GENEME

SARS-CoV-2

Isothermal Amplification Detection KIT

FRANKD by GENEME

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with C+

Purpose

The FRANKD by GeneMe SARS-CoV-2 isothermal detection kit, is a kit designed for *in vitro* identification of the new SARS-CoV-2 coronavirus in one reaction.

Description

The FRANKD by GeneMe SARS-CoV-2 Isothermal Amplification 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 Bst isothermal fusion 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 *S* 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 FRANKD strips with lyophilized enzymes, positive and negative controls.

FRANKD works with the GeneMe CoVi19 TEST Sample Collection kit (swabbing sample kit). This kit contains a single-use swab and a sample collection tube with transport buffer for one patient.

The method used

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is one type of nucleic acid amplification test.

This method detects whether or not viral copy DNA (produced from viral RNA) is present in a sample from a patient. It does this by capturing and amplifying regions of the virus genetic material. Here by amplifying multiple regions of a gene encoding the Spike protein.

RT-LAMP is conducted at a constant temperature of about 60 °C, using additives to the testing sample (called primers and a special type of the enzyme called polymerase). RT-LAMP assays for COVID-10 start with the collection of samples from oropharyngeal swab.

FRANKD components

FRANKD Kit components

ITEM	QUANTITY	STORAGE CONDITIONS
8-well FRANKD strip	4 pieces	5 °C - 24 °C
Control Buffer Tube	4 pieces	5 °C - 24 °C

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

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

Other components necessary for proper use (not delivered together with FRANKD Kit, recommended by GeneMe)

ITEM	QUANTITY	STORAGE CONDITIONS
Singleschannel pipette HTL 50 µl	1 piece	5 °C - 24 °C
Tip 2-100 µl Biosphere	32/ 1 FRANKD Reagent Kit	5 °C - 24 °C
BIO RAD CFX Connect device or MyGo Pro RT-PCR machine or ABI 7500 FAST QUANTITY	1 piece	Room Temperature

Transportation

A WarmMark (temperature indicator) is attached to the package. The WarmMark is informing about exceeding the maximum recommended temperature (24 °C) for product storage and transportation.

Expiration date

8-well FRANKD strip - 6 months from production date.
FRANKD Buffer - 6 months from production date.
Single sterile swab - 3 years from production date.

Kit compatibility with thermocyclers

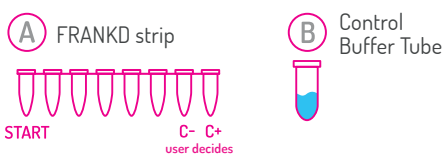
FRANKD is technologically compatible with all thermocyclers for real-time PCR. However, the temperature-time profile has been determined for the MyGo Pro real-time PCR. This is related to the duration of time for the fluorescence reads of the apparatus. For other thermocyclers, set the profile to obtain 30 readings and a total response time of 30 min. Fluorescence reading is performed as for intercalating dyes in the FAM channel (maximum absorption 498 nm and maximum emission 522 nm).

General information

There is no need for an RNA purification step. Recommended to usage all open reaction tubes as soon as possible to maintain the highest possible quality of components. If one PCR tube/ one part of Control Buffer will be taken to reaction, its needed to usage Kit Components before the expiry date in accordance with the manufacturer's recommendations.

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 any analyses in areas free from nucleases and using only pipettes with tips containing filters. Also, the FRANKD 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 or nasal sample, using a swab included in the Sample Collection Kit - according to the manual.
2. Choose one of the racks from the FRANKD box. Place the 8-well strip (A) on the rack. The test has 8-wells on the strip. All wells can be used for testing samples. User needs to decide in which wells in the strip to add negative and positive control. GeneMe recommends to run at least one negative and positive control per 1 FRANKD Reagent Kit.
3. Pick up the 8-well strip from the rack and open the appropriate testing tube lid (on the strip , starting with the testing tube lid nearest the label). Transfer 50 µl of buffer from the sample collection tube to the appropriate testing tube in the strip well, using the sterile tip and automatic pipette.
4. Close the testing tube lid and place the 8-well strip in the rack.
5. Repeat steps 3-4 for all samples that needs to be processed.
6. Add 50 µl of Control Buffer (B) and transfer it to negative control tube 7 and positive control tube 8.

IMPORTANT! For each control you MUST use a separate sterile pipette tip!

7. Place the strip in the rack.
8. Place the strip in the machine in the appropriate orientation according to the sample settings in the machine software.
9. Set the temperature and time profile on the machine, including the fluorescence measurement settings.
10. Run the amplification program.

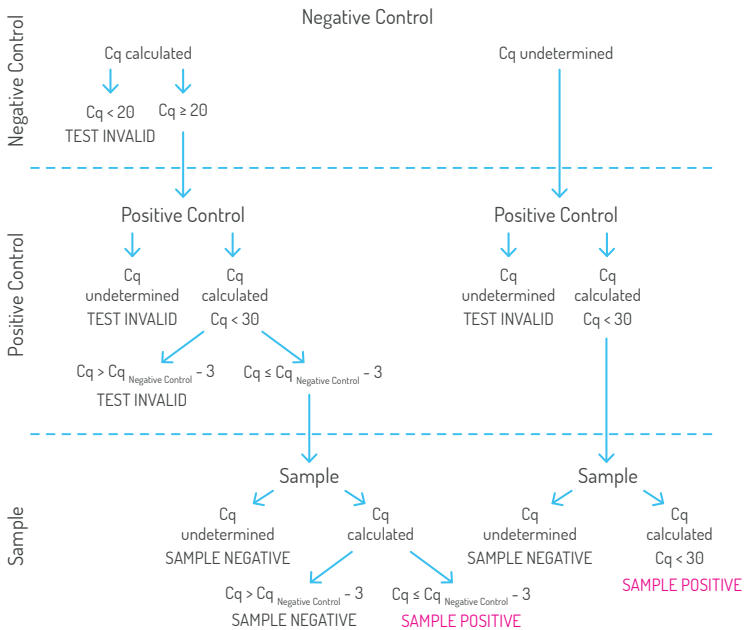
Amplification profile

	BIO RAD CFX	MYGO Pro	ABI 7500 FAST
TEMPERATURE	65 °C	65 °C	65 °C
TIME (EACH CYCLE)	48 s	60 s	60 s
CYCLES	30	30	30
FLUORESCENCE READING	Intercalating dye mode after each cycle (FAM channel)	Intercalating dye mode after each cycle (FAM channel)	Intercalating dye mode after each cycle (FAM channel)

The given profile has been optimized for the BIO RAD CFX Connect device, MyGo Pro RT-PCR machine and for ABI 7500 FAST machine. For other devices, the profile should be set to obtain 30 fluorescence readings with an incubation time window of 30 mins. The reading should be set as for the SYBR green intercalating dye after each cycle (every 1 min). When setting the cycle time you should consider the fluorescence reading time by reducing the cycle time by that amount. In the case of the Bio-Rad CFX Connect thermal cycler, the reading time lasts 12 seconds, therefore we set the cycle time 60 - 12 = 48 s. The total reaction time should be 30 min. In case of MyGo Pro there is no additional reading time.

Interpretation of results

The FRANKD results interpretation should start from the analysis of the negative control and then the user should follow the decision graph below:



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.
3. Do not eat, drink, smoke, use cosmetics, or touch contact lenses where reagents are present and human samples are handled.
4. All samples should be handled as potentially infectious, 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))
5. Samples should be processed in accordance with national and local biosafety regulations.
6. If SARS-CoV-2 infection is suspected based on current clinical and epidemiological test criteria samples should be taken with appropriate infection control measures.
7. 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. FRANKD only works with GeneMe CoVi19 TEST Sample Collection kit (swabbing sample kit).
3. 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 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 prevalence. False-negative test results are more likely when the incidence 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 of the effects of common drugs on colds, on reactions, 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). It is recommended to use fresh sample for FRANKD directly. In other case, frozen samples in FRANKD Buffer (kept for max. 7 days in -20 °C) may be analysed.
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 they cannot be excluded.
11. GeneMe independently assessed the sensitivity and specificity *in silico* and adopted the WHO assessment.
12. Patients shall not drink, eat or smoke minimum 30 minutes before swabbing.
13. Before proccesing the sample please check the turbidity and viscosity of the swab sample. Turbid and viscous samples can influence the fluorescence and therfore the results. In case of very turbid samples we recommend 10×, 100× and 1000× dillutions of swab samples before procedding testing of FRANKD. However this action will also lower the LOD of FRANKD.

14. RT-LAMP tests for COVID-19 can only tell if a person is currently infected with this particular coronavirus.
15. It can't provide information on other diseases or symptoms and does not tell staff if a patient has been previously infected with the virus or if patient has any immunity to the virus.

Performance characteristics

1. Limit of Detection (LOD)

The study showed a sensitivity of 1 x 10^{-6} ng RNA virus, which corresponds to 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).

Figure 1. The amplification curves of SARS-CoV-2 with FRANKD. Curves from left to right: 1-6 and the flat line is a negative control.

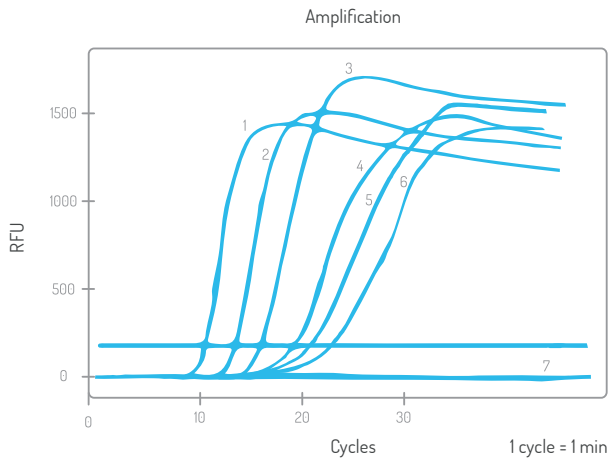


Table 1. The list of tested SARS-CoV-2 RNA dilutions.

FLUORESCENT DYE	SAMPLE	SARS-COV-2 RNA DILUTION	CQ	TIME [MINUTES]
SYBR	1	0.1 ng	10.33	10
SYBR	2	0.01 ng	12.90	13
SYBR	3	0.001 ng	15.06	15
SYBR	4	0.0001 ng	18.01	18
SYBR	5	0.00001 ng	22.10	23
SYBR	6	10^{-6} ng	23.25	24
SYBR	7	0 (negative control)	NOT DETECTED	NOT DETECTED

2. Cross-reactivity

Organisms (bacteria, viruses) usually inhabiting the respiratory system have been isolated and tested by the FRANKD test. No cross-reactivity was observed for any of the tested pathogens. The tested pathogens are listed in Table 2 and the Amplification curves for selected pathogens is presented in Figure 2.

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

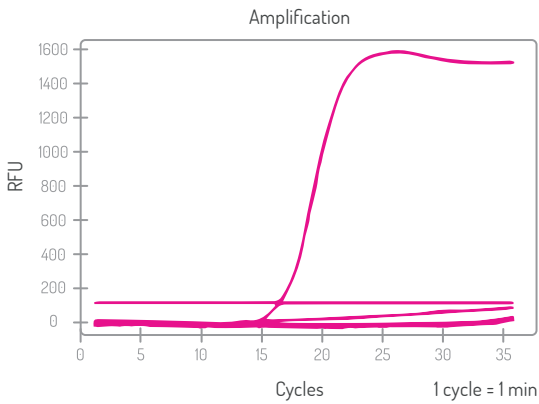


Table 2. The list of tested pathogens for potential cross-reactivity.

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 July 2, 2020). 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

Residual material from clinical swabs in transport medium routinely collected from patients were tested using the FRANKD by GENEME SARS-CoV-2 kit. The test was carried out using a directly transported reaction medium without the need for a RNA purification step. Residual material from the swab was vortexed for 5 s and then 50 µl were taken for the reaction using FRANKD.
Real-time RT-PCR (Anatolya GeneWorks) was used as the reference method for comparing the results. This RT-PCR test detects two different SARS-CoV-2 genes (*E* and *Orf1ab)* to confirm the result and 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 FRANKD Isothermal Amplification Diagnostic Kit for SARS CoV-2 was successfully validated in clinical performance studies. Validation using clinical samples gave the same results as real-time RT-PCR in all negative samples, and confirmed 58/60 positive results obtained by the reference RT-PCR method.

		RT-PCR	
		Positive	Negative
FRANKD	Positive	TP 58	FP 0
	Negative	FN 2	TN 60

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 FRANKD 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 FRANKD 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

96,97% diagnostic sensitivity FRANKD was determined for this panel (95% CI 88,47% - 99,59%).

FRANKD
by GENEME

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GeneMe warrants to the purchaser this product is free from defects in workmanship or materials for a period of 6 months from the date of production, under normal use, provided that the product has been kept in appropriate storage conditions and used in accordance with the instruction of use. The sole and exclusive remedy under this limited warranty is replacement of defective products or parts thereof. Replacement products or parts thereof will be furnished solely on an exchange basis and are obtainable only by the purchaser. The purchaser shall return the defective product, or part thereof, properly packaged, postage or shipping costs prepaid to GeneMe. Loss or damage during shipment shall be at the risk of the purchaser. Geneme does not give any express or implied warranties or representation on the accuracy levels of the product.

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

IVD

5°C 24°C

24

6 months from production date

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