GENEME

SARS-CoV-2

DIRECT RAPID RT-PCR
Detection KIT

SAVD

by GENEME

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Purpose

The SAVD by GeneMe SARS-CoV-2 Direct Rapid RT-PCR detection kit is a test designed for fast and accurate *in vitro* identification of the new SARS-CoV-2 coronavirus in one reaction. Please read this entire document before carrying out any tests.

Description

The SAVD by GeneMe SARS-CoV-2 Direct Rapid Detection Kit is designed for the *in vitro* identification of the new coronavirus SARS-CoV-2, in a single reaction. The presence of an innovative and patented *Pwo* polymerase and specific primers in the kit has enabled the creation of a highly specific and sensitive SARS-CoV-2 rapid detection kit. The specifically designed primers are 100% compatible with the SARS-CoV-2 genomic RNA sequence of gene *N* deposited in the NCBI database. Amplification of the targeted nucleic acids is observed by an increase of fluorescence signal during the reaction. The kit contains four 8-well SAVD strips with lyophilized enzymes and reagents.

SAVD works with the GeneMe CoVi19 TEST Sample Collection kit (swabbing sample kit) and CoVi19 Saliva Collection kit. The kits differ depending on the sample being analyzed - throat swab or saliva sample (please read below).

SAVD components

ITEM	QUANTITY	STORAGE CONDITIONS
8-well SAVD strip	4 pieces	+ 5 to + 12 °C
Positive Control Tube	4 pieces	+ 5 to + 12 °C
Control Buffer Tube	4 pieces	+ 5 to + 24 °C
Normalization Buffer Tube	32 pieces	+ 5 to + 24 °C

CoVi19 TEST Sample Collection Kit components (packed individually and delivered together with SAVD Kit)

ITEM	QUANTITY	STORAGE CONDITIONS
Single use sterile swab	1 piece	+ 2 to + 30 °C
Sample collection tube & buffer	1 piece	+ 5 to + 24 °C

CoVi19 Saliva Sample Collection Kit components
(packed individually and delivered together with SAVD Kit)

ITEM	QUANTITY	STORAGE CONDITIONS
Single use sterile straw	1 piece	+ 2 to + 30 °C
Sample collection tube & buffer	1 piece	+ 5 to + 24 °C

Additional storage information: The product should be kept in a dry place and hidden from direct sunlight.

Transportation

A WarmMark (temperature indicator) is attached to the package. The WarmMark indicates if the storage temperature has exceeded the maximum recommended temperature (12 °C) for product storage and transportation.

Expiration date

8-well SAVD strip - expires 6 months from production date.
SAVD Buffer - expires 6 months from production date.
Sterile swab - expires 3 years from production date.
Sterile straw - expires 3 years from production date.

Kit compatibility with thermocyclers

SAVD is compatible with all thermocyclers for real-time PCR.

Fluorescence reading is performed as for intercalating dyes in the FAM channel – SYBR Green dye (maximum absorption 498 nm and maximum emission 522 nm).

General information

In the event of using pure SARS-CoV-2 RNA as a matrix in the above test, it is very important to use tools and reagents free from RNases. In addition, it is recommended to carry out analyses in areas free from nucleases and using only pipettes with tips containing filters. Also, the SAVD test cannot be used as a method for analysing SARS-CoV-2 virus directly harvested from the cell line.

Procedure

1. Collect a deep throat swab or a saliva sample, using the instructions included in the Sample Collection Kit. Repeat this for as many samples as you wish to process in the test run.

2. Choose one of the racks and take out one 8-well strip from the SAVD box. Place the 8-well strip on the rack.

3. First, prepare the negative control. Only one negative control needs to be prepared for every run of tests in the PCR machine, whether you are processing a single 8-well strip, or multiple strips in a run.The negative control tube should always be the first tube used in the (first) 8-well strip of any test run (eg. if you're running one strip in a run, you should place the negative control in the first well of the strip; if you're running 3 strips, you should place a negative control in the first well of the FIRST strip - there is no need to place it in the first wells of the other strips).

NOTE: The negative control validates a particular LOT of the product. In a case of running more than one LOT during one run of the machine, each of the LOTs needs to be validated with a corresponding negative control.

4. To prepare the negative control: Pick up the 8-well strip from the rack and open the lid of the tube in position 1 (the negative control tube is tube 1 in part (A) of the diagram above).

5. Add 50 µl from the Control Buffer Tube (C) (see diagram above) to the tubel in position 1 on the 8-well strip. Do this using a NEW sterile tip on the automatic pipette provided. After adding the Control Buffer, close the lid of the tube.

6. Place the 8-well strip back in the rack.

7. Now prepare the sample tubes: The first sample tube is in position 2 on the (first) 8-well strip, and is the next tube situated after the negative control tube (this first sample tube is tube 2 in part (A) of the diagram above).

8. Transfer 50 µl of patient sample from the Sample Collection Tube to the Sample Normalization Buffer (B) using a NEW sterile tip on the automatic

pipette provided.

9. Pick up the (first) 8-well strip from the rack and open the lid of the first sample tube (position 2) on the 8-well strip.

10. Transfer 50 ul normalized sample from the Sample Normalization Buffer Tube (B) to the first sample tube (position 2) in the 8-well strip, using a NEW sterile tip on the automatic pipette provided. Then close the lid of that sample tube and place the 8-well strip back in the rack.

11. Repeat steps 8-10 with all the remaining samples that you wish to process together in the same run of the PCR machine. For each new sample from a new Sample Collection Tube, use the next available tube on the 8-well strip. Each sample must be placed in a separate tube on the 8-well strip, and must be transferred with a NEW sterile tip on the automatic pipette provided. If you are processing more than 6 samples in a run, you will need to use additional 8-well strips from the SAVD box, as required.

12. You must always make sure that there is at least one tube remaining available in the final 8-well strip that you'll use, after all the samples have been placed, in order for the positive control to be placed into this tube.

13. When you placed all the samples in the strips according to the procedures above, then prepare the positive control. Only one positive control needs to be prepared for every run of tests in the PCR machine, whether you are processing a single 8-well strip, or multiple 8-well strips in a run. The positive control tube should always be the last tube used in the (final) 8-well strip of any test run (PCT-Positive Control Tube) - eg. if you're running one strip in a run, you should place the positive control in the last well of the strip; if you're running 3 strips, you should place a positive control in the last well of the LAST strip - there is no need to place it in the last wells of the other strips).

NOTE: The positive control validates a particular LOT of the product. In a case of running more than one LOT during one run of the machine, each of the LOTs needs to be validated with a corresponding positive control.

14. To prepare the positive control: Pick up the 8-well strip from the rack (or pick up the final 8-well strip used, if multiple 8-well strips were used), and open the tube lid of the next tube situated after the last used sample tube (the positive control tube is marked 'PCT' in part (A) of the diagram above; in this diagram example, only one 8-well strip is being used in the run, containing six samples in tubes 2 to 7 and number 8 is 'PCT' and a positive control).

15. Add 50 µl from the Control Buffer Tube (C) to the Positive Control Master Tube (D), using a NEW sterile tip on the automatic pipette provided (see diagram above).

16. Then transfer 50 µl of the mixture from the Positive Control Master Tube into the next tube situated after the last used sample tube on the 8-well strip, using the same sterile tip on the automatic pipette provided. After you transferred the sample, please close the lid. This last used tube in the (final) 8-well strip is the 'PCT', which is the positive control tube.

    IMPORTANT! For each control tube (positive or negative), and for each sample tube, you MUST use a separate sterile pipette tip.

17. After you're done, place the 8-well strip back in the rack.

18. Now, take the 8-well strip(s) out of the racks and place them in order in the machine (in the appropriate orientation according to the sample settings in the machine software).

19. Set the temperature and time profile on the machine (or using the machine software on the computer), including the fluorescence measurement settings.

20. Close the machine and run the amplification program.

Amplification profile

The given profile has been validated on the BIO-RAD CFX96, Thermofisher's Quant Studio 1, and MyGo Pro RT-PCR machines. For other devices, further validation should be done. The reading should be set as for the SYBR green intercalating dye after each cycle.

MyGo Pro

TEMPERATURE	TIME	RAMP	CYCLES
50 °C	3 min	4 °C/s	1
95 °C	30 s	5 °C/s	1
95 °C	3 s	3 °C/s	35
58 °C	22 s *	1,50 °C/s	

* fluorescence reading – intercalation dye mode, SYBR Green dye

BioRad CFX96

TEMPERATURE	TIME	RAMP	CYCLES
50 °C	3 min	Default	1
95 °C	30 s	Default	1
95 °C	3 s	Default	35
58 °C	15 s	Default	

Interpretation of results

The correct test procedure and the ability to interpret the results are only possible if the appropriate signals are obtained for the controls in the reaction. When analyzing SAVD data, please use the decision matrix below:

Interpretation of the controls:

TYPE	NEGATIVE CONTROL	POSITIVE CONTROL
SIGNAL	NO SIGNAL Cq undetermined	Cq < 25
INTERPRETATION	VALID	VALID

Interpretation of the testing samples:

TYPE	SAMPLE	SAMPLE
SIGNAL	Cq undetermined	Cq < 30
INTERPRETATION	NEGATIVE	POSITIVE

General information and precautions

1. For *in vitro* diagnostic (IVD) use.
2. Follow standard infection control precautions. All patient samples and positive controls should be considered as potentially infectious and treated appropriately, using safe infection control procedures. See Provisional Biosafety Guidelines for the transfer and processing of SARS-CoV-2-related samples (e.g https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19))
3. Do not eat, drink, smoke, use cosmetics, or touch contact lenses where reagents are present and human samples are handled.
4. Samples should be processed in accordance with national and local biosafety regulations.
5. If SARS-CoV-2 infection is suspected based on current clinical and epidemiological test criteria, samples should be obtained using appropriate infection control measures.
6. The characteristics of analytical effectiveness were determined on laboratory RNA samples of SARS-CoV-2 virus and on samples of the upper and lower respiratory tract (presumably positive and negative).

Limitations

1. All users, analysts and anyone reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should be able to perform and interpret the result before performing the test independently themselves.
2. SAVD only works with GeneMe CoVi19 TEST Sample Collection kit.
3. Clinical test performance was determined based on SARS-CoV-2 RNA laboratory samples and clinical samples of upper and lower respiratory tract samples (such as nasopharyngeal or oropharyngeal swabs).
4. Negative results do not exclude the possibility of SARS-CoV-2 infection and should not be used as the sole basis for treatment or other clinical decisions. The time to reach the maximum viral load during infection due to SARS-CoV-2 has not been determined. Multiple samples (types and time points) may need to be taken from the same patient to detect the virus.

5. A false-negative result may occur if the sample is incorrectly collected, transported, or treated. False-negative results can also occur if there are amplification inhibitors in the sample or if there are not enough virus RNA molecules in the sample. Positive and negative predictive values are highly dependent on disease prevalence. False-negative test results are more likely when the prevalence of the disease is high. False-positive test results are more likely when the prevalence of the disease is moderate to low.

6. Do not use any reagents or test components beyond their expiration date.

7. If the virus mutates in the target region, SARS-CoV-2 may not be detected. Inhibitors or other types of interference may give a false-negative result. Interference studies on the effects of commonly prescribed drugs have not been conducted.

8. The impact that epidemiology and the clinical spectrum of SARS-CoV-2 infections may have on the test results is not fully known. For example, clinicians and laboratories may not know the optimal types of samples to collect, and when during infection these samples most likely contain levels of viral RNA that can be most easily detected.

9. GeneMe did not independently assess the stability of the fresh sample and frozen samples. GeneMe followed the standard practices recommended by the World Health Organization (WHO).

10. GeneMe did not test for interfering substances. We do not anticipate intervention by commonly used endogenous substances. No interference tests have been performed on this test, but this cannot be excluded.

11. GeneMe independently assessed the sensitivity and specificity in silico and adopted the WHO assessment.

12. Patients should not drink, eat or smoke for a minimum 30 minutes before swabbing.

13. Before processing the sample please check the turbidity and viscosity of the swab sample. Turbid and viscous samples can influence the fluorescence and therefore the results. In case of very turbid samples we recommend x10, x100 and x1000 dilutions of swab samples before proceeding with SAVD testing. However, this action will also lower the Limit of Detection of SAVD.

Performance characteristics

1. Limit of Detection (LOD)

The study was performed by NHS Scotland on purified SARS-CoV-2 RNA. The study showed LOD of about 10 copies of the SARS-CoV-2 virus per reaction. Reaction size was set at 50 (50/µl) microliters, equal to 200 copies of SARS-CoV-2 per milliliter (200/ml).

2. Cross-reactivity

Organisms (bacteria, viruses) usually inhabiting the respiratory system have been isolated and tested by the SAVD test. No cross-reactivity was observed for any of the tested pathogens. The tested pathogens are listed in Table 1 and the Amplification curves for selected pathogens is presented in Figure 1.

Figure 1. Amplification curves for SARS-CoV-2 (growing curve) and other Coronaviruses (flat lines).

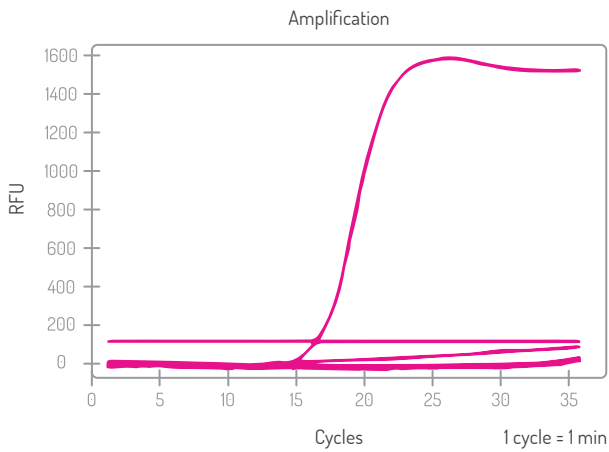

Table 1. The list of tested pathogens for potential cross-reactivity.

NO.	SAMPLE	CQ	TIME [MINUTES]
1.	SARS-CoV-2	16.32	17
2.	Human Coronavirus NL63	NOT DETECTED	NOT DETECTED
3.	Human Coronavirus 283E	NOT DETECTED	NOT DETECTED
4.	Human Coronavirus OC43	NOT DETECTED	NOT DETECTED
5.	Human Coronavirus 223E	NOT DETECTED	NOT DETECTED
6.	Human Coronavirus 229E	NOT DETECTED	NOT DETECTED
7.	*Streptococcus pyogenes* ATCC 19615	NOT DETECTED	NOT DETECTED
8.	*Haemophilus influenzae* ATCC 33391	NOT DETECTED	NOT DETECTED
9.	*Bordetella parapertussis* ATCC 15311	NOT DETECTED	NOT DETECTED
10.	Klebsiella pneumoniae ATCC 13883	NOT DETECTED	NOT DETECTED
11.	*Staphylococcus aureus* ATCC 12600	NOT DETECTED	NOT DETECTED
12.	*Pseudomonas aeruginosa* ATCC 10145	NOT DETECTED	NOT DETECTED
13.	Respiratory Syncytial virus ATCC VR-1540	NOT DETECTED	NOT DETECTED
14.	Epstein-Barr Virus	NOT DETECTED	NOT DETECTED
15.	Rhinovirus ATCC VR 283	NOT DETECTED	NOT DETECTED
16.	Influenza A H1N1 A/Virginia/ATCC/2009.	NOT DETECTED	NOT DETECTED

3. *In silico* specificity of primers

GeneMe performed the oligonucleotide primer alignment for the upper respiratory tract panel in accordance with FDA EUA recommendations, and with all publicly available SARS-CoV-2 sequences (as of January 13, 2021). All matches showed 100% identity for the available SARS-CoV-2 sequences and no significant match with the sequences of other upper respiratory tract pathogens.

4. Clinical Efficacy

Clinical swabs (80) in transport medium routinely collected by GeneMe Service Laboratories from patients were tested using the SAVD by GENEME. The test was

carried out using a directly transported reaction medium without the need for an RNA purification step. Real-time RT-PCR (PrimerDesign) was used as the reference method for comparing the results. This RT-PCR was carried out using purified RNA from the swab (100 µl of the swab was taken for the RNA isolation process). In this experiment, the SAVD was successfully validated in clinical performance studies. Validation using clinical samples gave the same results as real-time RT-PCR in 19 from 20 positive samples, and confirmed all negative (60) results obtained by the reference RT-PCR method.

Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were determined on the basis of RT-PCR sample testing as the reference method and FRANKD as the test method. Based on the above results, the diagnostic specificity of the SAVD test was defined as the ability to detect real healthy people, i.e. the ratio of true negative results to the sum of true negative and false positive results, with the equation:

SPECIFICITY = (TN / TN + FP) × 100

100% diagnostic specificity SAVD was determined for this panel (95% CI 94,04% - 100,00%).

The diagnostic sensitivity of the test is defined as the ratio of true positive results to the sum of true positive and false negative results, i.e. the ability of the diagnostic test to detect people who are suffering from the disease, with the equation:

SENSITIVITY [%] = (TP / TP + FN) × 100

95% diagnostic sensitivity SAVD was determined for this panel (95% CI 88,47% - 99,59%).

SAVD

by GENEME

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Upon receipt of the product, either directly from GeneMe or GeneMe authorised distributor, the purchaser shall examine it for material and performance defects* and the suitability for the purpose expressly stated in the IFU without undue delay, but not later than 14 calendar days from the date of delivery the product to the purchaser (when the products have been purchased directly from Geneme) or to the authorized distributor (when the products have been purchased from authorized distributor). In the described above situation, the purchaser shall give GeneMe (when purchased directly) or authorised distributor (when purchased from authorized distributor) immediate written notice of any defects, within 14 days from the date of delivery, or upon usage of a maximum of five percent of the delivery whichever is first. After this 14-days period, notification of any defects shall be made within 14 days of the date of identification defects by the purchaser and shall be precisely specify the type and extent of the defect in writing and shall include comprehensive details of any product transportation, product LOT number, run files from any PCR machine used, and a full and detailed description of storage conditions and any variations of those conditions. Any such notices of defects must be received by GeneMe (when purchased directly) or by GeneMe authorised distributor (when purchased from authorized distributor) within the warranty period.

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*A performance defect is a substantive deviation from the performance range as detailed in the IFU.

CE IVD 5°C 12°C 32

6 months from production date

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