoV-2 monitoring and spread.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** has been validated in laboratories for next generation sequencing (NGS). Physicians then may use the related genotyping data in conjunction with patient clinical data to adapt their patient management decisions. Laboratories shall be required to report all results and mutants to the appropriate public health authorities.

Principles of the assay

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is a reverse transcription polymerase chain reaction multiplex test which includes ninety-eight pairs of primers, reverse and forward, designed to detect SARS-CoV-2 RNA from individuals already tested SARS-CoV-2 positive.

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 98 targets takes place simultaneously in the same thermal cycling program in two distinct wells.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for next generation sequencing and analysed with a downstream analysis software to list SARS-CoV-2 mutations and issue a report with a list of mutations which can be filtered with available public reference knowledge, and display the most relevant variant identification. Physicians then may use the related genotyping data in conjunction with patient clinical data to support clinical decisions.

Assay components

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is provided in one model of 48 reactions (REF 159A48 – GTIN 05407007960217). The specified number of reactions is the number of reactions for each target.

Box contents

Label	Reagent	Description
Master Mix 2X	PCR Premix	2X solution containing Taq DNA polymerase, RT-PCR buffer, dNTP mix (daTP, dCTP, dGTP, dTTP) and ROX passive reference dye
RT Mix	RT Mix	Mix of 2 enzymes mix for reverse transcription
WG Primers Pool#1	Primer Mix	Pool of 109 not consecutive primers to cover the entire SARS-CoV-2 viral genome
WG Primers Pool#2	Primer Mix	Pool of 109 not consecutive primers to cover the entire SARS-CoV-2 viral genome
H_2O	Water	PCR grade nuclease-free water
Positive Control	Positive Control	Positive control RNA (whole genome SARS-CoV-2)
Negative Control	Negative Control	PCR grade nuclease-free water

*Table 1 : Components Included with the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.0***

Reagent storage and handling

Volumes and storage of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is listed in the following table.

Label	Volume for 48 Rn.	Color cap	Storage
Master Mix 2X	1 * 1320 µL	Green	-25°C to - 15 °C
RT Mix	1 * 26 µL	Pink	-25°C to - 15 °C
WG Primers Pool#1	1 * 190 µL	Yellow	-25°C to - 15 °C
WG Primers Pool#2	1 * 190 µL	Yellow	-25°C to - 15 °C
H_2O	1 * 280 µL	Blue	-25°C to - 15 °C
Positive control	1 * 20 µL	Clear	-25°C to - 15 °C
Negative control	1 * 20 µL	Black	-25°C to - 15 °C

*Table 2 : Volumes and storage conditions of the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** 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

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.

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

Note: Any laboratory validated instrument for RNA extraction and purification using magnetic-bead technology shall work with the test (i.e. bioMérieux EMAG®, Perkin Elmer ChemagicTM 360, ThermoFisher Scientific MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, Qiagen QIAamp Viral RNA Mini Kit, Biopur® Mini Spin Plus Kit and Magtivio MagSi-NA Pathogens).

Instruments

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

- ThermoFisher Scientific ProFlex™ PCR System (model 3 x 32-well (Catalog #4484073) or 96-well (Catalog #4484075) and associated specific material.

Note: Any laboratory validated thermal cycler with enough ramp rate of ≥ 1°C/s shall be sufficient.

Master mix preparation

- Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer.
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Pipetting Robot (optional).
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- 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.
- 0.5 ml or 1.5 ml RNase- and DNase-free PCR tubes.
- Ice/Icebox or even cooling blocks.
- 32 well or 96 well plate cooler (optional).
- 32 well or 96 well PCR plates.
- Plate thermo seals.
- Plate centrifuge.
- 0.2 mL thin walled 8 tube & domed cap.

Amplicons quality control

The test was validated with the use of

- Agilent ScreenTape D1000 and Reagents D1000 for Agilent TapeStation 4150 (capillary electrophoresis reagent).
- Invitrogen E-Gel EX reagents for 0.8–2% agarose gel in 0.5x TBE electrophoresis buffer.

Warnings and precautions

- The assay is for in vitro diagnostic (IVD) use.
- This product has been tested only for the amplification 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.
- When a positive control is used, it 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 according laboratory guidelines, 3) sample preparation area- isolation of the RNA/ DNA from sample and control, and 4) Amplification area- amplification and detection of nucleic acid targets.
- 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.
    - 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 directly used with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1:***
    a) PCR master mix preparation
    b) PCR reaction setup
    c) PCR run
    d) Quality control and analysis of the amplicon
    e) Next generation sequencing (NGS)
    f) Analysis of NGS raw data
    g) Laboratories reporting to the appropriate public health authorities.

If the downstream PCR and library preparation reactions of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** 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

As poor RNA quality might result in failure to amplify the targets, performing a new RNA isolation from initial upper respiratory specimen detected positive in a previous PCR run and with a Ct value < 28 should be considered.

Do not vortex specimens as this will fragment the RNA and lead to failure of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** tests.

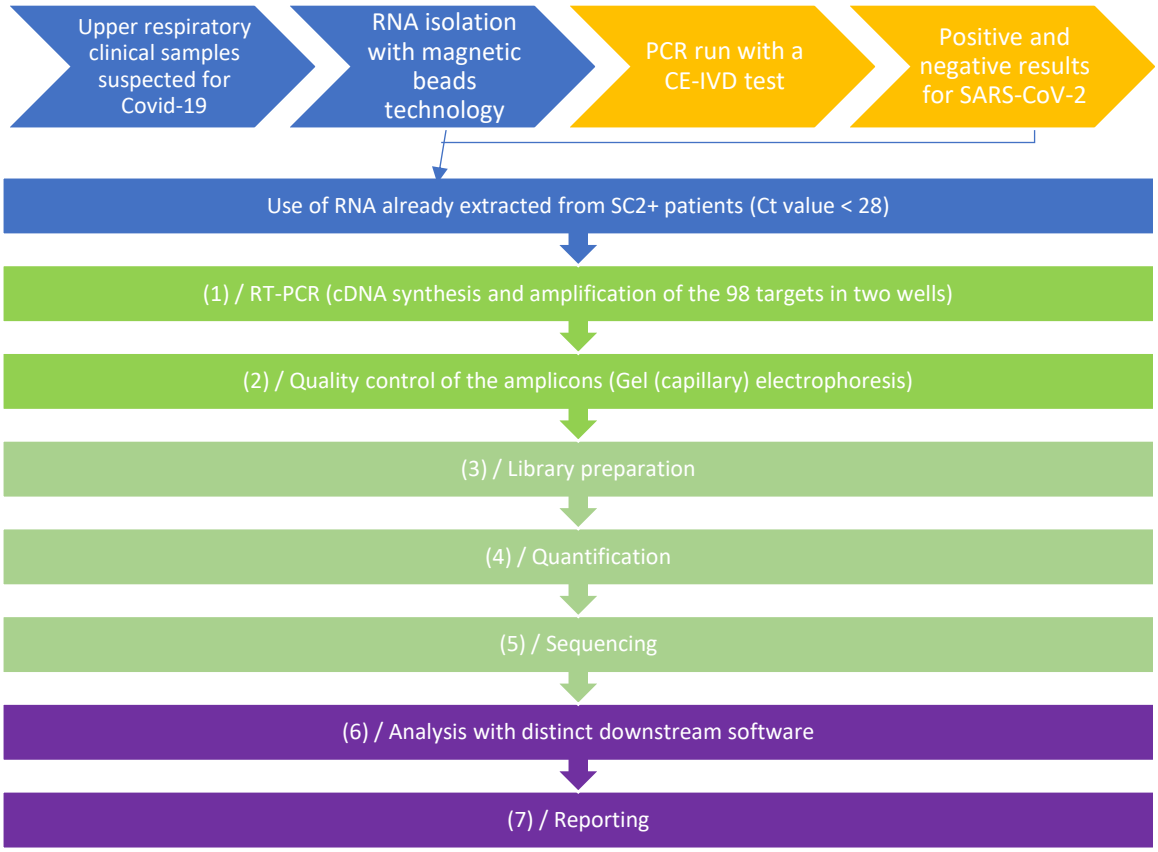

Figure 1 : Complete workflow for SARS-CoV-2 whole-genome sequencing using next generation sequencing

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** is composed of the steps (1) (PCR reaction setup, PCR run) and (2) (quality control of the amplicons) with a turnaround time approximatively of 4 hours for 48 samples.

The turnaround time for the complete workflow which includes also the next generation sequencing (3—5) and the results using a standalone downstream analysis software (6—7)(Software as a Medical Device (SaMD)) is 30 hours for 48 samples.

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 *DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1* is extracted RNA from SARS-CoV-2 positive PCR with Ct value < 28.

PCR reaction setup

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

Vortexing the mix of "WG Primers Pool #1", "WG Primers Pool #2", "Master Mix 2X" and "RT Mix" reagents, with or without extracted RNA solution, could lead to failures. Please don't vortex.

1. Thaw the following reagents on ice: Master Mix 2x, RT Mix, WG Primers Pool#1, WG Primers Pool#2 and H_2O.
2. Briefly centrifuge (2000rpm, 10sec) the reagents to collect the contents.
3. Prepare the 2 master mixes, "Pool1" and "Pool2", according to the tables below. The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume for each master mix greater (n+1) than that required for the total number of reactions to be performed. The master mix shall be kept on ice. **DON'T VORTEX.**

Reagent	Volume / Reaction	
	Pool #1	Pool #2
Master Mix 2X	12.50 µL	12.50 µL
RT Mix	0.25 µL	0.25 µL
WG Primers Pool# *n*	3.60 µL	3.60 µL
H_2O	2.65 µL	2.65 µL
Total Volume	19.00 µL	19.00 µL

Table 4 : Master mix preparation for each Pool

4. Add **6 µL** of extracted RNA solution to a single PCR tube. **DON'T VORTEX.**
5. Do not add more than one sample of extracted RNA into a single qPCR tube. **Each PCR tube shall have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a PCR machine for the amplification.**

Note : Prepare a volume of master mix greater (n+2) if you use a Positive Control.

Quality controls

Controls that are provided with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** 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 extraction and 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)] is needed to ensure the RT-PCR reaction setup and reagent integrity and is used to validate the RT-PCR plate results. The positive control is used during PCR.

PCR cycling condition

Set up the following thermal cycling program.

Stage	Cycle	Ramping rate (°C/s)	Temperature (°C)	Time (min:sec)
1	1	2.0	50	10:00
2	1	2.0	95	5:00
3	1	2.0	98	00:30
4	35	2.0	95	00:15
		2.0	63	05:00
5	1	2.0	4	HOLD

Table 5: PCR cycling program

Sample quality control

The PCR step shall achieve detectable amounts visible in quality control using gel or capillary electrophoresis to avoid unnecessary subsequent workload and reagents costs for downstream sequencing.

To maximize the quality of the sequencing reactions, analyze each PCR product at least for size (in base pairs (bp)) using one of the following options:

- Run the PCR products on an agarose gel and compare them to a "DNA Mass Ladder" containing DNA fragments of known sizes and amounts, or
- Perform a capillary electrophoresis.

Note:

- You may see variability in the yield and purity of the PCR products. This reflects variability in the viral load of the original plasma samples.
- The user shall use a calibrated (capillary) gel electrophoresis for the sample quality control.
- The user shall select the correct reagents and consumables and operate as specified in the manufacturer instructions for use.

Interpretation 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 interpretation of patient results. If any of the controls are not valid, the patient results cannot be interpreted.

Patient samples

The visual inspection of the quality control result from (capillary) gel electrophoresis is usually a visual band which means the amplification of SARS-CoV-2 target sequences (amplicons) was successful.

In some cases, the visual band may not be visible. This means the amplification of the PCR Step was not successful. See "Troubleshooting guide", below, for interpretation of inappropriate results.

Note: The expected molecular size of the amplicons is approximatively 400 bp for both "Pool#1" and "Pool#2" using the agarose gel, and is approximatively 300 bp for the pooled libraries using the gel elecrtropherosis.

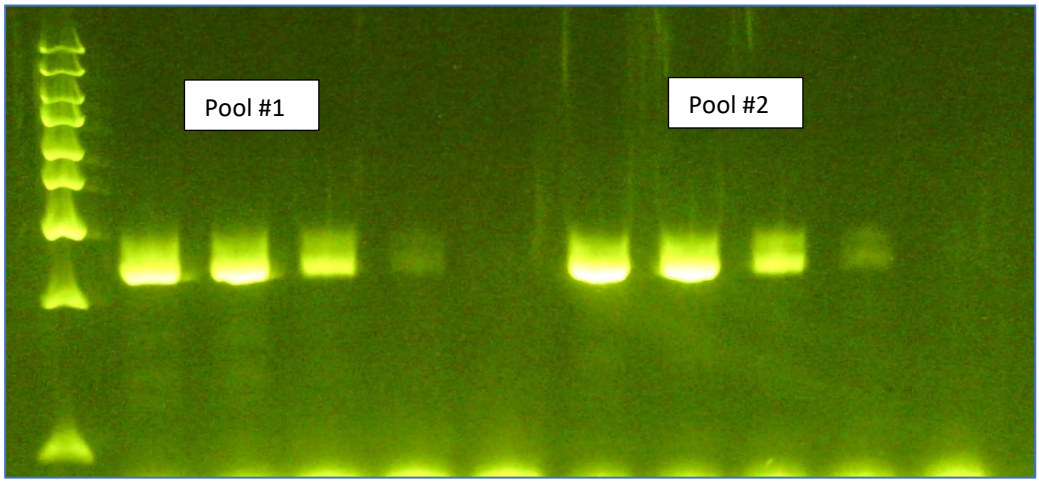

Figure 2 : Example of an agarose gel (2%) of a successful PCR for Pool#1 and Pool#2 Whole Genome

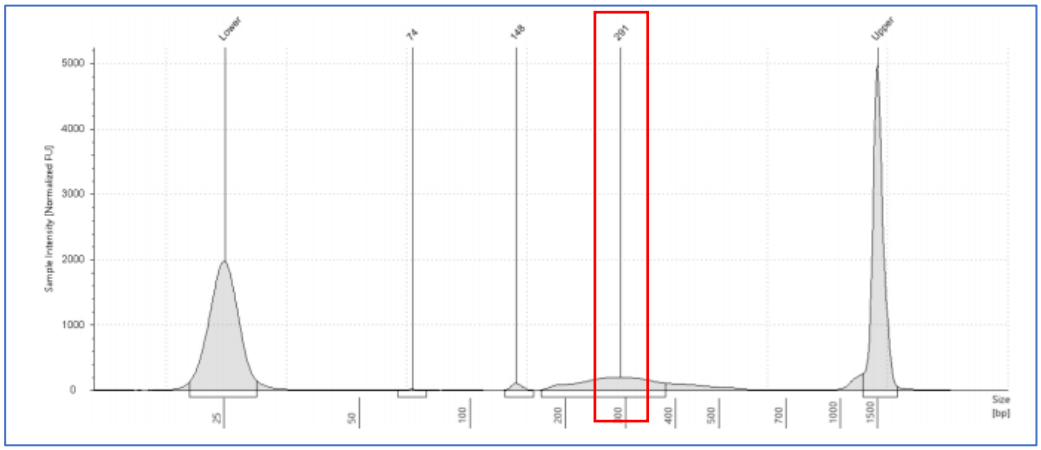

Figure 3 : Example of a capillary gel elecrtopherosis of a successful PCR for both Pool#1 and Pool#2 Whole Genome

Controls

The water control (NTC) should not lead to a visual band. A band for a water control may indicate cross-contamination. See "Troubleshooting guide", below.

In case of a cross-contamination of the water control, it is likely that all samples within the same run are affected by this cross-contamination. Thus, the result interpretation for downstream whole-genome genotyping or clade characterization may lead to wrong results and subsequently to inadequate clinical decisions.

Troubleshooting guide

Use the following troubleshooting table to diagnose and solve problems. The troubleshooting recommendations assume that all the DeepChek Assay reagents are stored according the specifications and that the directions in this guide have been followed correctly.

Comments and suggestions

Positive controls are negative for one or both "Pool #n" targets (Pool #1 and Pool #2)	
a. Pipetting error or omitted reagents	Check pipetting scheme and the setup of the affected target reaction. Repeat the PCR of the affected target (Pool #1, Pool #2 or both).
b. Inappropriate storage of Assay components	Store the DeepChek Assay at –25 to –15°C. See "Reagent Storage and Handling". Avoid repeated freezing and thawing.
c. Inappropriate thermal cycling program	Check the PCR protocol set-up for correctness. Check the thermal cycler calibration. Make sure that the PCR reactions were set up on ice or on a cold block. Refer to thermal cycler instructions for use.
Negative controls are positive	
Cross-contamination	Replace all critical reagents. Repeat the experiment with new reagents. Always handle samples, assay components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
Absent or low signal in samples but positive controls okay	
a. Poor DNA quality or inadequate concentration	Check your (capillary) gel electrophoresis preparation. You may dilute your DNA product according your (capillary) gel electrophoresis procedure. Follow the instructions for use if using a capillary electrophoresis instrument. Rerun your (capillary) gel electrophoresis.
b. Sample prepared too long before analysis leading to DNA evaporation	Ensure that your (capillary) gel electrophoresis is started immediately after sample preparation. Repeat the PCR of the affected sample target.
c. Poor agarose gel conditions or capillary electrophoresis instrument reagents used are incorrect or unproper	Use new (capillary) gel electrophoresis reagents. Rerun your (capillary) gel electrophoresis.
No band signal for one or few samples	
a. The PCR didn't work	Take the corresponding RT-PCR product. Run the (capillary) gel electrophoresis. If still no band signal, start again the whole protocol
b. Degraded RNA isolation from initial upper respiratory specimen or Ct value of positive sample > 28	Check again the Ct value of the SARS-CoV-2 detection rRT-PCR assay. If > 28 : sample with low viremia is more difficult to amplify for whole-genome sequencing. Repeat once the PCR of the affected sample target. If < 28 : Consider doing either: • Thaw a new aliquot of extracted RNA • Do a new RNA isolation for the initial upper respiratory specimen Start again the whole protocol.

	Comments and suggestions
c. Limitation variant	If you get repeatedly a negative result, and exclude use errors, it could be a variant binding the reverse transcription binding sites. Contact our technical support.

Note: As if processed in a normal laboratory workflow, a sample showing two consecutive full negative PCR (no amplification of Pool #1 nor Pool #2) is then considered as "no result obtained". You shall assess the Ct value of the SARS-CoV-2 detection assay and the RNA quality. You can contact ABL Technical Support for further assistance.

Post PCR

Next Generation Sequencing

The main volume of the product output is then used for next generation sequencing (NGS). The NGS workflow can be different as the NGS technics and NGS analyzers vary. NGS sequencing instruments are general laboratory use devices. Sequencing and library preparations reagents are general laboratory use products.

While the validation studies, we used the Illumina iSeq 100 Sequencing System (#20021532) and the following combination of reagents: ABL ***DeepChek NGS Library preparation*** (catalog #116AX, 24 or 48 or 96 tests), ABL ***DeepChek Assay Adapters*** (catalog #124AX, 1-24, 1-48 or 1-96) and Illumina iSeq 100 Reagent (catalog # 20021533, 300 cycles).

Note: The external clinical study was conducted with Illumina MiSeq NGS analyzer and related MiSeq Reagent Kit, v2 (catalog # MS-102-2003, 500 cycles) with no need to perform the fragmentation step with the ABL DeepChek NGS Library preparation (catalog #116AX, 24 or 48 or 96 tests).

Downstream NGS Analysis Software

The sequencing raw data are then uploaded in a specific downstream software tailored for SARS-CoV-2 whole genome analysis and interpretation. This software is a standalone medical device. The software itself can be CE-IVD marked and shall rely on recognized and updated public sources about SARS-CoV-2 circulating strains and SARS-CoV-2 lineage definitions and latest knowledge about the clinical impact of genomic variations.

For the validation studies and the external clinical validation study, the next generation sequencing raw data , outputs from Illumina iSeq100 and MiSeq NGS analyzers, were uploaded in a specific downstream software tailored for SARS-CoV-2 analysis which is a standalone medical device: ABL ***DeepChek CoV2 software (RUO)*** (licence and module (catalog #S-12-023 (CVL) and #S-12-023 (CVM)).

Limitations

- The performance of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** 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 ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***.
- The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** 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 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.
- The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** was validated with a new RNA isolation from the initial upper respiratory specimen used for the prior PCR positive SARS-CoV-2 detection.
- Using as an input for the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** PCR the RNA already extracted from the first RNA isolation is possible but the RNA integrity could be damaged, especially if storing conditions of the extracted RNA are not optimal.
- The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** was validated with frozen samples PCR positive SARS-CoV-2 with Ct value < 28.

- The lack of a mutation on a Variant Calling Report issued by a downstream next generation sequencing raw data analysis software does not preclude the possibility of genetic mutation.
- 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.
- False-positive results may arise from cross contamination during specimen handling, preparation, assay set-up or product handling.
- If the virus mutates in the test target regions, 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.
- 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.

Performance characteristics

Non-clinical performance evaluation

Non clinical were conducted to establish the analytical performance of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***.

Protocol of the studies

Measurement procedure

The test shall amplify 98 targets of the SARS-CoV-2 genome. If the PCR amplicons of sample were of quality (agarose gel signal intensity), then they were prepared for sequencing and sequenced.

We used the ABL NGS Library Preparation kit (catalog #116A96 and #124A96) with a downstream NGS sequencing instrument (iSeq100, Illumina, USA; One-Channel SBS Chemistry, iSeq100 Flow Cell).

For next generation sequencing (NGS), it was required to get a SARS-CoV-2 lineage identification. For the assays cut-off, we used the following criteria: minimal median coverage of 1000 reads for the amplicons and a Phred Quality Score Q30 >80% for the NGS run as reported by the Illumina Sequencing Analysis Viewer (instrument software version 2.4.7).

Sequencing output files were uploaded in a specific downstream software tailored for SARS-CoV-2 analysis which is a standalone medical device: ABL DeepChek CoV2 software (RUO) (licence and module (catalog #S-12-023 (CVL) and #S-12-023 (CVM)). The software reported the number of reads per amplicon and identified the most possible SARS-CoV-2 lineage over 12 lineages identified at the time of the studies (April 2021).

Specimen Collection and Handling

Our analytical performance approach was different to the routine procedure in terms of storing as we used leftover frozen clinical frozen 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). We performed in-silico analyses to simulate potential interferences.

Samples preparation and testing

For the testing of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***, we used contrived samples which were prepared by spiking a known concentration of a reference control into negative nasopharyngeal swab samples.

For SARS-CoV-2, three reference controls were used : the 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) ("Wild-Type") and 2 lyophilized purified RNA of coronavirus SARS-CoV-2 B.1.1.7 and B.1.351 lineages grown in Vero C1008 cells (Vircell AMPLIRUN® SARS-CoV-2 B.1.1.7 RNA CONTROL (catalog #MBC138-R, 1mL, lot #21MBC138001-R, 16000 cp/µL) ("UK") and AMPLIRUN® SARS-CoV-2 B.1.351 RNA CONTROL (catalog #MBC139-R, 1mL, lot #21MBC139001-R, 13000 cp/µL)) ("SA"). We used other reference controls for the cross-reaction study. The list of reference controls are listed in the study table.

Except Vircell AMPLIRUN® SARS-CoV-2 contrived samples, all 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 ThermoFisher Scientific ProFlex™ 3 x 32-well PCR System (Catalog #4484073) according to this instructions for use document.

After amplification, a quality control was done for the PCR products (2% agarose gel with Invitrogen E-Gel EX and gel electropherosis with TapeStation 4150; Agilent; ScreenTape D1000; Reagents D1000). We performed then the NGS library preparation and NGS sequencing steps. Initial concentration of pooled NGS libraries were measured before sequencing (ThermoFisher Scientific, Qubit 4 Fluorometer, catalog #Q33226). All the NGS outputs were analyzed by the DeepChek software using the same software version and reference knowledge databases.

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. A total of 12 concentration levels of the Wild-Type SARS-CoV-2 reference control, 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, at the lowest level, 0.0000115 $TCID_{50}$/mL SARS-CoV-2 for the ProFlex™ 3 x 32-well PCR System (ThermoFisher Scientific, Catalog #4484073). To confirm it, 20 replicates were tested at 0.0000115 $TCID_{50}$/mL and 0.000115 $TCID_{50}$/mL on the ProFlex™ 3 x 32-well PCR System (ThermoFisher Scientific, Catalog #4484073).

Ccn. SARS-CoV-2 $TCID_{50}$ / mL	**Tested**	**Agarose gel with good signal intensity**		**Downstream sequencing**			
		Pool #1	Pool #2	Mean initial ccn. of pooled NGS libraries after purification step	Clade 19B identification with > 1000 reads	Mean reads mapped to SARS-CoV-2	
						Mean count	**Mean %**
0.000115	**20**	**20**	**20**	**14.80 ng/µL**	**100% (20/20)**	**97563**	**52 %**
0.0000115	20	0	0	2.39 ng/µL	0% (0/20)	< 1000	n.a.

Table 6: Confirmatory LoD for **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1** *with ProFlex™ PCR System and downstream sequencing analzyzer (iSeq100, Illumina, iSeq100 Flow Cell) and downstream analysis software (DeepChek CoV2 software, ABL)*

The concentration level for the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** with observed rates greater than or equal to 95% was 0.000115 $TCID_{50}$/mL for SARS-CoV-2 with the ProFlex™ 3 x 32-well PCR System (Catalog #4484073).

Analytical specificity

Firstly, 18 clinical samples which were used for variant detection with the ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) (EUROFINS GENOMICS EUROPE) were tested with ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

Secondly, we tested 3 contrived samples each for the two reference controls of the SARS-CoV-2 variants of concern (VOC) for the UK and SA lineages, B.1.1.7 and B.1.351, respectively, with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

Among the tested samples, 16 had specific clade (UK or SA): 100% were adequately amplified, sequenced and characterized, either as B.1.1.7 or B.1.351, by the DeepChek CoV2 software following the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

Inclusivity (analytical reactivity)

In-silico primer analysis was performed to evaluate the inclusivity of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.*** As of the 21st of April 2021, there were 148,952 sequences (including the NC_045512.2 reference sequence) at that date matching the criteria, for a total of 121,358 unique sequences available after removal of identical sequences and sequences with length < 29,000 bp.

The BLASTn (NCBI) tool generated numerous records from which we filtered out both low identity and low coverage alignments. The results are summarized in the following table.

Primers identity	Mean	Median	5th Percentile	95th Percentile
100% identity	98.40%	98.62%	96.33%	100.00%
>=80% identity	98.96%	99.08%	97.24%	100.00%

Table 7 : In-Silico inclusivity study summary for all 121,358 unique sequences after removal of low identity and low coverage alignments

In summary, the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** has excellent coverage of all reported circulating SARS-CoV-2 strains in the Inclusivity dataset**.**

Cross-reactivity (*In Silico*)

NCBI Nucleotide BLAST (blastn) was used to query all primers sequences in the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** against the complete genome sequences in the NCBI database for the microorganisms listed in the following table.

Microorganism	GenBank Accession #	Number of amplicons detected*
Human coronavirus 229E	NC_002645.1	0
Human coronavirus OC43	NC_006213.1	0
Human coronavirus HKU1	NC_006577	0
Human coronavirus NL63	NC_005831.2	0
SARS-coronavirus	NC_004718.3	0[(a)]
MERS-coronavirus	KJ556336.1	0
Adenovirus type 1	MH183293.1	0
Adenovirus type 2	J01917.1	0
Adenovirus type 3	AY599836.1	0
Human Metapneumovirus (hMPV)	KJ627437.1	0
Parainfluenza virus 1	KX639498.1	0
Parainfluenza virus 2	KM190939.1	0
Parainfluenza virus 3	NC_001796.2	0
Parainfluenza virus 4	JQ241176.1	0
Influenza A	GCF_000928555.1	0
Influenza B	NC_000907.1	0
Enterovirus (e.g. EV68)	NC_001472.1	0
Respiratory syncytial virus	NC_001803.1	0
Rhinovirus	NC_009996.1	0
Chlamydia pneumoniae	NC_005043.1	0
Haemophilus influenzae	NZ_LN831035.1	0
Legionella pneumophila	NZ_LR134380.1	0
Mycobacterium tuberculosis	NC_000962.3	0
Streptococcus pneumoniae	NZ_LN831051.1	0
Streptococcus pyogenes	NZ_LN831034.1	0
Bordetella pertussis	NC_018518.1	0
Mycoplasma pneumoniae	NZ_CP010546.1	0
Pneumocystis jirovecii (PJP)	CAKM01000281.1	0
Candida albicans	SC5314	0
Pseudomonas aeruginosa	NC_002516.2	0
Staphylococcus epidermis	NZ_CP035288.1	0
Streptococcus salivarius	NZ_CP009913.1	0

Table 8 : In silico cross-reactivity testing between the sequences primers of the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1** *and representative common respiratory microorganisms*

([a]): 8 primers pairs on 98 can amplify and detect small portions of SARS coronavirus. However, these won't lead to amplifications as the forward and reverse primers don't align to the same reference sequence, the forward and reverse primers are on different strands and the amplicon size is longer than 200bp. Thus, the SARS-CoV is not circulating currently and the DeepChek CoV2 software filtering system would remove corresponding SARS-CoV reads. (b): found single potential off-target in the 7th chromosome of the human genome. Using the primer pair 33_LEFT and 62_RIGHT, it could result in a 33 bp amplicon. It is however not expected to be amplified unless contamination occurs. Thus, DeepChek CoV2 software filtering system would remove corresponding reads.

In summary, all pathogens were determined to have no cross-reactivity with the primers pairs used for the the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

Cross-reactivity (Wet testing)

The possible cross-reactivity with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** for each microorganism listed in the table below was determined using the isolate (per reference and indicated concentration).

Microorganism	ID	Test Ccn.	Replicates detected / total	Agarose gel band for SARS-CoV-2 amplification
Human coronavirus NL63	ZeptoMetrix #0810228CFHI	1.41×10^5 $TCID_{50}$/mL	(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	$1 \times 10^{5.15}$ $TCID_{50}$/mL	(0/3)	Not detected
Influenza B	ZeptoMetrix # 0810255CF	$1 \times 10^{6.10}$ $TCID_{50}$/mL	(0/3)	Not detected
Respiratory syncytial virus	ZeptoMetrix # 0810040ACF	5.01×10^5 $TCID_{50}$/mL	(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	(3/3)	Detected
***Pneumocystis jirovecii* (PJP)**	ZeptoMetrix # 0801698	1.56×10^8 CFU/mL	(0/3)	Not detected
Candida albicans	ZeptoMetrix # 0801504	6.24×10^8 CFU/mL	(0/3)	Not detected
Pseudomonas aeruginosa	ZeptoMetrix # 0801519	8.4×10^9 CFU/mL	(0/3)	Not detected
Staphylococcus epidermis	ZeptoMetrix # 0801651	1.21×10^{10} CFU/mL	(3/3)	Detected
Streptococcus salivarius	ZeptoMetrix # 0801896	8.17×10^8 CFU/mL	(0/3)	Not detected

Table 9 : Cross-reactivity results with the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1**

Mycoplasma pneumoniae and Staphylococcus epidermidis showed a band on quality control with the agarose gel electrophoresis. After NGS library preparation, no sequences were analyzed as SARS-CoV-2.

For cross-reactivity wet testing, the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** did not react (i.e., good agarose gel band for Pool#1 or Pool#2) for most of the tested microorganisms that are commonly found in upper respiratory specimens and tested at the indicated concentration.

Clinical performance evaluation

Clinical sensitivity

Clinical evaluation of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** was performed using sixty (60) leftovers of nasopharyngeal swab samples from patients suspected of COVID-19 by their health care provider: 30 PCR SARS-CoV-2 negative and 30 PCR SARS-CoV-2 positive samples, with a Ct value less than 28 (french national guidance for performing SARS-CoV-2 whole-genome sequencing).

The specimens were collected by qualified personnel from patients with signs and symptoms of an upper respiratory infection. The testing was done at a French private pathology laboratory that characterized the samples for SARS-CoV-2 detection using the ***UltraGene Combo2Screen SARS-CoV-2 Assay (V2)*** (ABL, Catalog #139BX) (CE-IVD). Using the RNA already extracted, positive samples were processed for SARS-CoV-2 variant detection by the ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) (EUROFINS GENOMICS EUROPE) and for SARS-CoV-2 whole genome sequencing using the the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)***.

Samples were extracted with the ThermoFisher CheMagic and tested using the Roche LightCycler 480 II instrument for real-time RT-PCR assays.

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 new extracted RNA were processed blindly with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** according to its instructions for use document.

	SARS-CoV-2 clade identification with > 1000 reads	**Mean**			
		Ct value Target E	Ct value Target N	Initial ccn. of pooled NGS libr. after purif.	Reads mapped to SARS-CoV-2
30 positive samples processed with ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***	30/30 (100%)	20.0	19.2	17.54 ng/µL (*)	48141(#)

Table 10 : Descriptive statistics of the 30 PCR positive SARS-CoV-2 clinical samples processed by **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1**

(*): mean initial concentration of pooled NGS libraries after purification step was 1.16 ng/µL for negative samples. None of the negative samples was assigned a SARS-CoV-2 clade identification (less than 1000 reads were generated). (#): the 60 samples and controls were processed using the same flowcell cartdrige.

The clinical performance of the ***DeepChek Assay*** in nasopharyngeal swab samples is reported in the tables below.

Nasopharyngeal swab specimens in VTM		***CE-IVD assay comparator***		
		Positive (*)	Negative	Total
DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1	Positive	30	0	30
	Negative	0	30	30
	Total	**30**	**30**	**60**

Table 11 : Clinical performance of the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1** *in nasopharyngeal swab specimens*

(*) The ability to report a clade (wild-type, UK, SA, BR) was assumed as "Positive" as the nature of the two assays was different. At the time of the studies, no CE-IVD comparator was available for SARS-CoV-2 whole genome sequencing for genomic variations.

The positive (PPA) and negative (NPA) percent agreements between the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** and the CE-IVD comparator assay are: PPA = 30/30 = 100% (95% C.I. = 88.65% - 100%) and NPA = 30/30 = 100% (95% C.I. = 88.65% - 100%).

The variant detection results obtained with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** were compared with the CE-IVD predicate ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2).

ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2)	***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***	
	Wild-Type	Variant
Wild-Type	16	1
Variant	1	13

Table 12 : Agreement between the ViroBOAR Spike 1.0 RT-PCR Kit (SARS-CoV-2) and the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1**

The agreement between the two tests (93.5%) was perfect (Cohen's kappa = 0.87).

Clinical reproducibility

The 30 PCR SARS-CoV-2 positive samples were tested by the French pathology laboratory for SARS-CoV-2 whole genome sequencing. The RNA already extracted was processed with GeneAmp PCR System 9700 (Applied Biosystems, catalog # N805-0200) and Illumina MiSeq NGS analyzer and related MiSeq Reagents Kit, v2 (catalog # MS-102-2003, 500 cycles)). Downstream sequencing outputs analysis was done using ABL ***DeepChek CoV2 software (RUO)*** (licence and module (catalog #S-12-023 (CVL) and #S-12-023 (CVM)). On the 30 positive samples, one was in failure (suspicion of RNA degradation). The same sample which was processed for a new RNA isolation was successful with ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

We compared head to head the 29 results obtained by the French pathology laboratory for SARS-CoV-2 whole genome sequencing (different NGS analyzer, different reagents, but same technology (SBS)) and the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1***. We obtained 100% results accuracy both for SARS-CoV-2 clade identification and defining mutations used for clade identification (Nexstrain SARS-CoV-2[1]).

External clinical validation

An external clinical validation of ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** was conducted at a French private pathology laboratory accredited by public health authorities for SARS-CoV-2 whole genome sequencing to investigate specific situations (i.e.: clusters), to identify the share of the different variants on a territory and to identify new variants.

From the 1st of March 2021 to the 16th of April 2021, 283 clinical samples were processed. The mean Ct value of the samples processed was 20.41. A total of 28 samples (9.9%) were in failure (no SARS-CoV-2 clade identification). The main suspicion for these failures is the RNA degradation as no new RNA isolation was done. The clinical sensitivity was 90.1%. However, 7 samples in failure (2.5%) were above the recommended assay cut-off (Ct value < 28). The clinical sensitivity was 92.5% if the operator followed strictly the instructions for use.

Among the 255 successful samples, the SARS-CoV-2 clades identified as variants of concern (VOC) (n=102, 40.0%) were identified with 29.4% (20H/501Y.V2)(SA), 5.9% (20J/501Y.V3)(BR) and 4.7% (20I/501Y.V1) (UK1). Among the other SARS-CoV-2 clades identified, the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1*** detected one of the first cases in France for the Indian double mutant (20A/484Q (B.1.617)). A subset of 10 samples (extracted RNA) were also sent for results validation by the French SARS-CoV-2 CNR (Centre National de Référence des virus respiratoires): 100% results accuracy was reached with the CNR whole genome sequencing workflow and the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.***

[1] https://covariants.org/shared-mutations

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

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Negative control
	Catalog number		Positive control
	Use by		Temperature limitation
	Manufacturer		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

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