Nano-Check™ COVID-19 IgG/IgM Antibody Test

For prescription use only. For *in vitro* diagnostic use.

1. INTENDED USE

Nano-Check™ COVID-19 IgG/IgM Antibody Test is a rapid immunoassay for the qualitative detection of human IgG/IgM antibodies to SARS-CoV-2 in human whole blood (venipuncture), plasma (Heparin/EDTA) and serum sample collected in CLIA certified laboratory or by healthcare workers at the point of care. The Nano-Check™ COVID-19 IgG/IgM Antibody Test 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 Nano-Check™ COVID-19 IgG/IgM Antibody Test 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, to perform moderate and high complexity tests.

2. SUMMARY AND EXPLANATION OF THE TEST

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) not previously seen in humans. The SARS-CoV-2 virus causes respiratory illness (like the flu) with symptoms such as cough, fever, fatigue, and in more severe cases, difficulty breathing or shortness of breath. The COVID-19 disease was first identified in 2019 in Wuhan, China, and has since spread rapidly around the world, resulting in the ongoing 2019–2020 novel coronavirus pandemic.
Test results of Nano-Check™ COVID-19 IgG/IgM Antibody Test should not be used as the sole basis to diagnosis. The test results should be interpreted by the physician along with other test results and patient's clinical symptoms. IgM antibodies against SARS-CoV-2 are generally found in human blood several days after viral infection, even though the rate and kinetics of serological response have not been clearly defined. IgG antibodies against SARS-CoV-2 appear later and gradually replace the IgM antibody. The sensitivity of antibody detection is directly related to the time after infection when blood samples are collected.

3. PRINCIPLE

The Nano-Check™ COVID-19 IgG/IgM Antibody Test is an immune chromatography assay for the qualitative detection of human IgG/IgM antibodies against SARS-CoV-2 using human blood, plasma and serum sample. The membrane strip contains two test lines and one control line; antibody against human IgM and antibody against human IgG for test lines, goat anti-chicken IgY antibody for the control line. A dye pad is placed at the end of the membrane containing gold colloidal particle coupled with recombinant SARS-CoV-2 antigen and gold colloidal particle coupled with chicken IgY.
When a sample is applied into the sample well, the IgM antibodies against SARS-CoV-2 present in the sample bind to the specific SARS-CoV-2 antigen coupled with gold particles. The immune complexes move along the nitrocellulose membrane through the test lines and bind to anti-human IgM immobilized on the test line. Unbound immune complexes pass through the test line and chicken IgY coupled with gold particles are captured by anti-chicken IgY in the control line. The IgM test line appears pinkish red color, indicating positive result for the SARS-CoV-2 IgM antibody. In the same way, the IgG antibodies against SARS-CoV-2 present in the sample bind to the specific SARS-CoV-2 antigen coupled with gold particles. The immune complexes move along the nitrocellulose membrane through the test lines and bind to anti-human IgG immobilized on the test line. Unbound immune complexes pass through the test line. The IgG test line appears pinkish red color, indicating positive result for the SARS-CoV-2 IgG antibody. The absence of colored band at two test lines indicates a negative result.
The control line (Con) is present in the test window for self- procedure validation control. This colored control band always appears at the control line position (Con) in valid test result. Any test result is not valid unless the control line appears in the test window.

4. REAGEANTS and MATERIALS

Provided

- 20 Test devices in sealed aluminum foil pouch with desiccant
- 20 Disposable transfer pipettes, 10 μL
- 1 Bottle of developer solution
- 1 Instructions for Use

Required but not provided

- Whole blood, Serum, or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Micropipette and tip, 5 μL

5. STORAGE AND STABILITY

The test kit should be stored at 2°C - 30°C in the original sealed pouch for the duration of shelf life.

6. PRECAUTIONS

- For *in-vitro* diagnostic and professional use only.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer device for each clinical sample tested.
- Do not use test kit if the pouch is damaged or improperly sealed.
- Do not use test kit beyond expiration date.

7. SPECIMEN COLLECTION AND PREPARATION

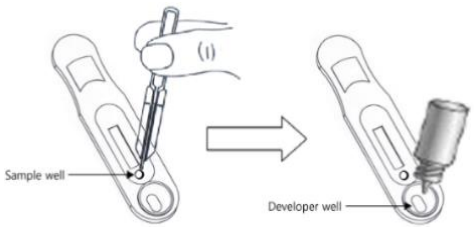
- This test can be used for whole blood, serum, and plasma samples. If serum samples are to be used, collect the blood in a tube without anticoagulant and allow clotting for at least 30 minutes before centrifugation. Whole blood or plasma samples using heparin or EDTA as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variation in these products may exist between manufacturers and, at times, from lot-to-lot.
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection. Plasma or serum samples may be

refrigerated for 24 hours at 2-8ºC. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20ºC or colder.

- Refrigerated or frozen serum or plasma specimen should reach room temperature and be homogeneous prior to testing

8. TEST PROCEDURE AND PROTOCOL

1. Collect specimen according to instructions in "Specimen Collection and Preparation".
2. Test device and sample should be brought to room temperature (20°C-30°C) prior to testing.
3. Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.
4. When using transfer pipette for 10 µL of whole blood or using micropipette for 5 µL of plasma or serum sample, allow the tip of pipette to touch lightly on the pad underneath the sample well and dispense the contents.
5. Apply two drops of developer solution (50~70 µL) to the developer well by squeezing the bottle.
6. Read the results at 10 minutes visually. Do not read result after 15 minutes after adding developer solution.



Specimen addition Developer dropping

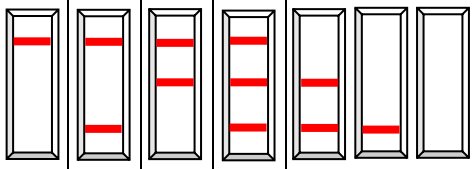
9. INTERPRETATION OF RESULTS

1. *Negative*: A single pinkish red colored band at the control line with the absence of other bands at test lines is a negative result for IgG/IgM antibodies against SARS-CoV-2 virus. Negative result does not indicate the absolute absence of IgG/IgM antibodies against SARS-CoV-2 virus in specimen. Instead, it only indicates that the specimen contains the antibody titer at below the detectable level.

2. *Positive:*

   - IgM only positive: Appearance of pinkish red colored bands at the control line and the IgM test line indicate that the SARS-CoV-2 IgM antibody was detected.
   - IgG only positive: Appearance of pinkish red colored bands at the control line and the IgG test line indicate that the SARS-CoV-2 IgG antibody was detected.
   - IgM and IgG positive: Appearance of pinkish red colored bands at the control line, the IgG test line and the IgM test line indicate that the SARS-CoV-2 IgG and IgM antibodies are detected.

3. *Invalid:* If no colored line appears in the control region (Con), the test result is invalid. The test result is inconclusive, and the assay should be repeated with a new test device.

	VALID				INVALID
Con IgG IgM					
IgG	–	–	+	+	Any result without control line
IgM	–	+	–	+	

10. LIMITATIONS

- The test is for professional and *in-vitro* diagnostic use only.
- This test has not been reviewed by the FDA.
- Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- 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.
- Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
- IgM antibodies may not be detected in the first few days of infection.
- As with all diagnostic tests, a definite clinical diagnosis should not be made based on the results of a single test. The test result should be used in conjunction with other clinical information such as clinical signs and symptoms and other test results to diagnose SARS-CoV-2 infection. Confirmation of test results should only be made by a physician along with clinical symptoms and laboratory findings.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Do not use finger stick sample.
- Not for the screening of donated blood.

11. QUALITY CONTROL

The presence of a pinkish red colored band in the Control area of the window acts as an internal control to ensure that an adequate volume of sample has been added. In the absence of this Control band, the test is invalid and must be repeated. Good laboratory practice recommends the use of control materials to ensure proper kit performance. When quality control specimens are available from commercial sources, controls should be assayed using the same procedures followed when running patient samples. Controls should minimally be run before using each new lot or shipment of Nano-Check™ COVID-19 IgG/IgM Antibody Test, at regular intervals afterwards and any time the validity of the test results are questioned. All users should follow local, state and federal regulations regarding quality control procedures.

12. PERFORMANCE CHARACTERISTICS

1. *Clinical Performance*

1.1 Positive clinical specimens: Plasma/Serum

Fifty-five (55) positive serum or plasma samples collected from patients tested positive for SARS-CoV-2 by RT-PCR method for

SARS-CoV2 infection were tested on Nano-Check™ COVID-19 IgG/IgM Antibody Test. In addition, 100 negative serum or plasma samples collected prior to October 2019 were tested with the Nano-Check™ COVID-19 IgG/IgM Antibody Test.

Fifty-two (52) out of the 55 positive samples were positive with IgG or IgM or both, and three (3) were negative, and ninety-eight (98) out of the 100 negative samples were negative. Nano-Check™ COVID-19 IgG/IgM Antibody Test had Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of 94.55% (95% CI: 85.15-98.13%) and 98.00% (95% CI: 93.00-99.45%), respectively. The Overall Percent Agreement (OPA) was 96.77 % (95% CI: 92.67-98.61%).

Nano-Check™ COVID-19 IgG/IgM Antibody Test		Comparator/RT-PCR		Total
		Positive	Negative	
Positive	IgM+/IgG+	35	0	35
	IgM+/IgG-	1	2	3
	IgM-/IgG+	16	0	16
Negative	IgM-/IgG-	3	98	101
Total		55	100	155

Positive Percent Agreement (PPA)= 52/55 (94.55%), 95% CI: 85.15% to 98.13%
Negative Percent Agreement (NPA)= 98/100 (98.00%), 95% CI: 93.00% to 99.45%

Based on the positive agreement study above, PPA results by days post onset of symptom were further assessed.

Days*	Number of Sample	Number of Positive ** (PPA, %)	95% CI
0-10	5	3 (60)	23.1-88.2
11-14	18	18 (100)	82.4-100
15-21	31	30 (96.8)	83.8-99.4
>21	1	1 (100)	20.7-100
Total	55	52 (94.5)	92.7-98.6

* Days from Symptom Onset to Blood Collection
** Number of positive of Nano-Check™ COVID-19 IgG/IgM Antibody Test

1.2 Positive clinical specimens: Whole Blood/Plasma/Serum

Matrix comparison study for the Nano-Check™ COVID-19 IgG/IgM Antibody Test was performed using plasma, serum and whole blood. The spiked sample panels were prepared by spiking with COVID-19 positive 5 samples comprising different antibody property into each of 5 whole blood samples, which was treated with Sodium heparin, K2 EDTA, and a plain tube. The spiked sample panels were then serially diluted up to an end-point and run on the Nano-Check™ COVID-19 IgG/IgM Antibody Test. The data demonstrated that there were 98.8% agreement in sample type equivalency test using spiked sample panel. Therefore, Nano-Check™ COVID-19 IgG/IgM Antibody Test performance is equivalent to sample types: heparinized whole blood, heparinized plasma, EDTA treated whole blood, EDTA treated plasma, and serum.

2. *Assay Cross Reactivity*

Cross-reactivity of the Nano-Check™ COVID-19 IgG/IgM Antibody Test was evaluated using human serum or plasma samples which contain antibodies to the pathogens listed below. All anti-serum samples were negative, and no false positive result was found with following:

Cross reactant	Number of samples	Positive	Negative
Influenza A virus IgG	6	0	6
Influenza A virus IgM	3	0	3
Influenza B virus IgG	6	0	6
Influenza B virus IgM	3	0	3
Epstein-Barr virus	5	0	5
Cytomegalovirus	5	0	5
Hepatitis A virus IgG	4	0	4
Hepatitis A virus IgM	1	0	1
Hepatitis B virus IgM	1	0	1
Hepatitis C virus	3	0	3
HIV 1/2	3	0	3
Mononucleosis	3	0	3
HSV-2	1	0	1
Mycoplasma	1	0	1
Rubella IgG	5	0	5

3. *Interference & specificity test*

Potentially interfering substances were spiked into SARS-CoV-2 antibody positive or negative samples. The substances at the following level do not interfere with the performance of the Nano-Check™ COVID-19 IgG/IgM Antibody Test. No false positive or negative result was found with following:

	Substances	Concentration
Endogenous substances	Human serum albumin	100 mg/mL
	Hemoglobin	50 mg/mL
	Triglyceride	100 mg/mL
	Bilirubin	500 ug/mL
	Cholesterol	20 mg/mL
	Biotin	3000 ng/mL
	Fibrinogen	1 mg/mL
	Rheumatoid Factor	200 IU/mL

The following medicines and chemicals were proven to be not interfering to Nano-Check™ COVID-19 IgG/IgM Antibody Test.

Acetaminophen	Tobramycin	Dexamethasone
Acetylsalicylic acid	Mucin	Ibuprofen

13. REFERENCES

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2. Lauer SA, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Intern Med 2020. doi:10.7326/M20-0504.
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