IVD

FREND™ COVID-19 total Ab

Qualitative assay for COVID-19 total antibodies

REF FRCOA 020

IVD For *in vitro* diagnostic use only

℞ For prescription use only. For Emergency Use Authorization only.

Intended Use

The FREND™ COVID-19 total Ab is a fluorescence immunoassay (FIA) using the FREND™ System intended for the qualitative detection of total antibody to SARS-CoV-2 in human dipotassium EDTA plasma. The FREND™ COVID-19 total Ab is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The FREND™ COVID-19 total Ab should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C §263a, that meet requirements to perform moderate or high complexity tests.

Results are for the detection of SARS-CoV-2 antibodies. Total antibody to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of FREND™ COVID-19 total Ab early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for FREND™ COVID-19 total Ab may occur due to cross-reactivity from pre- existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using second, different total antibody assay.

The FREND™ COVID-19 total Ab is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation of test

COVID-19 is a respiratory disease caused by a novel coronavirus called SARS-CoV-2. The symptoms for patients have included mild to severe respiratory illness with fever, cough, and difficulty breathing.[1-2] The test result shows the presence of SARS-CoV-2 antibodies, which are generally detectable in the blood several days after symptom onset.[3-4] The antibody test provides information specific to an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. It is not yet known if the presence of antibody confers immunity. Positive results should be confirmed by a second serology test. Final recommendations and patient management should be determined by healthcare professional based on clinical symptoms and other diagnostic tests.

Principle of the assay

The FREND™ cartridge utilizes micro-fluidics lateral flow technology where the analyte of interest in the sample forms immune complexes while moving through the fluidics pathway in the cartridge.

A specimen is added to sample dilution tube and mixed. A well-mixed sample of 35 µL is transferred to the sample inlet of a single use FREND™ COVID-19 total Ab cartridge. The cartridge is then placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. IgM and IgG if present in the sample will bind to SARS-CoV-2 Nucleocapsid fluorescent beads which then bind to the anti-IgM and anti-IgG in the test zones, respectively forming individual complexes. Fluorescent control beads are captured at the reference zone. The reaction and analysis time is approximately 3-4 minutes. The anti-coronavirus total antibody qualitative measurement is based on the ratio of fluorescence detected by the FREND™ System at the FREND™ Test and Reference zones. The magnitude of the fluorescent ratio is proportional to the presence and absence of total antibody in the sample.

The FREND™ System is a bench-top fluorescence reader containing a touchscreen user interface. The System has a slot that accepts the FREND™ COVID-19 total Ab test cartridge (which contains the reagents and sample) and is programmed to analyze the test when the sample has fully reacted with the on-board cartridge reagents. Results of the test are displayed on the screen and can be printed on an optional printer. The FREND™ System has been previously cleared for use with other FREND™ cartridges through the 510k process. Please see k162378 (FREND™ PSA Plus reagent cartridge), k162754 (FREND™ Vitamin D test system), k153577 (FREND™ Testosterone test system), k152422 (FREND™ Free T4 test system) and k131928 (FREND™ TSH test system).

Material provided

Q'ty	Contents	Catalogue number
20	Cartridges	FRCOA 020
20	Dilution tubes	
40	Disposable pipette tips	
01	Code chip	
01	Package insert	

Components required but not included with the test

The following materials are not provided with the reagent but are required to perform COVID-19 total antibody analysis using the FREND™ COVID-19 total Ab on the FREND™ System.

-FREND™ System including calibrated pipette,
-QC Cartridge and QC Code chip manufactured by NanoEntek.

Warning and Precautions

- For *in vitro* diagnostic use under the FDA Emergency Use Authorization
- For Prescription Use only
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by authorized laboratories.
- This test has been authorized only for the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The FREND™ COVID-19 total Ab cartridges are intended for *in vitro* diagnostic use only.
- The FREND™ COVID-19 total Ab cartridges are only to be used on the NanoEntek FREND™ System.
- The FREND™ COVID-19 total Ab cartridges and dilution tubes are disposable, single use devices. Do not reuse!
- Allow sealed cartridges to come to room temperature for 15-30 minutes prior to use.
- Cartridge and dilution tubes should not be frozen.
- The humidity in the laboratory must be 10-80% range when running tests.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen when transferring the sample to the dilution tube. Use another new pipette tip when transferring are diluted, sample to the cartridge.
- Avoid high humidity, direct sunlight or heat when storing cartridges and dilution tubes.
- Testing of contaminated samples may cause erroneous results.
- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package insert and User manual

● Keep the cartridge sealed in the pouch until ready for use.
● Use the cartridge immediately after opening the pouch.
● Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.
● Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the laboratory may be derived from human materials. Use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
● Wash hands thoroughly and often after handling reagent cartridges or samples.
● Do not ingest the silica gel packet found in the cartridge pouch.
● Used cartridges and diluent tubes should be disposed in accordance with local or national regulations.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at refrigerator temperature storage (2-8℃). Reagent stability has been demonstrated for nine months from the date of manufacture.
The expiration date is clearly indicated on the product box and the cartridges.

Specimen collection and handling

The assay can be performed using K_2EDTA plasma. No special patient preparation is necessary. Collect the appropriate venous blood sample in accordance with standard laboratory procedures. After collection, centrifuge the sample for 10 minutes at 3,000 rpm within 2 hours of collection and immediately separate the plasma from the packed cells.
It is recommended to use samples immediately. However, if testing is not done immediately, samples may be stored at 2-8℃ for up to 6 hours prior to testing. If testing is not performed within 6 hours, store at -20℃ or below. Samples can be stored frozen for up to 30 days prior to testing.

Repeated freeze-thaw cycles should be avoided. Turbid samples containing particulate matter such as fibrin clots or visible strands should be-centrifuged before being tested. Prior to assay, slowly bring frozen samples to room temperature and mix gently but thoroughly before testing.
For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter. When pipetting into the cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedure

● Reagent preparation
There is no reagent preparation required to run the FREND™ COVID-19 total Ab cartridge on the FREND™ System. However, the cartridge and dilution tube should be removed from the

refrigerator and incubated at room temperature for 15-30 minutes prior to testing.

● Code chip installation

A lot-specific Code chip is supplied with each kit of FREND™ COVID-19 total Ab. When using a new lot of reagent, the Code chip of the same lot must be installed in the FREND™ System. Please refer to the FREND™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions are as follows:

(1) Insert the FREND™ System electrical cord into an appropriate outlet.

(2) Insert the Code chip into the Code chip slot at the rear of the system following the arrows.

(3) Press the 'Setup' button on the 'Main' screen.

(4) Press the 'Code chip' button on the 'Setup' screen.

(5) The information embedded on the FREND™ COVID-19 total Ab Code chip is automatically saved on the FREND™ System.

(6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.

(7) Press the 'Item' button on the 'Setup' screen.

(8) Check the FREND™ COVID-19 total Ab cartridge lot number and the installation date of the Code chip.

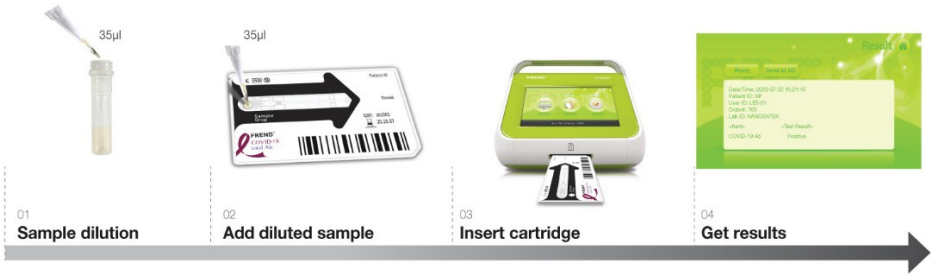
(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

● Specimen processing

Allow the tubes and the sealed pouches containing the FREND™ COVID-19 total Ab cartridges and dilution tubes to come to room temperature for 15-30 minutes prior to use.

If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing. Testing should not begin on frozen samples until they have reached room temperature.

● Assay procedure



Have the FREND™ COVID-19 total Ab cartridge and samples ready for testing.

(1) Record the Sample ID on the cartridge in the designated area.

(2) Using the micropipette, transfer 35 µL of the sample to a sample dilution tube and mix by inverting the sample gently for 3-5 times. Using the mixed sample and a new pipette tip, transfer 35 µL into the sample inlet.

(3) Press the 'Test' button on the 'Main' screen of the FREND™ System.

(4) The system moves to the Patient ID screen automatically.

(5) Type the Patient ID and press the 'Enter' button to begin the test.

(6) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.
Caution: Please check the direction of the cartridge before insertion and assure the insertion is complete. Caution: Please insert the cartridge into the FREND™ System after loading the sample into the cartridge.

(7) When the reaction in the cartridges is complete, the FREND™ System will automatically begin the reading.

(8) When the reading has been completed, the cartridge will automatically be expelled and the results displayed.

Caution: Do not remove power from the FREND™ System while a cartridge is in the reading chamber.
This may cause a system error.

(9) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.

(10) For more detailed instructions, please refer to the 'FREND™ System User manual'.

Control materials

● FREND™ System check

It is recommended for operators to use the QC Cartridge daily for the maintenance of the FREND™ System. Install QC Code chip in the FREND™ System before using the QC Cartridge. The QC Cartridge confirms the proper function of the FREND™ System including:
-Step 1 Laser power
-Step 2 Laser alignment
-Step 3 Calculate ratio
Please use the QC Cartridge and QC Code chip provided with the FREND™ System.

● Internal control

FREND™ COVID-19 total Ab cartridge contains built in control features. Fluorescence signal in the Reference zone of each cartridge shows: (1) that enough sample volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this Reference zone signal is missing or lower than the threshold, FREND™ System considers it as an incorrect or failed test, and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors, in part, for
(1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

● Positive and negative controls

Control materials are available for purchase from NanoEntek (product name: COVID-19 total Ab Control LQ, cat.# FIC-COALQ) and are not supplied with the kit. Refer to the COVID-19 total Ab Control LQ Package Insert for Instructions. Good laboratory practice suggests that positive and negative controls are run routinely to ensure that test reagents are working and that the test is correctly performed. External positive and negative controls should be used in accordance with local, state, federal accrediting organizations, or your laboratory's standard quality control procedures, as applicable. If controls fail, repeat the control testing procedure and if expected results are not obtained, do not proceed with clinical testing and contact NanoEntek service.

- Control Procedure

1. Take out the COVID-19 total Ab Control LQ from the refrigerator and incubate at room temperature for approximately 20 minutes.
2. Mix control materials gently and load 35µl to the cartridges.
   *Do not use the dilution tube when testing the control.
3. Check the result.

Control	total Ab	PASS/FAIL
Negative	Negative	PASS
	Positive	FAIL
Positive	Negative	FAIL
	Positive	PASS

Interpretation of results

Positive result
A positive result is an indication of the presence of antibodies to the SARS-CoV-2 virus.

Negative result
A negative test indicates that total antibody to the SARS-CoV-2 virus were not detected.

FREND System results display
The FREND™ System provides the qualitative detection of total antibody to the SARS-CoV-2 virus. Report options are indicted below: Note the following precautions:

-Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Testing with a molecular assay should be performed to evaluate for acute infection in symptomatic individuals.
-Results from antibody testing are not an indication of SARS-CoV-2 infection. Presence of SARS-CoV- 2 antibodies are an indication of an adaptive immune response to the virus.
-Positive results may indicate past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E. Positive results should be confirmed using a second serology test.
-Not for the screening of donated blood.

Please read the section 'Result interpretation' and 'Limitation of procedure' carefully before you use the FREND™ COVID-19 total Ab and the FREND™ System.

Displayed results	Description
	COVID-19 Ab "Positive"
	COVID-19 Ab "Negative"

Limitation of the procedure

- For use under an Emergency Use Authorization Only
- This test is only to be used in CLIA certified laboratories that meet requirements to perform moderate or high complexity tests and not in point-of-care or at-home testing settings.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- This test must be read using only the FREND™System and cannot be read visually.
- Immunocompromised patients who have COVID-19 may have a delayed antibody response or produce levels of antibody which may not be detected as positive by the assay.
- The assay procedure and results interpretation must be followed closely when testing for the presence of SARS-CoV-2 virus specific antibodies. For optimal test performance, proper sample collection is critical. Failure to follow the procedure may give inaccurate results.
- The results obtained with this test should only be interpreted in conjunction with clinical findings,

and the results from other laboratory tests and evaluations.

- Heterophilic antibodies in serum specimens may cause interference in the immunoassay. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing.
- This test should not be used to diagnose or exclude acute infection. Testing with a molecular assay should be performed to evaluate for acute infection in symptomatic individuals.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole

basis for patient management decisions.
- A negative or non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody detected by the test.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second, alternative serology test to confirm an immune response.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
- This test should not be used for screening of donated blood.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The FREND™ COVID-19 total Ab Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

However, to assist clinical laboratories using the FREND COVID-19 total Ab, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[a] using the FREND™ COVID-19 total Ab will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will use the FREND™ COVID-19 total Ab as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instrument, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the product are not permitted.
- Authorized laboratories that receive the FREND™ COVID-19 total Ab will notify the relevant public health authorities of their intent to run the assay prior to initiating testing.
- Authorized laboratories using the FREND™ COVID-19 total Ab will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will collect information on the performance of the FREND™ COVID-19 total Ab and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to you (ivdst@nanoentek.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the assay of which they become aware.
- All laboratory personnel using the FREND™ COVID-19 total Ab must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the FREND™ COVID-19 total Ab in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the the FREND™ COVID-19 total Ab.
- NanoEntek America Inc., authorized distributors, and authorized laboratories using the FREND™ COVID-19 total Ab will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[a]The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests" as "authorized laboratories".

Performance evaluation

Analytical Performance

Cross-reactivity

Cross-reactivity testing was performed on samples collected from the United Stated of America. Blood samples (EDTA plasma) from subjects with high rates of vaccination were purchased from Gulf Coast Regional Blood Center 1400 La Concha Lane Houston, Texas 77054 on August 20, 2019 before the outbreak of COVID-19.

Method		Samples Collected Prior to COVID-19 Outbreak
FREND™ COVID-19 total Ab	Positive	0
	Negative	120
Total		120 (100%)

Interference

The interference evaluation test of FREND™ COVID-19 total Ab was conducted according to CLSI Guidelines EP7-A2 using one lot.

No interference in the testing of the FREND™ COVID-19 total Ab with 4 interfering substances was observed at the stated concentrations.

Endogenous substances	Concentration tested
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3 g/dL

Total protein	12 g/dL

Clinical Performance

Clinical agreement study

Ninety-one (91) clinical samples (K_2EDTA plasma) were collected at Kangwon National University Hospital, Department of diagnostic examination, 156 Baeknyeong-ro, Chuncheon-si, Gangwon 24289, Korea from April 7th to April 10th 2020. Patients were confirmed for SARS-CoV-2 by rtPCR with the Allplex™ 2019-nCoV Assay (Seegene, Inc.). The results were as follows.

Time Stratification of Data for Korean Samples

		75 Positive Confirmed by RT-PCR			
Days Between		0~7 days	8~14 days	>15 days	UNK
FREND COVID-19 total Ab	Positive	34	21	17	0
	Negative	3	0	0	0
Total		37	21	17	0
PPA		(34/37) 91.89%	(21/21) 100%	(17/17) 100%	(0/0) 0%
95% CI		78.70%-97.20%	84.54%-100%	81.57%-100%	N/A

Negative Percent Agreement was 15/16, 95% CI (71.67% – 98.89%)

An additional 56 clinical samples (44 SARS-CoV-2 PCR* confirmed and 12 negative, pre-COVID-19), purchased from a commercial vendor (MRN Diagnostics, LLC) in the United States were tested at an independent clinical laboratory with the FREND COVID-19 total Ab. The results are presented in the table below.

*Note: samples were obtained from several clinical sources using different EUA PCR methods.

Time Stratification of Data for US Samples and Tested by Independent Lab

		44 Positive Confirmed by RT-PCR			
Days Between		0~7 days	8~14 days	>15 days	UNK
FREND COVID-19 total Ab	Positive	0	2	33	9
	Negative	0	0	0	0
Total		0	2	33	9
PPA		(0/0) 0%	(2/2) 100%	(33/33) 100%	(9/9) 100%
95% CI		N/A	34.24%-100%	89.57%-100%	70.09%-100%

Negative Percent Agreement: 100.0% (12/12), 95%CI (75.75%-100%)

An additional 200 clinical samples (100 SARS-CoV-2 PCR confirmed positive and 100 negative, pre-COVID- 19), purchased from MRN Diagnostic, LLC in the United States were tested at the NanoEntek laboratory with the FREND™ COVID-19 total Ab. The results are presented in the table below.

Time Stratification of Data for US Samples and Tested by NanoEntek

Days Between		100 Positive Confirmed by RT-PCR			
		0~7 days	8~14 days	>15 days	UNK
FREND COVID-19 total Ab	Positive	0	2	98	0
	Negative	0	0	0	0
Total		0	2	98	0
PPA		(0/0) 0%	(2/2) 100%	(98/98) 100%	(0/0) 0%
95% CI		N/A	34.24%-100%	96.23%-100%	N/A

Negative Percent Agreement: 100.0% (100/100), 95%CI (96.30%-100%)

For the combined US and Korean testing, there were 347 samples: 219 PCR confirmed positive SARS- CoV-2 and 128 negative/pre-COVID-19. The results of the testing are presented in the table below:

Days Between		219 Positive Confirmed by RT-PCR			
		0~7 days	8~14 days	>15 days	UNK
FREND COVID-19 total Ab	Positive	34	25	148	9
	Negative	3	0	0	0
Total		37	25	148	9
PPA		(34/37) 91.89%	(25/25) 100%	(148/148) 100%	(9/9) 100%
95% CI		78.70%-97.20%	86.68%-100%	97.47%-100%	70.09%-100%

Overall Negative Percent Agreement: 99.2% (127/128); 95% CI (95.71%-99.86%)

Independent Clinical Agreement Validation:

The FREND COVID-19 total Ab from NanoEntek was tested on August 19, 2020 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the FREND COVID-19 total Ab. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers. All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the FREND COVID-19 total Ab. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown in the Tables below.

	Comparator Method Antibody Positive			Collected pre-2020 Antibody Negative		
FREND COVID-19 total Ab	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
(IgM / IgG) +	29			1		30
(IgM / IgG) -	1			69	10	80
Total	30			70	10	110

Measure	Estimate	Confidence Interval
(IgM / IgG) Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
(IgM / IgG) Specificity	98.8% (79/80)	(93.3%; 99.8%)
Combined Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
Combined Specificity	98.8% (79/80)	(93.3%; 99.8%)
Combined PPV for prevalence = 5.0%	80.3%	(39.4%; 96.0%)
Combined NPV for prevalence = 5.0%	99.8%	(99.1%; 100%)
Cross-reactivity with HIV+	0.0% (0/10), not detected	

Limitations of the Study:

Sensitivity and specificity estimates in this report may not be indicative of the real world performance of the NanoEntek Inc FREND™ COVID-19 total Ab test.

These results are based on serum and plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.

The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

References

1. Coronavirus Disease 2019 (COVID-19) Frequently Asked Questions, FDA
2. Q&A on coronaviruses (COVID-19), WHO
3. SARS-CoV-2: virus dynamics and host response, Yu Chen, Lanjuan Li, Lancet, March 23, 2020, https://doi.org/10.1016/S1473-3099(20)30235-8
4. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, Kelvin Kai-Wang To, *et al.*, The Lancet Infectious Diseases, March 23, 2020, https://doi.org/10.1016/S1473- 3099(20)30196-1

Glossary of symbols

	Caution, warning, Consult accompanying documents		*In vitro* diagnostic medical device
	Catalogue number / Reference number		Temperature limitation
	Lot number / Batch number		Contains sufficient for <n> tests
	Use by YYYY-MM-DD or YYYY-MM		Do not reuse
	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		For prescription use
	CE marking		Irritant

ivdst@nanoentek.com
www.nanoentek.com

Manufactured by
NanoEntek, Inc.
851-14 Seohae-ro, Paltan-myeon, Hwaseong-si, Gyeonggi-do, 18531, Korea
Tel.:+82-2-6220-7942, Fax.:+82-2-6220-7999

NanoEntek America, Inc.
220 Bear Hill Road, Suite 102, Waltham, MA 02451, USA
Tel.:+1-781-472-2558, Fax.:+1-781-790-5649

EC REP MT Promedt Consulting
GmbH Altenhofstrasse 80, 66386 St.
Ingbert, Germany

Revised on 2021.12.13