NowCheck

COVID-19 IgM/IgG Test

I2901-2E

For *in vitro* diagnostics use only

PRINCIPLE

NowCheck COVID-19 IgM/IgG Test is a rapid chromatographic immunoassay for the qualitative detection of specific antibodies to SARS-CoV-2 present in human serum, plasma or whole blood. This test is for *in vitro* professional diagnostic use and intended as an aid to diagnosis of SARS-CoV-2 infection in convalescent phase of patient with clinical symptoms with SARS-CoV-2 infection. It provides only an initial screening test result. More specific alternative diagnosis methods should be performed in order to obtain the confirmation of SARS-CoV-2 infection.

NowCheck COVID-19 IgM/IgG Test has three pre-coated lines for the device on the surface of the nitrocellulose membrane: "C" Control line, "G" and "M" Test lines. The control line and two test lines in the result window are not visible before applying any specimens. Monoclonal anti-COVID-19 NP antibody and purified chicken IgY are coated on the control line region, while monoclonal anti-human IgG antibody and monoclonal anti-human IgM antibody are coated on the "G" and "M" test line region. Recombinant COVID-19 nucleocapsid protein and recombinant COVID-19 structural protein with colloidal gold particles are used as detectors for "M" and "G" test lines. Monoclonal anti-chicken IgY antibody conjugatd with colloidal gold particles is used as detectors for "C" control line. During the test, SARS-CoV-2 antibodies in the specimen interact with recombinant COVID-19 nucleocapsid protein and recombinant COVID-19 structural protein conjugated with colloidal gold particles making antibody antigen gold particle complex. This complex migrates on the membrane via capillary action until the "M" and "G" test lines, where it will be captured by the monoclonal anti-human IgG antibody or monoclonal anti-human IgM antibody. A violet test line would be visible in the result window if SARS-CoV-2 antibodies are present in the specimen. The intensity of violet test line will vary depending upon the amount SARS-CoV-2 antibodies present in the specimen. If SARS-CoV-2 antibodies are not present in the specimen, then no color appears in the test line. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working.

MATERIALS PROVIDED

	Reagent	25 Tests/Kit
①	Test device	25
②	Assay diluent bottle	1
③	Capillary tube (20 µl)	25
④	Film	1
⑤	Instructions for use	1

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Timer
2. Micropipette

STORAGE AND STABILITY

1. Store the kit at room temperature, 2~30°C / 36-86°F, out of direct sunlight.
2. Kit materials are stable until the expiration date printed on the outer box.
3. Do not freeze the kit.
4. It is recommended to perform the test immediately after removing the test device from the foil pouch because the test device is sensitive to humidity and temperature.

PRECAUTIONS

1. Do not re-use the test kit.
2. Do not use the test kit if the pouch is damaged or the seal is broken.
3. Do not use the buffer of another lot.
4. Do not smoke, drink, or eat while handling specimen.
5. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after the tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout testing procedures.
9. Dispose of all specimens and materials used to perform the test as biohazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
10. Desiccant in foil pouch is to absorb moisture and keep humidity from affecting products.
11. Good laboratory practice recommends the use of the control materials. Users should follow the appropriate federal state, and local guidelines concerning the frequency of assaying external quality control materials.

COLLECTION AND PREPARATION OF SAMPLE

[Whole blood]

• **Capillary whole blood**

1. Capillary whole blood should be collected aseptically by fingertip.
2. Clean the area to be lanced with an alcohol swab.
3. Squeeze the end of the fingertip and pierce with a sterile lancet.
4. Using a tube, collect the 20 µl of capillary whole blood to the black line of the tube.
5. The capillary whole blood must be tested immediately after collection.

• **Venous whole blood**

1. Collect the venous whole blood into the commercially available anticoagulant tube such as Heparin, EDTA, or Sodium citrate by venipuncture.
2. If venous whole blood in an anti-coagulant tube is stored in a refrigerator at 2-8°C/36-46°F, the specimen can be used for testing within 1-2 days after collection.
3. Do not use hemolyzed blood specimens.

[Serum]

1. Collect the whole blood into the commercially available plain tube, NOT containing anti-coagulants such as Heparin, EDTA, or Sodium citrate by venipuncture and leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get the serum as a supernatant.
2. If serum in the plain tube is stored in a refrigerator at 2-8°C/36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen stored for more than 1 week can cause non-specific reactions. For prolonged storage, it should be at below -40°C/-40°F.
3. They should be brought to room temperature prior to use.

[Plasma]

1. Collect the venous blood into the commercially available anti-coagulant tube such as Heparin, EDTA, or Sodium citrate by venipuncture and centrifuge blood to get the plasma specimen.
2. If plasma in an anti-coagulant tube is stored in a refrigerator at 2-8°C/36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen stored for more than 1 week can cause non-specific reactions. For prolonged storage, it should be at below -40°C/-40°F.
3. They should be brought to room temperature prior to use.

CAUTION

* Use separate disposable materials for each specimen in order to avoid cross-contamination which can cause erroneous results.

INTERPRETATION OF THE RESULT

1. A colored band will appear in the top section of the result window to show that the test is working properly. This band is control line (C).
2. Two colored bands will appear in the lower section of the result window. These bands are each test line of IgM/IgG (M, G).
3. Even if the control line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as positive.

*** NowCheck COVID-19 IgM/IgG Test may cross-react with antibody against SARS-CoV-1.**
*** Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.**
*** Positive results should be considered in conjunction with the clinical history, RT-PCR results, and other data available.**

PERFORMANCE CHARACTERISTIC

1. Clinical evaluation

Residual serum from 31 positive patients confirmed by real-time PCR (PowerChek™ 2019-nCoV Real-time PCR kit, KCDC approved, FDA EUA) method and 74 negative person confirmed by real-time PCR (PowerChek™ 2019-nCoV Real-time PCR kit, KCDC approved, FDA EUA) method.

1) Diagnostic Sensitivity

Sensitivity (n=31)		NowCheck COVID-19 IgM/IgG Test		
		IgM+	IgG+	IgM+ and/or IgG+
Time post symptom onset	0-7 days	1/1 (100%) [95% CI: 20.65%-100%]	1/1 (100%) [95% CI: 20.65%-100%]	1/1 (100%) [95% CI: 20.65%-100%]
	8-14 days	2/3 (66.67%) [95% CI: 20.77%-93.85%]	3/3 (100%) [95% CI: 43.85%-100%]	3/3 (100%) [95% CI: 43.85%-100%]
	≥ 15 days	24/27 (88.89%) [95% CI: 71.94%-96.15%	26/27 (96.3%) [95% CI: 81.72%-99.34%]	26/27 (96.3%) [95% CI: 81.72%-99.34%]

2) Diagnostic Specificity

Specificity (n=74)	NowCheck COVID-19 IgM/IgG Test		
	IgM-	IgG-	IgM- and IgG-
RT-PCR SARS-CoV-2 negative samples	73/74 (98.65%) [95% CI: 92.73%-99.76%]	74/74 (100%) [95% CI: 95.07%-100%]	73/74 (98.65%) [95% CI: 92.73%-99.76%]

The NowCheck COVID-19 IgM/IgG Test showed 96.77% (30/31) of sensitivity and 98.65% (73/74) of specificity, compared to the Real-time PCR method.

ANALYTICAL PERFORMANCE

1. Limit of Detection: The limit of detection of NowCheck COVID-19 IgM/IgG Test for SN titer was represented as below.

SN titer (Detection limit of NowCheck COVID-19 IgM/IgG Test)
1: 40

2. Cross-Reactivity: There was no cross-reactivity about various anti-sera listed below. But, Anti-SARS-CoV showed little cross-reactivity.

Category	Potential Cross-Reactive Specimen
Respiratory Diseases	Anti-Influenza A
	Anti-Influenza B
	Anti-Influenza A+B
	Anti-RSV
	Anti-MERS CoV
	Anti-HCoV 229E
	Anti-HCoV HKU1
	Anti-HCoV NL63
	Anti-HCoV OC43
	Anti-Haemophilus Influenzae
	Anti-Mycoplasma pneumoniae
	Anti-Tuberculosis
	Anti-Rubella virus
Sexually Transmitted Infections	Anti-HIV
	Anti-Treponema Palladium (Syphilis)
Hepatitis	Anti-HAV (Hepatitis A Virus)
	Anti-HBV (Hepatitis B Virus)
	Anti-HCV (Hepatitis C Virus)
	Anti-EBV (Epstein-Barr virus)
Vector-borne Diseases	Anti-JEV (Japanese Encephalitis Virus)
	Anti-ZIKA virus
	Anti-Chikungunya virus
	Anti-Dengue virus
	Anti-TBE (Tick Borne Encephalitis virus)
	Anti-WNV (West Nile Virus)
	Anti-Filariasis
	Anti-Brucella
	Anti-Leishmania
	Anti-Chagas
	Anti-Malaria (P.vivax)
	Anti-Malaria (P.falciparum)
Food-borne Diseases	Anti-Toxoplasma
Enteric Diseases	Anti-Salmonella typhi
Other infectious Diseases	Anti-CMV (Cytomegalovirus)
Vaccinated	Influenza vaccined

3. Interference study: There was no interference for potential interfering substances listed below.

Exogenous Factor	Interfering Substances	Test Conc.
Relevant Medicines	Zanamivir (Influenza)	5 mg/ml
	Oseltamivir (Influenza)	10 mg/ml
	Artemether-lumefantrine (Malaria)	50 µM
	Doxycycline hyclate (Malaria)	70 µM
	Quinine (Malaria)	150 µM
	Lamivudine (Retroviral medication)	1 mg/ml
	Ribavirin (HCV)	1 mg/ml
	Daclatasvir (HCV)	1 mg/ml
	Tenofovir (HIV)	1 mg/ml
Antiparasitic Medication	Metronidazole	720 µmol/L
	Tinidazole	1 mg/ml
	Niclosamide	1 mg/ml
	Praziquantel	1 mg/ml
Anti-tuberculosis Medication	Rifampicin	80 µmol/L
	Isoniazid	300 µmol/L
Anti-inflammatory Medication	Acetaminophen	1100 µM
	Acetylsalicylic acid (Aspirin)	200 µM
	Ibuprofen	3 mM
	L-ascorbic acid	350 µM
Antibiotics	Erythromycin	200 µM
	Ciprofloxacin	50 µM
	Kanamycin	200 µM
	Ampicillin	250 µM
Dietary substances	Caffeine	600 µM
	Ethanol	90 mM
	Biotin	1200 ng/ml
Lipid	Triglycerides	90 mM
	Cholesterol	100 µg/ml
Colorimetric or chemical	Bilirubin (Unconjugated)	15 µg/ml
	Bilirubin (Conjugated)	5 µg/ml
	Hemoglobin	200 mg/ml
Anticoagulants	EDTA	3.4 µmol/L
	Heparin	3000 U/L
	Sodium citrate	3.8% (w/v)

Endogenous Factor	
Human anti-mouse Antibody	Elevated IgG
Anti-Nuclear Antibody (ANA) Homogeneous	Elevated IgM
Anti-Nuclear Antibody (ANA) Centromere	human antibodies to E.coli
Anti-Nuclear Antibody (ANA) Nucleolar	Pregnant women serum
Anti-Nuclear Antibody (ANA) Speckled	Human serum albumin
Rheumatoid Factor	Serum total proteins

4. Matrix Equivalency: The matrix and anticoagulants do not affect the detection of COVID-19 IgG and IgM in contrived serum, plasma (Heparin, EDTA, or Sodium citrate), venous whole blood (Heparin, EDTA, or Sodium citrate), and capillary whole blood (collected in EDTA-treated tubes) samples.

LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
2. This test detects the presence of anti-SARS-CoV-2 IgM/IgG in the specimen and should not be used as the sole criteria for the diagnosis of SARS-CoV-2 infection.
3. Test results must be considered with other clinical data available to the physician.
4. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
5. Neither the quantitative value nor the rate of anti-SARS-CoV-2 IgM/IgG concentration can be determined by this qualitative test.
6. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
7. A negative result may occur if the concentration of antibody in a specimen is below the detection limit of the test or if the specimen is collected or transported improperly, therefore a negative test result does not eliminate the possibility of SARS-CoV-2 infection, and should be confirmed by viral culture or a molecular assay or ELISA.
8. Positive test results do not rule out co-infections with other pathogens.
9. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
10. Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.

EXTERNAL QUALITY CONTROL

1. Positive and negative controls are optional contents (NowCheck COVID-19 IgM/IgG Control(Cat No. **RB2901CD**)) and these controls can be provided as a means on additional quality control to demonstrate a positive or negative reaction.
2. Quality controls should be treated and tested the same as patient specimens.
3. It is recommended that positive and negative controls be run:
   - once for each new lot.
   - once for each untrained operator.
   - as required by test procedures in this instructions and in accordance with local, state and federal regulations or accreditation requirements.

NOTIFICATION FOR COVID-19 ANTIBODY TESTS

1. 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 or past or present infection with SARS virus (no. 6).
2. Not for the screening of donated blood.
3. The test procedure should be conducted in ambient temperature and pressure.
4. Results of these tests should be appropriately recorded in a test report.

BIBLIOGRAPHY OF SUGGESTED READING

[1] Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Interim guidance. WHO.2020
[2] Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR.2020
[3] Diagnosis and treatment of pneumonia caused by new coronavirus (trial version 4) National Health Commission. 2020
[4] Guo L et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clinical Infectious Disease. 2020
[5] Zhao J et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Disease. 2020

Doc. No.: I2901-2E
Issued date : Sep. 11, 2020

IVD CE

Authorized Representative
EC REP MT Promedt Consulting GmbH
Altenhofstrasse 80 D-66386 St. Ingbert Germany
Tel: +49-6894-581020 I Fax: +49-6894-581021

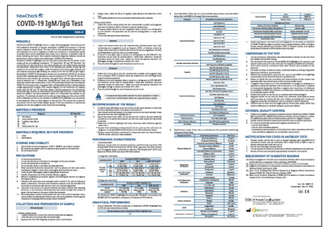
BIONOTE
22, Samsung 1-ro 4-gil, Hwaseong-si, Gyeonggi-do 18449, Republic of Korea
Tel: +82-31-211-0516 I Fax: +82-31-8003-0618 www.bionote.co.kr

NowCheck

COVID-19 IgM/IgG Test

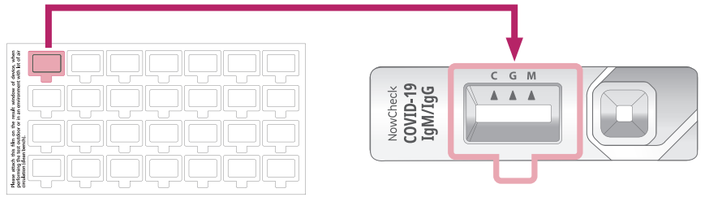
STEP 1.

Carefully read instruction for using the NowCheck COVID-19 IgM/IgG Test



STEP 2.

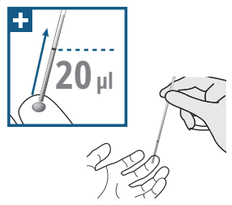
Attach the provided film on to the test device



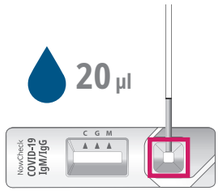
TEST PROCEDURE

[Using Capillary whole blood]

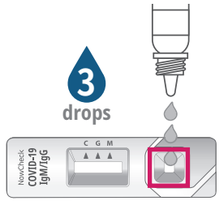
1 Specimen collection
Using a capillary tube, collect the 20 µl of capillary whole blood to the black line of the capillary tube.



2 Adding of Specimen
Add the collected capillary whole blood into the sample hole of the test device.



3 Dropping of buffer
Add 3 drops (90 µl) of buffer vertically into the sample hole of the test device.

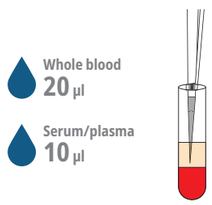


4 Reading Time
Read the test result in 10 ~15 min. Do not read test results after 15 min.

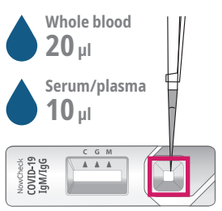
CAUTION
- Do not read test results after 15 min. It may give false results.

[Using serum/plasma/whole blood]

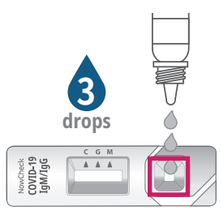
1 Specimen collection
Using a micropipette, collect the 10 µl of serum, plasma or 20 µl of whole blood.



2 Adding of Specimen
Add the collected serum, plasma or whole blood into the sample hole of the test device.



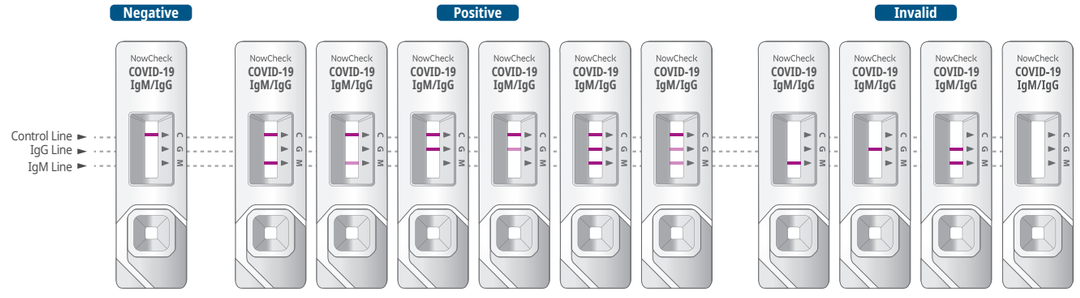
3 Dropping of buffer
Add 3 drops (90 µl) of buffer vertically into the sample hole of the test device.



4 Reading Time
Read the test result in 10 ~15 min. Do not read test results after 15 min.

CAUTION
- Do not read test results after 15 min. It may give false results.

TEST RESULT



1. A colored band will appear in the top section of the result window to show that the test is working properly. This band is control line (C).
2. Two colored bands will appear in the lower section of the result window. These bands are each test line of IgM/IgG (M, G).
3. Even if the control line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as positive.

*** NowCheck COVID-19 IgM/IgG Test may cross-react with antibody against SARS-CoV-1.**
*** Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.**
*** Positive results should be considered in conjunction with the clinical history, RT-PCR results, and other data available.**

SYMBOL

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Contains sufficient for <n> tests		Indicates that the product is fragile and to handle it with care
	Consult instructions for use		Do not re-use	LOT	Batch code To indicate the lot number
REF	Reference number		Use by		Indicates to discard it separately from other household waste
	Date of manufacture To indicate the date of manufacture		Caution! Indicates a situation, which if not avoided could result		Keep away from sunlight
	Note		Indicates that you should keep the product dry		Do not use if package is damaged
CE	Fulfill the requirements of Directive 98/79/EC on *in vitro* diagnostic medical devices				