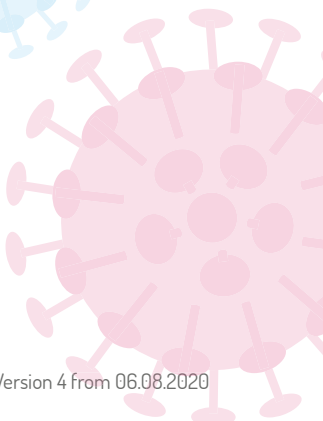
GENEME

SARS-CoV-2

Isothermal Amplification Detection KIT



FRANKD by GENEME

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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 polymeras 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	Room temperature
Control Buffer Tube	4 pieces	Room temperature

CoVi19 TEST Sample Collection Kit components (delivered separately from the FRANKD Kit)

ITEM	QUANTITY	STORAGE CONDITIONS
Single use sterile swab	1 piece	Room temperature
Sample collection tube & buffer	1 piece	Room temperature

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 Bio-Rad CFX Connect thermocycler. 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 RNase. 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. Acquire deep throat or a nasal swab with the use of the GeneMe CoVi19 TEST Sample Collection kit according to the manual.
2. Choose one of the racks from the FRANKD box. This rack contains an 8-well strip. The label attached to the 8-well strip determines the side from which samples should be added first. The first 6 wells on the strip (counting from the label) are for testing samples and the last 2 wells are for controls.
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 - one 8-well strip allows you to test 6 samples.
6. Pick up the strip from the rack and open the control tubes lids (positions 7 and 8 on the 8-well strip).
7. Add 50 µl of Control Buffer to the control wells on the strip (positions 7 and 8 , furthest away from the strip-well label) using the sterile tip and automatic pipette provided. For each control you MUST use a separate sterile tip.
8. Close the control tube lids and place the strip in the rack.
9. Repeat steps 3-8 for every testing strip you would like to use (one kit contains four 8-well testing strips).
10. Make sure all the tube lids are closed. Then remove all bubbles in the testing tubes by tapping the strip on a hard surface. Do not flick the individual testing sample tubes.
11. Place the strip in the machine in the appropriate orientation determined by the strip label, and according to the sample settings in the machine software.
12. Set the temperature and time profile on the machine, including the fluorescence measurement settings.
13. Run the amplification program.

Amplification profile

	BIO RAD CFX	MY GO Pro
Temperature:	65 °C	65 °C
Time (each cycle):	**48 s**	**60 s**
Cycles:	30	30
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 the BIO RAD CFX Connect device. 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.

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. The positive control should give a fluorescence signal in less than 25 mins, while the negative control should give fluorescence below the threshold level. A positive result for the test sample occurs when the fluorescence increases above the threshold in less than 30 min. The result is interpreted as negative when no increase in fluorescence above the threshold is achieved in 30 mins.

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).
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.

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. The effective sensitivity obtained at the proposed analysis time (30 min) indicates the detection of about 1000 PFU after 18 minutes.

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.

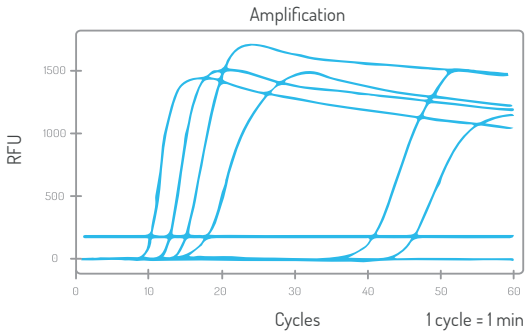

Table 1. The list of tested SARS-CoV-2 RNA dilutions.

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)	N/A	N/A

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).

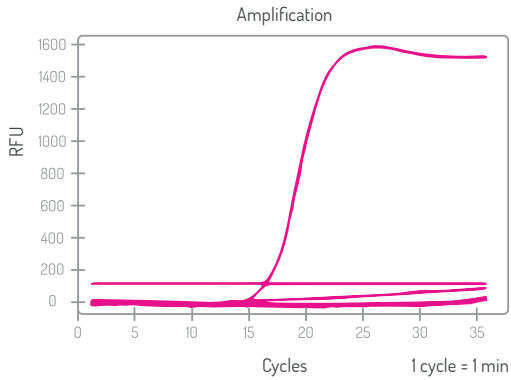

Table 2. The list of tested pathogens for potential cross-reactivity.

NO.	SAMPLE	CQ	TIME [MINUTES]
1.	SARS-CoV-2	16.32	17
2.	Human Coronavirus NL63	N/A	N/A
3.	Human Coronavirus 283E	N/A	N/A
4.	Human Coronavirus OC43	N/A	N/A
5.	Human Coronavirus 223E	N/A	N/A
6.	Human Coronavirus 229E	N/A	N/A
7.	*Streptococcus pyogenes* ATCC 19615	N/A	N/A
8.	*Haemophilus influenzae* ATCC 33391	N/A	N/A
9.	*Bordetella parapertussis* ATCC 15311	N/A	N/A
10.	Klebsiella pneumoniae ATCC 13883	N/A	N/A
11.	*Staphylococcus aureus* ATCC 12600	N/A	N/A
12.	*Pseudomonas aeruginosa* ATCC 10145	N/A	N/A
13.	Respiratory Syncytial virus ATCC VR-1540	N/A	N/A
14.	Epstein-Barr Virus	N/A	N/A
15.	Rhinovirus ATCC VR 283	N/A	N/A
16.	Influenza A H1N1 A/Virginia/ATCC/2009.	N/A	N/A

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 5s 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 (100µl 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:

SPECIFICITY = (TN / TN + FP) X 100

100% diagnostic specificity FRANKD was determined for this panel.

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) X 100

97% diagnostic sensitivity FRANKD was determined for this panel.

FRANKD

by GENEME

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