COVID-19 NAb

Rapid COVID-19 Neutralizing Antibody (NAb) "Quantitative" Test

A Rapid Immunochromatographic Test for the Quantitative Detection of Neutralizing Antibodies (NAb) Titer post COVID-19 Vaccination or after SARS-CoV-2 Infection in Human Finger-prick Blood, Serum or Plasma

CE REF 1170SQ-25

For Professional In Vitro Diagnostic use only ***Read Instructions before use***

INTENDED USE

TestNOW® - COVID-19 NAb Test Device is Rapid immunochromatography *in vitro* test for the Quantitative detection of circulating Neutralizing Antibodies (NAb) Titer against SARS-CoV-2 that block the interaction between the Receptor Binding Domain (RBD) of the viral Spike Protein (S) with the Angiotensin Converting Enzyme-2 (ACE2) cell surface receptor. **TestNOW® - COVID-19 NAb** Test detects Neutralizing Antibodies (NAb) in whole blood, serum and plasma that neutralize the RBD-ACE2 interaction after SARS-CoV-2 infection or post COVID-19 vaccination. **TestNOW® - COVID-19 NAb** is a Point-Of-Care finger-prick whole blood Quantitative test for checking the efficacy of COVID-19 vaccines and monitoring the levels of protective, neutralizing antibodies Titer longitudinally to help determine duration of immunity.

SUMMARY

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, or 2019-nCoV) is an enveloped non-segmented positive-sense RNA virus. It is the cause of coronavirus disease 2019 (COVID-19), which is contagious in humans. SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a Receptor Binding Domain (RBD), which is responsible for recognizing the cell surface receptor, Angiotensin Converting Enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication. Infection with the SARS-CoV-2 or post COVID-19 vaccination initiates an immune response, which includes the production of antibodies in the blood. The secreted antibodies provide protection against future infections from viruses, because they remain in the circulatory system for months to years and will bind quickly and strongly to the pathogen to block cellular infiltration and replication. These sub-group of IgG antibodies are named Neutralizing Antibodies (NAb). Clinical study has shown that Serum IgG against RBD best correlates with virus-neutralizing activity and disease severity[1].

PRINCIPLE

TestNOW® - COVID-19 NAb Test Device utilizes the principle of "Sandwich" Immunochromatography. Mouse anti-human IgG antibodies are immobilized on the nitrocellulose membrane as Test line (T) in the test window of the device. As the test sample flows through the membrane within the test device, the colored Receptor Binding Domain (RBD)-colloidal gold conjugate forms complex with specific Neutralizing Antibodies (NAb) against SARS-CoV-2, if present in the sample. This complex moves further on the membrane to the test line region where it is captured by the anti-human IgG antibodies coated on the membrane, leading to formation of a colored band, which indicates a positive test result. The intensity of colored band in the test line region is Neutralizing Antibody (NAb) concentration-dependent, higher the concentration of Neutralizing Antibody (NAb) in the tested sample, the stronger the colored band is. Absence of this colored band in the test window indicates a negative test result. A built-in control line (C) will always appear in the test window when the test has performed properly, regardless of the presence or absence of Neutralizing Antibodies (NAb) against SARS-CoV-2 in the specimen.

MATERIALS PROVIDED

1. **TestNOW® - COVID-19 NAb** Test device (Kit Size: 25 Tests/Box)
2. Sample Buffer (One Bottle of 6.5 ml)
3. **UniSampler™** Device (26 Collection Tubes + 26 Blood Collectors)
4. RFID Card - 1
5. Instructions for use – 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer or clock
2. Safety Lancet
3. Alcohol Swab
4. Disposable gloves and disinfectant
5. Biohazard waste container
6. Micropipette for Serum or Plasma testing
7. ***RapiRead™*** CUBE Reader (CE Marked) – To be purchased separately

STORAGE

1. Store the test device at 4° to 30°C in the original sealed pouch.
2. The expiration date indicated on the pouch is based on the storage conditions.
3. The test device should remain in its original sealed pouch until ready for use. After opening, the test device should be used immediately. Do not reuse the device.

SPECIMEN COLLECTION AND PREPARATION

1. The whole blood, serum or plasma specimen should be collected under standard laboratory conditions.
2. Heat inactivation of specimens, which may cause hemolysis and protein denaturation, should be avoided.
3. The test works best on fresh whole blood / serum / plasma samples. If testing cannot be performed immediately, serum / plasma may be stored at 2-8ºC up to 3 days in case of delay in testing. For long-term storage, serum / plasma specimens can be frozen at -20ºC for 3 months or -70ºC for longer period. Repeated freezing and thawing of the specimen should be avoided.
4. Sodium azide can be added as a preservative up to 0.1% without affecting the test results.

QUALITY CONTROL

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.

PROCEDURE

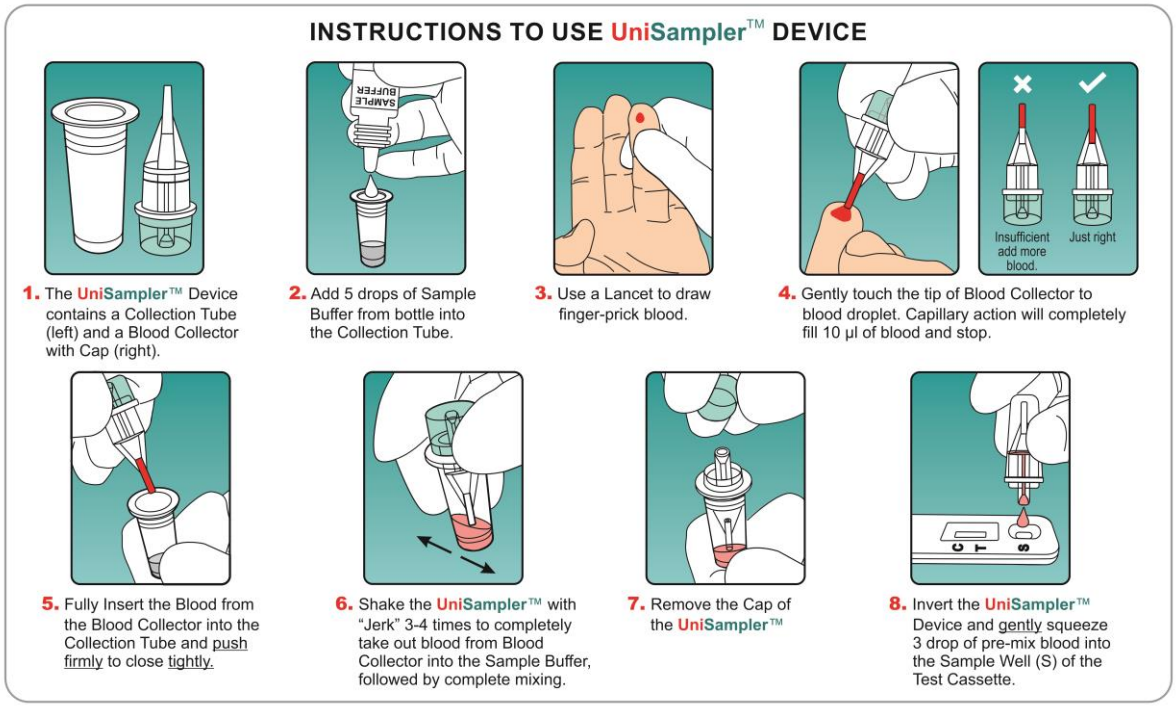
1. Bring the kit components to room temperature before testing.
2. Open the pouch and remove the Test Device. Once opened, the test device must be used immediately.
3. Label the test device with sample identification (ID).
4. Wash your hand thoroughly and dry completely.
5. Rub and Wipe your ring or middle finger of non-dominant hand.

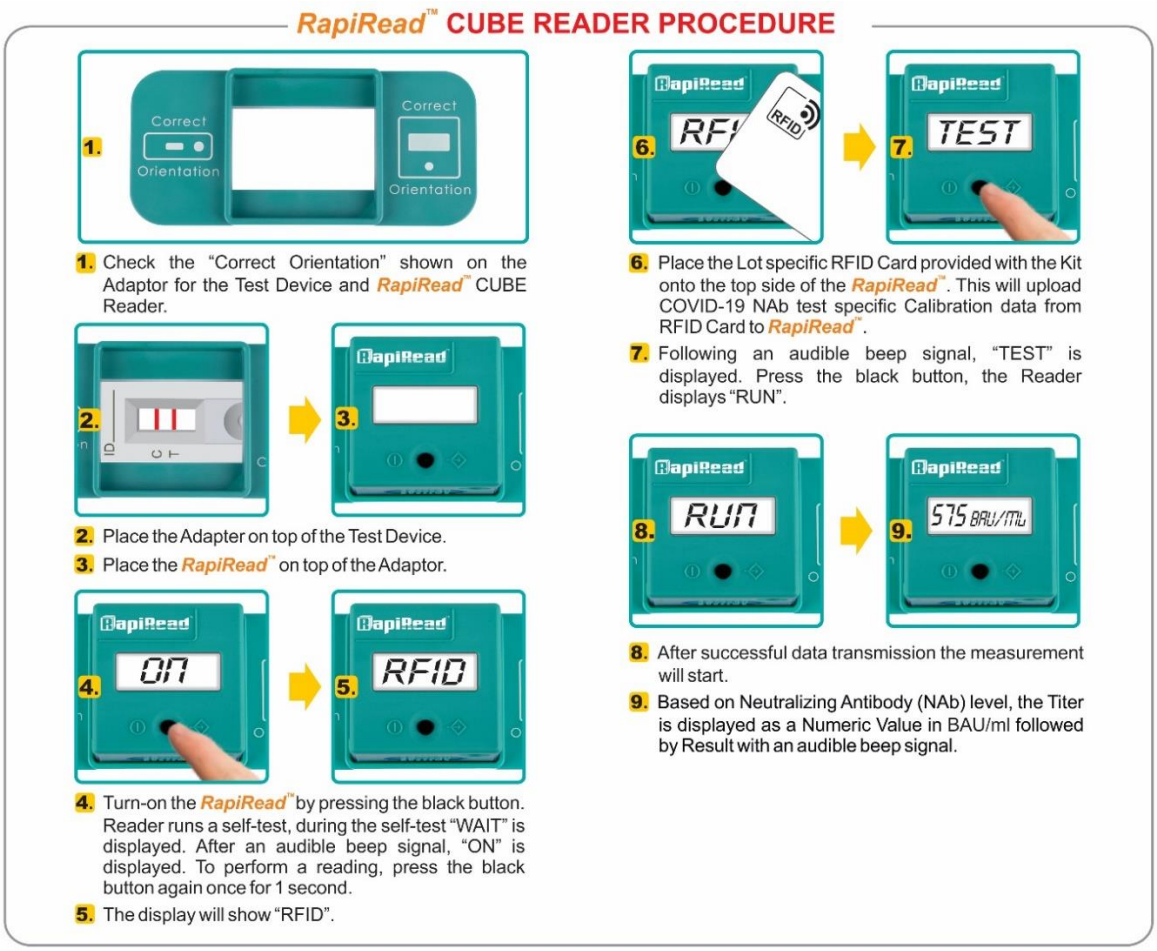
6. Using safety lancet puncture the side of your finger.
7. Collect 10 µl blood using Blood Collector (See instructions below) and perform testing immediately.
8. After applying 3 drops of pre-mix blood into the sample well (S), read and record the results at 15 Minutes by ***RapiRead™*** CUBE Reader. For Timer Protocol, please consult ***RapiRead™*** CUBE Reader Product Manual or Brochure.

Important Note: ***Result after 15 minutes may not be accurate.***

SERUM / PLASMA PROTOCOL

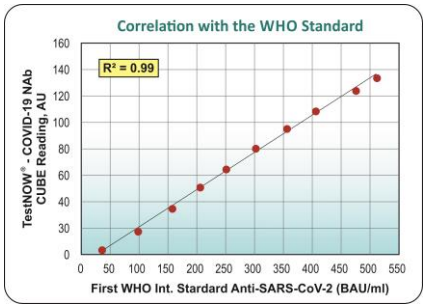
TestNOW® - COVID-19 NAb has been designed for human finger-prick blood. However, Serum or plasma samples can be used for testing. Instead of taking finger prick blood with blood collector, apply 5µl of Serum or Plasma into the Collection Tube using Micropipette and follow "Instructions to Use **UniSampler™** Device".





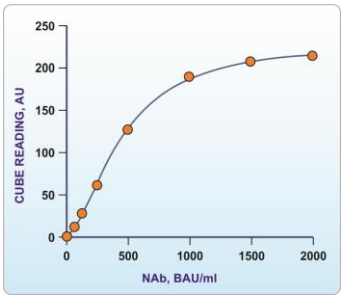
CORRELATION WITH THE WHO STANDARD

TestNOW® - COVID-19 NAb Test has been standardized against "First WHO International Standard for anti-SARS-CoV-2 Immunoglobulins" (NIBSC Code: 20/136) [2, 3, 4] with excellent Correlation Coefficient (R^2 = 0.99).



STANDARD CURVE

A typical standard curve is illustrated on right side. The CUBE Reading AU is automatically converted into Binding Antibody Units (BAU)/ml in ***RapiRead***™ Reader.



INTERPRETATION OF RESULTS

The ***RapiRead***™ CUBE analyzer automatically determines the final result by comparing the AU for each sample against a pre-established calibration curve. Neutralizing Antibody Titer Results are expressed in BAU/ml (concentration of total SARS-CoV-2 Neutralizing Antibodies in BAU/ml Unit). Results obtained with the **TestNOW® - COVID-19 NAb** Test are interpreted as follows:

Neutralizing Antibody Titer (Numeric Result in BAU/ml)	Result Message	Result Interpretation	Degree of Immunity
< 50 BAU/ml	NEGATIVE	Negative for SARS-CoV-2 Neutralizing Antibodies	NO IMMUNITY
≥ 50 – < 250 BAU/ml	LOW +	Positive for SARS-CoV-2 Neutralizing Antibodies. Low Titer	LOW IMMUNITY
≥ 250 – < 500 BAU/ml	MEDIUM +	Positive for SARS-CoV-2 Neutralizing Antibodies. Medium Titer	MEDIUM IMMUNITY
≥ 500 BAU/ml	HIGH +	Positive for SARS-CoV-2 Neutralizing Antibodies. High Titer	HIGH IMMUNITY

PERFORMANCE CHARACTERISTICS

The clinical study comparing with RT-PCR and Abbott Architect SARS-CoV-2 IgG Positive and Negative convalescent plasma samples was conducted:

		Reference Neutralizing Antibody (NAb) ELISA Positive	Reference Neutralizing Antibody (NAb) ELISA Negative
	Number of Samples	120	75
TestNOW® - COVID-19 NAb Test	Positive	119	1
	Negative	1	74

Clinical Sensitivity = 119 / 120 = 99.17% (CI 95%: 95.44% - 99.98%)
Positive Predictive Value (PPV) = 119 / (120 +1) = 98.35%

Clinical Specificity = 74 / 75 = 98.67% (CI 95%: 92.79% - 99.97%)
Negative Predictive Value (NPV) = 74 / (74 + 1) = 98.67%

Accuracy:
Total Confidence Rate: (119+74) / (119+74+1+1) *100 = 98.97%

POST COVID-19 VACCINE NAb TITER

16 persons who either received Pfizer or Modorna COVID-19 vaccine were tested with **TestNOW® - COVID-19 NAb** Test, 2 Weeks Post 1st and 2nd shots, and their longitudinal Neutralizing Antibodies (NAb) Titers are show below:

Person No.	Sample	COVID-19 Vaccine Type	NAb Titer (BAU/ml)			
			2 Weeks Post 1st shot	Result	2 Weeks Post 2nd shot	Result
1	Finger-prick Blood	Pfizer	449.1	MEDIUM +	1242.7	HIGH +
2	Finger-prick Blood	Pfizer	380.2	MEDIUM +	1265.6	HIGH +
3	Finger-prick Blood	Pfizer	68.9	LOW +	732.9	HIGH +
4	Finger-prick Blood	Pfizer	83.6	LOW +	750.0	HIGH +
5	Finger-prick Blood	Pfizer	68.4	LOW +	708.5	HIGH +
6	Finger-prick Blood	Pfizer	225.1	LOW +	1086.6	HIGH +
7	Finger-prick Blood	Pfizer	347.4	MEDIUM +	1395.2	HIGH +
8	Finger-prick Blood	Pfizer	95.8	LOW +	767.6	HIGH +
9	Finger-prick Blood	Pfizer	189.9	LOW +	1199.5	HIGH +
10	Finger-prick Blood	Pfizer	190.2	LOW +	1022.5	HIGH +
11	Finger-prick Blood	Pfizer	90.9	LOW +	1179.0	HIGH +
12	Finger-prick Blood	Pfizer	58.3	LOW +	795.5	HIGH +
13	Finger-prick Blood	Pfizer	100.3	LOW +	939.1	HIGH +
14	Finger-prick Blood	Moderna	253.0	MEDIUM +	1159.2	HIGH +
15	Finger-prick Blood	Moderna	387.0	MEDIUM +	1069.8	HIGH +
16	Finger-prick Blood	Moderna	284.5	MEDIUM +	758.7	HIGH +
			Mean = 204.5	**62.5% LOW + 37.5% MEDIUM +**	**Mean = 1004.5**	**100% HIGH +**

Study on Autologous Serum, Plasma, and Whole Blood Samples:
TestNOW® - COVID-19 NAb Test Device was also tested with 10 Negative convalescent serum and autologous plasma and whole blood samples, and 10 Positive convalescent serum and autologous plasma and whole blood samples. Consistent test results were obtained for all samples confirming that serum, plasma, and whole blood can all be used as testing samples for the **TestNOW® - COVID-19 NAb** Test Device.

PRECISION
Precision study was conducted by testing 30 each of Negative, Low, Medium and High positive plasma samples by 3 lots and duplicated by three technicians within a day. The results showed 100% agreement performance with no human error.

LIMIT OF DETECTION (LOD) & UPPER LIMIT OF QUANTIFICATION (ULOQ) – TEST LOT DEPENDENT
The **LOD** and **ULOQ** for **TestNOW® - COVID-19 NAb** Test against the "First WHO International Standard for anti-SARS-CoV-2 Immunoglobulins" (NIBSC Code: 20/136) are **25 – 50 BAU/ml** and **2,500 – 3,000 BAU/ml** respectively.

ASSAY SPECIFICITY

1. Other infectious diseases
TestNOW® - COVID-19 NAb Test Device has tested samples that were infected by the following diseases: Influenza A Virus, Influenza B Virus, Adenovirus, Rotavirus and Mycoplasma Pneumonia. All the samples showed no effect on the assay.

2. Blood compounds
TestNOW® - COVID-19 NAb Test Device has tested samples with high Rheumatoid Factor (RF), Bilirubin, Triglyceride and Hemoglobin. The results showed that these compounds had no effect on the specificity of the assay up to the listed concentration.

Rheumatoid Factor: 80 IU/ml	Bilirubin: 342 µmol/L	Triglyceride: 37 mmol/L	Hemoglobin: 10 mg/ml

3. Interference Studies - Common drugs
The following substances with the listed concentrations were added to negative, low, medium and high positive controls to reach the defined concentration. Each compound in each level of controls was tested in triplicates using one lot of **TestNOW® - COVID-19 NAb** Test Device.

Histamine Hydrochloride:	1.5 mg/mL	Interferon-α	150 µg/mL	Zanamivir	426 ng/mL
Ribavirin	6 mg/L	Oseltamivir	46.9 mg/L	Peramivir	132.7 µg/mL
Lopinavir	3.2 mg/mL	Ritonavir	159 µg/mL	Arbidol	2.0 µg/mL
Levofloxacin	9.2 mg/L	Azithromycin	9.2 mg/L	Ceftriaxone	240 mg/L
Meropenem	200 mg/mL	Tobramycin	12 mg/L		

The results showed that these drugs have no interference effect on the specificity of the assay.

PRECAUTIONS
1. For **Professional *In Vitro*** diagnostic use only.
2. Do not use the product beyond the expiration date.
3. Do not use the product if the pouch is damaged or the seal is broken.
4. Handle all specimens as potentially infectious.
5. Follow standard laboratory procedure and biosafety guidelines for the handling and disposal of potentially infectious material. When the assay procedure is completed, dispose specimens after autoclaving at 121º C for at least 20 min or treating with 0.5% Sodium Hypochlorite for 1-2 hours.
6. **TestNOW® - COVID-19 NAb** Test must be quantified with ***RapiRead™*** CUBE Reader only.
7. RFID Card is Lot Specific and cannot interchanged with another Lot.

LIMITATIONS
1. Although the test is very accurate in detecting Neutralizing antibody Titer, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but should only be made by the physician after all clinical and laboratory findings have been evaluated.

REFERENCES

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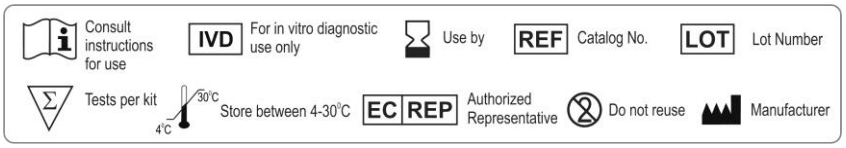
SHELF LIFE: 18 Months

2021-09-ver-5
(Lot # 21071470)

INDEX OF CE SYMBOLS



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