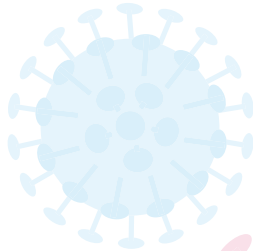




G E N E M E

SARS-CoV-2

Isothermal Amplification
Detection KIT



FRANKD

by G E N E M E

GeneMe Sp. z o.o.
ul. Piotrkowska 41c
80-180 Gdańsk
Poland

Version 11 from 16.11.2020
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.

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 |

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

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.

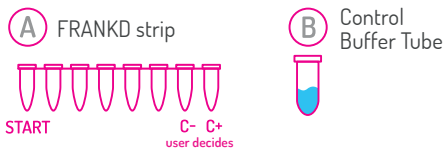
Kit compatibility with thermocyclers

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

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 box.
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 and MyGo Pro RT-PCR 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 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 FRANKD data please use the below decision matrix:

Interpretation of the controls:

| TYPE | NEGATIVE CONTROL (POSITION 7) | NEGATIVE CONTROL (POSITION 8) |
|----------------|---------------------------------------|--|
| SIGNAL | no signal $C_q = 30$ $C_q \geq 20$ | $C_q \leq C_{q_{\text{Negative Control}}} - 3$ |
| INTERPRETATION | VALID | VALID |

Interpretation of the testing samples:

| TYPE | SAMPLE | SAMPLE |
|----------------|--|--|
| SIGNAL | no signal $C_q = 30$ $C_q \geq C_{q_{\text{Negative Control}}} - 3$ | $C_q \leq C_{q_{\text{Negative Control}}} - 3$ |
| INTERPRETATION | NEGATIVE | POSITIVE |

Invalid test:

| | |
|--------------|---|
| INVALID TEST | Positive Control $\geq C_{q_{\text{Negative Control}}} - 3$ |
|--------------|---|

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-\(covid-19\)](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).
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 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 10×, 100× and 1000× dilutions of swab samples before proceeding testing of FRANKD. However this action will also lower the LOD of FRANKD.

Performance characteristics

1. Limit of Detection (LOD)

The study showed a sensitivity of 1×10^{-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/ μ l) 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.

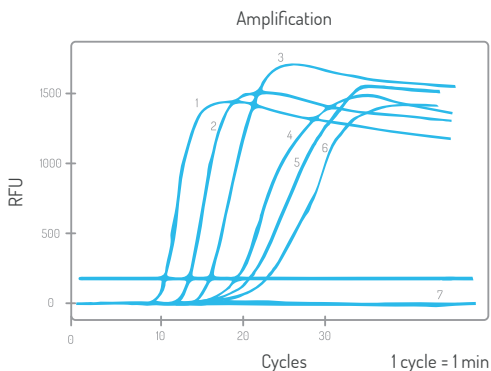


Table 1. The list of tested SARS-CoV-2 RNA dilutions.

| 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. *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.

3. 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).

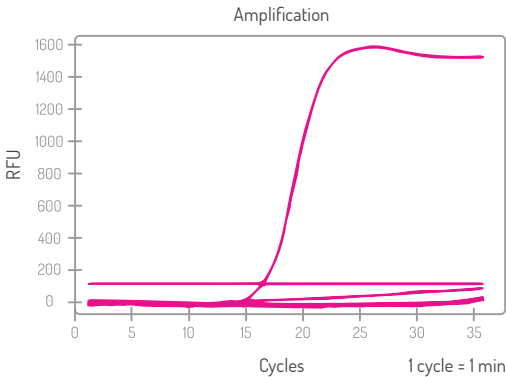


Table 2. The list of tested pathogens for potential cross-reactivity.

| 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. | <i>Streptococcus pyogenes</i> ATCC 19615 | NOT DETECTED | NOT DETECTED |
| 8. | <i>Haemophilus influenzae</i> ATCC 33391 | NOT DETECTED | NOT DETECTED |
| 9. | <i>Bordetella parapertussis</i> ATCC 15311 | NOT DETECTED | NOT DETECTED |
| 10. | <i>Klebsiella pneumoniae</i> ATCC 13883 | NOT DETECTED | NOT DETECTED |
| 11. | <i>Staphylococcus aureus</i> ATCC 12600 | NOT DETECTED | NOT DETECTED |
| 12. | <i>Pseudomonas aeruginosa</i> 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 |

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 μ l 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 *Orflab*) 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 trials. 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:

$$\text{SPECIFICITY} = (\text{TN} / \text{TN} + \text{FP}) \times 100$$

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:

$$\text{SENSITIVITY} [\%] = (\text{TP} / \text{TP} + \text{FN}) \times 100$$

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

FRANKD

by G E N E M E

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.

The warranties set out here apply to defects that appear under the conditions of operations provided for by the agreement and in particular do not apply in any of the following cases: (a) the products have been subject of replacement necessitated by accident, neglected, misused, relocation, unauthorized repair or modification of the product; (b) the products have been altered or repaired by anyone other than GeneMe without GeneMe's prior written consent; (c) the products have been damaged by circumstances beyond the reasonable control of GeneMe; (d) the products have been improperly used or maintained by the purchaser; (e) the products have been subject to conditions of use and/or maintenance not in conformity with GeneMe's instructions; (f) the products have been used by non-professional users; (g) the products have been damaged by: abuse, negligence in use, including using the product in a manner incompatible with the instruction of use, improper storage or transportation or handling.

Subject to the limitations resulting from the mandatory provisions of law GeneMe shall not be liable to the third party and the purchaser, its staff or its customers under contract, tort (including negligence) or statute for loss of revenue, loss of profit, loss of opportunity, loss of goodwill, loss of data or the cost of replacement goods and services, or any indirect, consequential or incidental loss.

GeneMe shall not be liable for any failure of this warranty if the GeneMe's obligation performance becomes impossible due to a force majeure. Force Majeure means an event out of any GeneMe's control, which occurs unexpectedly, extraordinarily, which makes it impossible to rationally carry out either GeneMe's obligations.

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, but not latest than 14 calendar days from the date of delivery of 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 noticed by 14 days from 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.

THE ABOVE LIMITED WARRANTY IS THE SOLE WARRANTY PROVIDED BY GENE ME. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, ARE PROVIDED WHATSOEVER. NO GENE ME AGENT OR EMPLOYEE MAY MODIFY, EXTEND OR ADD TO THE ABOVE LIMITED WARRANTY. NOTHING IN THIS WARRANTY SHALL OPERATE TO EXCLUDE OR LIMIT A GENE ME'S LIABILITY FOR FRAUD OR FRAUDULENT MISREPRESENTATION.

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.



 6 months from production date



GeneMe Sp. z o.o. | ul. Piotrkowska 41c | 80-180 Gdańsk, Poland

www.geneme.eu