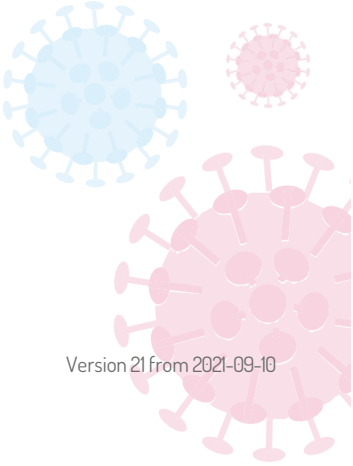
GENEME

FRANKD

SARS-CoV-2

Isothermal Detection KIT

IFU – Instructions for Use



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Purpose and description

The FRANKD by GeneMe is a SARS-CoV-2 isothermal detection kit, designed for *in vitro* identification of the SARS-CoV-2 coronavirus in a single reaction. The presence of an innovative and patented Bst isothermal fusion polymerase and specific primers made it possible to create a highly specific and sensitive test. The individually designed primers are 100% compatible with the SARS-CoV-2 genomic RNA sequence of *N* gene as deposited in the NCBI database. Amplification of target nucleic acids is observed by increasing the fluorescence signal during the reaction.

FRANKD is compatible with the GeneMe Sample Collection Kit (CoVi19 TEST Sample Collection Kit).

Please read this manual carefully before performing the test.

The method used

Reverse transcription isothermal amplification is one type of nucleic acid amplification tests (NAAT).

This method detects whether a viral copy of DNA (made from viral RNA) is present in a patient sample. It does this by capturing and enhancing regions of viral genetic material. Here by amplifying multiple regions of the gene encoding the Spike protein.

The isothermal NAAT is carried out at a constant temperature of approx. 60°C, with the use of additives to the tested sample (the so-called primers and a special type of enzyme called polymerase).

FRANKD components

FRANKD Detection Kit components

ITEM	QUANTITY	STORAGE CONDITIONS
8-well FRANKD Strip (with freeze-dried reagents)	4 pieces	+ 5°C to + 12°C
Control Buffer Tube	4 pieces	+ 5°C to + 24°C
Positive Control Tube	4 pieces	+ 5°C to + 12°C
Normalization Buffer Tube	32 pieces	+ 5°C to + 24°C

CoVi19 TEST Sample Collection Kit components (packed individually and delivered on request with FRANKD Detection Kit)

ITEM	QUANTITY	STORAGE CONDITIONS
Single use sterile swab	1 piece	+ 2°C to + 30°C
Sample Collection Buffer Tube	1 piece	+ 5°C to + 24°C

Expiration date

8-well FRANKD Strip - 6 months from the production date
Control Buffer Tube - 6 months from the production date
Positive Control Buffer - 6 months from the production date
Normalization Buffer Tube - 6 months from the production date
Single use sterile swab - 3 years from the production date
Sample Collection Buffer Tube - 6 months from the production date

Kit compatibility with thermocyclers

FRANKD is technologically compatible with real-time PCR thermocyclers. However, a temperature-time profile was determined for Bio-Rad CFX real-time PCR. This is related to the duration of the instrument's fluorescence readings. For other thermocyclers, set the profile to 60 readings and a total response time of 30 minutes. The fluorescence reading is as for the intercalation of dyes in the FAM

channel (maximum absorption 498 nm and maximum emission 522 nm).

General information

When using pure SARS-CoV-2 RNA as a template in the test, it is very important to use RNase-free tools and reagents. In addition, it is recommended that any analysis must be performed in nuclease-free areas and that only pipettes with filter tips must be used. The FRANKD test cannot be used as a method of analyzing SARS-CoV-2 virus directly taken from a cell line.

Before you start

Required equipment

- Thermocycler
- Vortex mixer
- 8-well mini-centrifuge
- Single-channel adjustable volume pipette (required volume range: 10 - 50 µl) or single-channel fixed volume pipette 50 µl (direct samples only)
- Pipette tips with filters
- Disposable gloves

Samples

The following procedure do not cover collecting the samples. Please prepare throat swab or nasopharyngeal swab before, by using the instructions in the respective Sample Collection Kit. It is recommended to store the samples refrigerated (+4°C to +8°C).

FRANKD Detection Kit can be used both with *direct samples* and with *isolated RNA samples*. ***Make sure you are following the appropriate procedure for the type of sample.***

Prepare your workplace

- Remember to dress properly before performing the test. It is mandatory to wear closed-toe shoes, long pants which fully covers the legs. Hair and shoes

shall be covered with the cap. It is necessary to wear a laboratory coat, face mask and gloves.

- Make sure your table, pipette and fresh gloves are sterile. Use appropriate disinfectants and paper towels. It is important that the workplace is dry before starting work.
- If possible, separate the workstation for working with the tests and samples from the workstation of the thermocycler and analysis of the results to prevent the spread of contamination.

Plan your work

- Determine the quantity of samples tested during a single run of thermocycler.
- Determine the appropriate number of FRANKD Detection Kits you need. It is recommended to use ONE LOT for the entire run (each FRANKD Detection Kit box has a LOT number assigned at the back).
- It is mandatory to use one positive and one negative control for each LOT used in a single run. ***Take this into account when calculating the number of samples and kits needed.***
- Plan the positions of negative and positive controls in the thermocycler. These positions can be specified according to user's preference under the condition that the following rules are applied:

    1. There should be at least one negative and one positive control in each run of a thermocycler.
    2. If more than one LOT of FRANKD Detection Kit is used in a single run – it is mandatory to use ONE positive and ONE negative control for each LOT used in a run.
    3. It is mandatory to follow the order when using the FRANKD Detection Kit:
        a. preparation of all negative controls,
        b. preparation of all samples,
        c. preparation of all positive controls.

    Having this in mind it is recommended to use the first well of the first strip from a single LOT for negative control and the last well of the last strip of a single LOT for positive control. All remaining wells should be used for samples.

Precautions

- Holding the strips in your hand may reduce the sensitivity of the test.
- Avoid removing the strips from the rack whenever possible.
- **Make sure you <u>do not touch</u> anything with your bare hands.**

If you have any doubts – please read this FRANKD Detection Kit IFU again.

Procedure

Negative control wells preparation

1. Open the FRANKD Detection Kit box. Remove the rack from the main box. Place one FRANKD 8-well strip on the rack.
2. Open the well that you intended for a negative control when planning work (please see *Before you start – Planning your work* section).
3. Open the Control Buffer Tube. Transfer 50 µl of Control Buffer to the open well. Do this by using a fresh sterile tip on an automatic pipette.
4. Close the well and the tube.
5. **Repeat steps 1-4 for each negative control.**

Sample wells preparation

*NOTE: If you are working with **isolated RNA samples** continue with step **6**. If you are working with **direct samples** skip steps 6-13 and continue with step **14**.*

Isolated RNA samples

6. Put your sample tubes (isolated RNA) on the table.
7. Open the first available well that you intended for a sample.
8. Open the Normalization Buffer Tube (these are the slightly larger tubes on the top row on the rack). Number on the rack should match the number of the first available well intended for sample (e.g., 1 match 1, 2 match 2 and so on).
9. Transfer 40 µl of normalization buffer to the open well. Do this by using a fresh sterile tip on an automatic pipette.
10. Open the sample tube. Transfer 10 µl of the isolated RNA to the open well. Do

this by using a fresh sterile tip on an automatic pipette.

11. Close the well and both tubes.
12. **Repeat steps 6-11 for each sample.**
13. When all sample wells are ready continue with step **24**.

Direct Samples

14. Put your sample tubes (direct samples) on the table.
15. Open the Normalization Buffer Tube (these are the slightly larger tubes on the top row on the rack). The number on the rack should match the number of the first available well intended for sample (e.g., 1 match 1, 2 match 2 and so on).
16. Mix sample in the sample tube by rotating 5 times.
17. Open the sample tube. Transfer 50 µl of the mixture to the open Normalization Buffer Tube. Do this by using a fresh sterile tip on an automatic pipette. **Pipette 5 times.**
18. Close both tubes.
19. **Repeat steps 14-18 for each sample.**
20. Open the first available well that you intended for a sample.
21. Open the matching Normalization Buffer Tube. Transfer 50 µl of normalized sample to the open well. Do this by using a fresh sterile tip on an automatic pipette.
22. Close the well and the tube.
23. **Repeat steps 20-22 for each normalized sample.**

Positive control wells preparation

24. Place one Positive Control Tube in the designated place in the rack.
25. Open the well (FRANKD 8-well Strip) that you intended for a positive control.
26. Open the matching Normalization Buffer Tube.
27. Open the Positive Control Tube. Transfer 50 µl of Normalization Buffer to the Positive Control Tube. Do this by using a fresh sterile tip on an automatic pipette.

28. Transfer 50 µl of the mixture from the Positive Control Tube to the open well (FRANKD 8-well Strip).
29. Close the well and the tubes.
30. **Repeat steps 24–29 for each positive control.**
31. Change the gloves before moving to the next step.

Preparing the PCR reaction

32. If the lyophilizate is completely dissolved in the test well continue with step **35**.
33. Take the prepared FRANKD 8-well Strip to hand avoiding holding the bottom part filled with liquid. Attach the FRANKD 8-well Strip to the running vortex while moving the strip over so that each well in turn touches the vibrating element and the liquid is completely mixed. Put the strip back to the rack. Repeat this step for each strip.
34. Transfer the prepared strips to the 8-well mini-centrifuge and centrifuge for a few seconds until all the liquid is on the bottom of the wells and the air bubbles are gone. Repeat this step for each strip.
35. Place the FRANKD 8-well Strips into the thermocycler, one by one (in the correct orientation according to the sample settings in the thermocycler software).
36. Set the temperature and time profile on the thermocycler (or use the thermocycler software on the PC), including settings for the fluorescence measurement.
37. Close the thermocycler and run the amplification program.

NOTE: The preparation time of the FRANKD test from flooding the first FRANKD Strip well to insertion into the thermocycler cannot be longer than 30 minutes. Failure to comply with these guidelines will reduce the sensitivity of the FRANKD test.

Amplification profile

	BIO RAD CFX	MYGO Pro
TEMPERATURE	67°C	67°C
TIME (EACH CYCLE)	18 s	30 s
CYCLES	60	60
FLUORESCENCE READING	Intercalating dye mode after each cycle (FAM channel)	Intercalating dye mode after each cycle (FAM channel)

The given profile has been optimized for BIO RAD CFX Connect and MyGo Pro RT-PCR devices. For other devices, the profile should be set to obtain 30 fluorescence readings with an incubation time window of 30 minutes. The reading should be set as for green intercalating SYBR dye after each cycle (every 30 s). When setting the cycle time, take the fluorescence reading time into account by reducing the cycle time by this value. For the Bio-Rad CFX Connect thermocycler, the reading time is 12 seconds, therefore we set the cycle time 30 - 12 = 18 seconds. The total reaction time should be 30 minutes. There is no extra reading time with MyGo Pro.

Interpretation of results

When analyzing the FRANKD data, please use the following decision matrix below:

Interpretation of the controls:

TYPE	NEGATIVE CONTROL	POSITIVE CONTROL
SIGNAL	Cq undetermined or Cq>50	Cq < 50
INTERPRETATION	VALID	VALID

Interpretation of the tested samples:

TYPE	NEGATIVE SAMPLE	POSITIVE SAMPLE
SIGNAL	Cq undetermined or $Cq > Cq_{negative\ control}$	Cq determined and $Cq < Cq_{negative\ control}$

General information and precautions

1. For *in vitro* diagnostic (IVD) use.
2. Follow standard infection control precautions. All patients' samples and positive controls should be considered as potentially infectious and treated appropriately using safe infection control procedures.
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 collected with 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 reporting of diagnostic results should receive training by a competent instructor to perform this procedure. They should be able to independently perform and interpret the result before taking the test.
2. FRANKD only works with the GeneMe CoVi19 TEST Sample Collection kit.
3. The performance of the test was determined using SARS-CoV-2 RNA laboratory samples and upper and lower respiratory tract clinical samples (such as nasopharyngeal or oropharyngeal swabs).
4. Negative results do not rule out SARS-CoV-2 infection and should not be used

as the sole basis of treatment or other clinical decisions. Time to arrive the maximum viral load during infection with SARS-CoV-2 has not been determined. It may be necessary to collect multiple samples (types and time points) from the same patient to detect the virus.

5. A false negative result may occur if the sample is incorrectly collected, transported or processed. False negative results can also occur if amplification inhibitors are present in the sample or if there are not enough viral RNA molecules in the sample. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely to occur when morbidity is high. False positive test results are more likely when the incidence is moderate or 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 produce a false negative result. The effects of medications and responses to colds have not been studied.
8. The impact of the epidemiology and clinical spectrum of SARS-CoV-2 infections on the study results is not fully understood. For example, clinicians and laboratories may not know the optimal types of samples to collect, and when infected, these samples most likely contain viral RNA levels that are easiest to detect.
9. GeneMe did not independently evaluate the stability of fresh and frozen samples. GeneMe followed standard practices recommended by the World Health Organization (WHO); direct use of a fresh sample for the FRANKD is recommended. Otherwise, samples frozen in FRANKD buffer (stored for a maximum of 7 days at -20°C) can be analyzed.
10. GeneMe did not test the interfering substances. We do not anticipate the intervention of commonly used endogenous substances. No interference tests were performed with this test but cannot be ruled out.
11. GeneMe independently assessed the sensitivity and specificity *in silico* and adopted the WHO assessment.
12. Patients should not drink, eat or smoke minimum 30 minutes before swabbing**.**
13. Before processing the sample, check the turbidity and viscosity of the swab sample. Cloudy and sticky samples can affect the fluorescence and therefore

the results. For very turbid samples, we recommend 10x, 100x and 1000x dilutions of the swabs prior to the FRANKD test. However, this action will also lower the LOD of the FRANKD.

14. Isothermal NAAT COVID-19 tests can only tell if a person is currently infected with this coronavirus. They cannot provide information on other diseases or symptoms, and do not inform staff as to whether the patient has been previously infected with the virus or whether the 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.

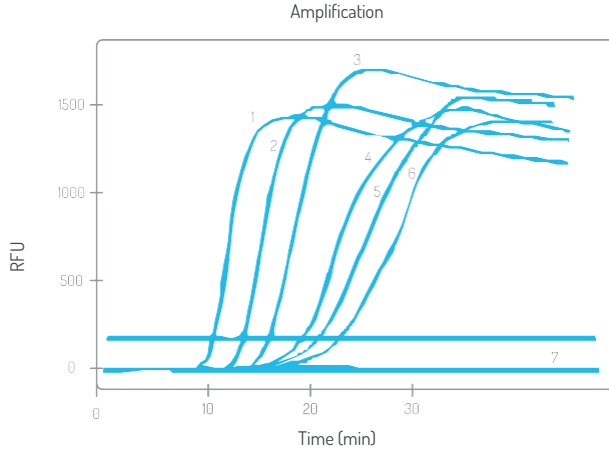

Table 1. The list of tested SARS-CoV-2 RNA dilutions.

FLUORESCENT DYE	SAMPLE	SARS-COV-2 RNA DILUTION	CQ	TIME (MINUTES)
SYBR	1	0.1 ng	20,66	10
SYBR	2	0.01 ng	25,8	13
SYBR	3	0.001 ng	30,12	15
SYBR	4	0.0001 ng	36,02	18
SYBR	5	0.00001 ng	45,2	23
SYBR	6	10^{-6} ng	46,5	24
SYBR	7	0 (negative control)	NOT DETECTED	NOT DETECTED

2. Cross-reactivity

Microorganisms (bacteria, viruses) usually found in the respiratory system were

isolated and tested with the FRANKD test. Cross-reactivity with any of the pathogens tested was not observed. The pathogens tested are listed in Table 2 and the amplification curves for selected pathogens are shown in Fig. 2.

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

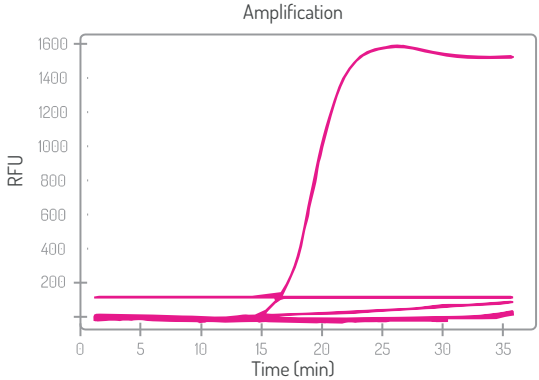

Table 2. The list of tested pathogens for potential cross-reactivity.

NO.	SAMPLE	CQ	TIME [MINUTES]
1.	SARS-CoV-2	32,64	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 oligonucleotide primer alignment for the upper airway panel in accordance with FDA EUA recommendations and all publicly available SARS-CoV-2 sequences (as of July 2, 2020). All matches showed 100% identity with the available SARS-CoV-2 sequences and no significant match with the sequences of other upper respiratory pathogens.

4. Clinical Efficacy

Residual material from clinical swabs in the transport medium routinely collected from patients were tested using the FRANKD Detection Kit. The test was performed using directly transported reaction medium without the need for an RNA purification step. Residual material from the swab was vortexed for 5 s, diluted 10 times in normalization buffer and then 50 µl were taken for the reaction using FRANKD. Real-time RT-PCR (Anatolya GeneWorks) was used as a method to compare the results. This RT-PCR test detects two different SARS-CoV-2 genes (*E* and *Orf1ab)* to confirm the result and was performed using purified RNA swab (100 µl of the swab taken for the RNA isolation process).

In this experiment, the FRANKD Detection Kit was successfully validated in clinical trials. Validation with 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 as the reference method and FRANKD as the research method.

Based on the above results, the diagnostic specificity of the FRANKD test was defined as the ability to detect genuinely healthy people, that is, the ratio of true negatives to the sum of true negatives and false positives, 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 a test is defined as the ratio of true positive to the sum of true positive and false negatives, that is, the ability of a diagnostic test to detect people 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

LIMITED PRODUCT WARRANTY

This warranty applies to products manufactured by GeneMe where such products have been purchased directly from GeneMe or a GeneMe authorised distributor. Any products coming into the possession of a user via another source are without warranty and should not be used under any circumstances.

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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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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This Agreement contains the entire agreement between Geneme and the purchaser relating to the product's warranty. This warranty shall be interpreted in accordance with polish law.

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

6 months from the production date

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