Artron

COVID-19 IgM/IgG Antibody Test

Instructions For Use

Format: Cassette
Specimen: Serum/Plasma/Whole Blood
Catalog Number: A03-51-322

CE

* Please read the instructions carefully before use

INTENDED USE

Artron COVID-19 IgM/IgG Antibody Test is a rapid, qualitative and convenient immunochromatographic *in-vitro* assay for the differential detection of IgM &/or IgG antibodies to SARS-COV-2 in human serum, plasma or whole blood samples obtained from patient with SARS-COV-2 infection. The device is designed to aid in the determination of recent or previous exposure to SARS-COV-2 virus, tracking the body's immunity status to the virus after SARS-COV-2 infection.

This assay only provides a preliminary result. A positive result does not necessarily mean a current infection but represents a different stage of the disease after infection. IgM positive or IgM&IgG both positive suggest recent exposure. Current infection should be confirmed by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) or viral gene sequencing. Negative results do not preclude SARS-COV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. False negative results can occur in elderly and immunocompromised patients. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. The test is intended for screening/monitoring/aid to diagnosis/staging/aid to staging of infection in populations exposed to SARS-COV-2. It is intended for professional use or point of care use and should only be used by qualified and experienced inspectors for use in clinical specimens and molecular biology experiments. They should be trained minimally in pathology practices but be fully trained in taking blood specimens.

SUMMARY AND PRINCIPLE OF THE ASSAY

Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) is the virus strain that caused an outbreak of a novel coronavirus disease (COVID-19), which has subsequently affected countries and regions worldwide. Severe disease onset might result in death due to massive alveolar damage and progressive respiratory failure. On March 11, 2020, the World Health Organization (WHO) has declared the global outbreak of COVID-19 a pandemic associated with substantial morbidity and mortality.

The principle of Artron COVID-19 IgM/IgG Antibody Test is an antibody-capture immunochromatographic assay for the simultaneous detection and differentiation of IgM & IgG antibodies to SARS-COV-2 virus in human serum, plasma, or whole blood samples. SARS-COV-2 -specific antigens are conjugated to a colloidal gold and deposited on the conjugate pad. Monoclonal anti-human IgM and monoclonal anti-human IgG are immobilized on two individual test lines (M line and G line) of the nitrocellulose membrane. The M line is closer to the sample well and followed by the G line. When the sample is loaded, the gold-antigen conjugate is rehydrated and the SARS-COV-2 IgM and/or IgG antibodies, if any in the sample, will interact with the gold conjugated antigen. The immunocomplex will migrate towards the test window reaching the test zone (G line & M line) where they will be captured by the relevant anti-human IgM (M line) and/or anti-human IgG (G line), forming a visible pink line, indicating positive results. If SARS-COV-2 antibodies are absent in the sample, no pink line will appear in the test lines (G line & M line), indicating a negative result.

To serve as an internal process control, a control line should always appear at Control Zone (C line) after the test is completed. Absence of a pink control line in the Control Zone is an indication of an invalid result.

PACKAGE CONTENTS

- Pouch contents: One test cassette with test strip, One desiccant;
- 25 capillary tubes for 25 tests (to collect fingerstick blood);
- Sample buffer 3 ml/vial for 25 tests

MATERIALS REQUIRED (BUT NOT PROVIDED)

- Alcohol swab
- Safety lancet (for fingerstick whole blood specimens)
- Blood collection device. (for other than fingerstick whole blood specimens)

- Precision pipette capable of delivering 10µl and/or 20µl with disposable tips (for other than fingerstick whole blood specimens)
- Gloves
- Clock or timer

WARNINGS AND PRECAUTIONS

- For professional *in-vitro* diagnostic use only.
- Do not reuse.
- Do not use if the product seal or its packaging is compromised
- Do not use after the expiration date shown on the pouch
- Do not mix and interchange different specimens
- This test should be performed at 2 to 30°C (17 to 86°F). If stored refrigerated, ensure that the Test units are brought to operating temperature before performing testing.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection while handling potentially infectious materials or performing the assay
- Wash hands thoroughly after finishing the tests
- Do not eat, drink, or smoke in the area where the specimens or test are handled
- Clean up spills thoroughly with appropriate disinfectants
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing procedures.
- Dispose of all specimens and used devices in a proper bio-hazard container. The handling and disposal of the hazardous materials should follow local, regional, or national regulations.
- Keep out of children's reach.

SPECIMEN PREPARATION

- **Whole Blood** samples may be collected by fingerstick or venipuncture, following routine facility procedures. In summary:

  Fingerstick whole blood:

  - Clean the area of finger to be lanced with the alcohol swab. Allow to dry.
  - Without touching the puncture site, rub down the hand towards the middle or ring finger fingerstick.
  - Puncture the skin with a sterile lancet and wipe away the first drop of blood.
  - Gently rub the hand from wrist to the lanced finger to form a full drop of blood over the puncture site
  - Collect the blood droplet using the included capillary tube
  - Fingerstick whole blood must be tested immediately after collection.

  Venous whole blood:

  - Collect venous whole blood in a tube with anticoagulant (Heparin/EDTA/CPDA-1)
- **Serum samples,** collect blood in a tube without anticoagulant and allow it to clot.
- **Plasma samples,** collect blood in a tube containing anticoagulant (Heparin/EDTA/CPDA-1)

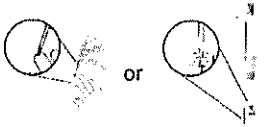
Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.

Whole blood in blood collection tubes with anticoagulant (EDTA/Heparin/CPDA1), serum/plasma in blood collection tubes may be stored at 2°C to 8°C for up to 7 days, if the sample needs to be transported or the tests cannot be performed immediately. Bring sample to attain room temperature (without heating) prior to use.

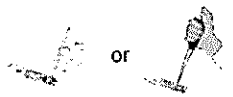
TEST PROCEDURES

1. Get the test cassette from the sealed pouch by tearing at the notch and place the cassette on a flat, dry surface

2. **For fingerstick or venous whole blood:**
   Using a capillary tube, collect the fingerstick whole blood or whole blood till to the black line. (20µl)
   For serum/plasma:
   Using a pipette, collect the serum/plasma (10µl).



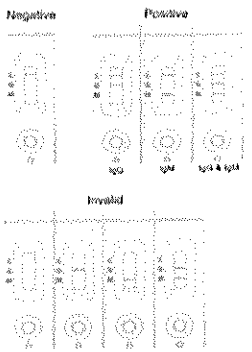
3. Add the collected serum/plasma/whole blood to the upper area (close to test window) of the sample well on the test cassette without air bubbles (hold the capillary tube/ pipette vertically and gently touch the end against the pad within the sample well for transferring).



4. Wait for 20-30 seconds, add 2 drops (around 90µl) of sample buffer to the sample well of the test cassette

5. Wait and read results in15-30 minutes.

   DO NOT INTERPRET RESULTS AFTER 30 MINUTES



RESULT INTERPRETATION

Negative
A pink colored band appears only at the control region (C), indicating a negative result for SARS-COV-2 infection.

Positive
Pink colored bands appear at the control region (C) and G and /or M region.
1) IgM and IgG Positive, visible bands at both M and G
2) IgM positive, a visible band at M region.
3) IgG positive, a visible band at G region

Invalid

No visible band at the control region (C). Repeat with a new test. If test still fails, please contact the distributor with the lot number

QUALITY CONTROL

Although the testing cassette contains an internal quality control (pink colored band in the control region), good laboratory practice recommends the daily use of an external control to ensure proper testing performance. Quality control samples should be tested according to the standard quality control procedure established by your laboratory.

STORAGE AND STABILITY

- The test should be stored at 2-30°C in its original package, avoid direct sunlight and freezing
- Opened test cassette should be used within 1 hour.
- Do not freeze the test device.
- Shelf life: 6 months

LIMITATIONS

- The instructions for use of the test should be followed during testing procedures
- Use in conjunction with the testing strategy outlined by public health authorities in your area.
- Humidity and temperature can adversely affect results.
- There is always a possibility that false results will occur due to the presence of interfering substances in the specimen or factors beyond the control of the manufacturer, such as technical or procedural errors associated with the testing.
- Use in conjunction with the testing strategy outlined by public health authorities in your area
- Negative results do not preclude SARS-COV-2 infection and should not be used as the sole basis for patient management decisions. IgM antibodies may not be detected in the first few days of infection, the sensitivity of the test early after infection is unknown
- Results are for the detection of SARS-COV-2 antibodies. IgM antibodies to SARS-COV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-COV-2 become detectable later following infection. At this time, it is unknown how long IgM or IgG antibodies may persist following infection.
- Positive results for both IgG and IgM could occur after infection and can be indicative of acute or recent infection (and successful immune response to a vaccine, once developed).
- False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes
- False negative results for IgM and IgG antibodies may occur due to high-dose hook effect. It is recommended to further dilute the specimens which are suspected of carrying high-dose hook effect and combine other test results along with comprehensive consideration of clinical history.
- False negative results for IgM and IgG antibodies may occur due to hypergammaglobulinaemia. It is recommended to further confirm the suspected case with clinical observations, patient history, and epidemiological information.
- Although Artron COVID-19 IgM/IgG Antibody Test applies multiple antigens to prevent missed detection. However, it still could not rule out the possibility of in false negative test results due to SARS-COV-2 variants.
- The performance of this device has not been assessed in a population vaccinated against COVID-19
- This test identifies antibodies to the spike protein of the SARS-COV-2 virus and is therefore unable to distinguish between previously infected individuals and the vaccinated individuals.
- The control line is only indicative of the sample flow and will not show the degradation of the critical active components.
- Laboratories are required to report all positive results to the appropriate public health authorities
- Although the test demonstrates superior accuracy in detecting antibodies against SARS-COV-2 virus, a low incidence of false results can occur. Therefore, other clinically available tests are required in case of questionable results. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

- **Analysis of Sensitivity and Specificity**

  Inactive COVID-19 IgM/IgG sensitivity panel including 3 SARS-COV-2 IgM positive sera (Strong, moderate and weak), 3 SARS-COV-2 IgG positive sera (Strong, moderate and weak), and one negative serum was applied to validate the analysis sensitivity of Artron COVID-19 IgM/IgG Antibody Test. Artron COVID-19 IgM/IgG Antibody Test could identify all the positive samples. No false positive or false negative results were observed. Samples with below seromarkers associated with unrelated SARS-COV-2 medical conditions were used to evaluate cross-reaction:

  - Rheumatoid factor(RF)
  - Anti-nuclear antibodies(ANA)
  - Coronavirus Seasonal
  - Coronavirus 229E
  - Coronavirus NL63
  - Coronavirus HKU1
  - Coronavirus OC43
  - Anti-Human immunodeficiency virus (HIV)
  - Human Hepatitis B virus(HBV) serum markers (HBsAg, anti-HBc IgG/IgM)
  - Anti-Human Hepatitis C virus(HCV)
  - Anti-Helicobacter pylori(HP)
  - Anti-Herpes simplex virus(HSV)
  - Anti-Cytomegalovirus(CMV)
  - Anti-Chikungunya virus (CHIKV)
  - Anti-Zika virus (ZIKV)
  - Anti-Mycoplasma
  - Anti-Dengue Virus(DENV)
  - Anti-Adenovirus(ADV)
  - Anti-Respiratory syncytial virus(RSV)
  - Anti-Influenza A
  - Anti-Influenza B
  - Anti-Parainfluenza virus 1/2/3
  - Anti-Epstein-Barr virus(EBV)
  - Anti-Haemophilus influenza
  - Anti-Streptococcus pyogenes
  - Anti-Mycobacterium tuberculosis(TB)
  - Anti-T. pallidum (TP)
  - Anti-Plasmodium

  Total 153 samples were applied to evaluate the analytical specificity of Artron COVID-19 IgM/IgG Antibody Test. The results showed that only one anti-CHIKV plasma gave very

equivocal signal on IgM band on the test, and Artron COVID-19 IgM/IgG Antibody Test had no cross reaction with all other 152 samples. The total specificity for all 153 samples was 99.35% No significant cross-reactivity was observed to most of above relevant serum markers. The results demonstrated that COVID-19 IgG/IgM Antibody Test has good analytical specificity

- **Interference Study**

  Analytes commonly found in OTC, prescription and/ or abuse drugs, chemical analytes, and endogenous substances below did not interfere with Artron COVID-19 IgM/IgG Antibody Test

Potential Interfering Substances	Concentration	Potential Interfering Substances	Concentration
Acetaminophen	20 g/dl	Albumin	6g/dl
Acetylsalicylic acid	65 µg/dl	Glucose	1000 mg/dl
Ascorbic acid	6g/dl	Conjugated bilirubin	100g/dl
Caffeine	6g/dl	Unconjugated bilirubin	40g/dl
Gentesic acid	1.8 g/dl	Hemoglobin	200 µg/dl
Phenylpropanolamine	20 mg/dl	Triglyceride	37mmol/ml
Salicylic acid	60 mg/dl	Cholesterol	100g/dl
EDTA	100 µg/dl	HAMA plasma	⅓ dilution
Benzoylecgonine	42 mg/dl	Rheumatoid factor	920IU/ml
Atropine	20 mg/dl	Anti-nuclear antibodies	1 399OD
Cannabinol	10 mg/dl		
Ethanol	0 4µg/dl		
Morphine	50µg/dl		

The interfering effects of Human IgM myeloma at 2mg/mL can cause false-negative IgM results, but not interfere with IgG results

- **Repeatability and Reproducibility**

  Tests showed positive results with all positive samples and showed negative results with negative samples. There was no significant difference observed to the same sample when repeatedly testing 10 tests in the same batch. No appreciable intra and inter lot variation were observed among different tests for each lot, different lots, different operators at different test sites in different time for the same sample.
  The results demonstrated that the repeatability and reproducibility of Artron COVID-19 IgM/IgG Antibody Test is satisfactory

- **Diagnostic Sensitivity and Specificity**

  1. **Diagnostic Sensitivity and Specificity of Sera/Plasmas/Venous Whole Blood Specimens**

     A total of 2155 samples including 762 of molecular diagnosis confirmed SARS-COV-2 positive sera/plasma/whole blood samples and 1393 of clinically true (collected before Nov. 2019) or RT-PCR confirmed SARS-COV-2 negative samples sera/plasma samples were used to evaluate Artron COVID-19 IgM/IgG Antibody Test from six evaluation centers. Out of all the 762 positive samples, Artron COVID-19 IgM/IgG Antibody Test identified 649 of IgM positive and 640 of IgG positive and 703 of SARS-COV-2 IgM&/or IgG positive samples. The diagnostic sensitivity for IgM is 85.17%, for IgG is 83.99%, and the combined sensitivity is 92.26% The diagnostic sensitivity of IgG for over 14 days after onset is 95.41%. The diagnostic sensitivity of IgG and IgM combined for over 14 days after onset is 97.49% The diagnostic specificity for IgM is 98.28%, for IgG is 99.78% and the combined specificity is 98 13%. The overall agreement for IgM and IgG is 93 64% and 94 20%, respectively The combined overall agreement is 96.06%

Table 1. Summary for all the test results of sera/plasma/venous whole blood

	Sensitivity				Sensitivity	Specificity
	Days from Onset					
	Asymptomatic infection	≤ 7 days	8-14 days	>14 days		
Isotype	(N=6	(N=79)	(N=198)	(N=479)	(N=762)	(N=1393)
IgM	66 67% (4) (22 28-95 67)	60 76% (48) (49 13-71 56)	90 40% (179) (85 42-94 12)	87 27% (418) (83 94-90 12)	85 17% (649) (82 45-87 62)	98 28% (1369) (97 45-98 89)
IgG (% CI)	66 67% (4) (22 28-95 67)	27 85% (22) (18 35-39 07)	79 29% (157) (72 98-84 71)	95 41% (457) (93 13-97 10)	83 99% (640) (81 19-86 52)	99 78% (1390) (99 37-99 96)
IgM + IgG (% CI)	66 67% (4) (22 28-95 67)	62 03% (49) (50 41-72 72)	92 42% (183) (87 81-95 70)	97 49% (467) (95 66-98 70)	92 26% (703) (90 13-94 05)	98 13% (1367) (97 28-98 78)

2. **Diagnostic Sensitivity of Seroconversion**

   The seroconversion evaluation sensitivity of the Artron COVID-19 IgG/IgM Antibody Test Cassette was evaluated on 51 serum samples from 8 individuals getting treatment at the Central Laboratory of Saitama Medical University Hospital in Japan The collected samples were chosen from the patients with the early infection, from one to three weeks after onset. All the studied cases are confirmed by RT-PCR. The anti-SARS-COV-2 antibodies levels of the patients were examined using Artron COVID-19 IgM/IgG Antibody Test. Artron COVID-19 IgG/IgM Antibody Test detected IgG seroconversion during 9-15 days after onset, in all 8 cases and the seroconversion rate was 100%.

Table 2. Summary for the positive rate in eight SARS-Cov-2 seroconverted patients

After onset (day)	Artron COVID-19 IgM/IgG Antibody Test			
	IgM		IgG	
	Negative	Positive	Negative	Positive
0-8	5	3	7	1
9-15	0	8	0	8
Positive rate (%)	IgM		IgG	
0-8	37.5		12.5	
9-15	100		100	

3. Diagnostic Sensitivity and Specificity of Fingerstick Whole Blood Specimens

A total of 482 samples were collected in point-of-care testing including 215 molecular diagnosis confirmed SARS-COV-2 positive fingerstick whole blood samples and 267 RT-PCR confirmed SARS-COV-2 negative samples. Of all these 215 positive samples, 53% (114/215) of the positive samples were from convalescent patients who were post onset more than 40 days, Artron COVID-19 IgM/IgG Antibody Test identified 116 IgM positive, 183 IgG positive cases and 213 of SARS-COV-2 IgM&/or IgG positive samples. Diagnostic sensitivity for IgM is 53.95%, for IgG is 85.12 %, and the combined sensitivity is 99.07 %. The diagnostic sensitivity for IgG post onset >14 days is 96.47%. One of the IgM false positive and one of the IgG false positive cases from a total of 267 negative samples were observed, which demonstrated that the diagnostic specificity for IgM is 99.63% and for IgG is 99.63%, the combined specificity is 99.25%

Table 3. Summary for the test results of fingerstick whole blood

	Sensitivity Days from Onset			Sensitivity	Specificity
	≤ 7 days	8-14 days	>14 days		
Isotype	(N=19)	(N=26)	(N=170)	(N=215)	(N=267)
IgM	100.00% (19)	100.00% (26)	41.76% (71)	53.95% (116)	99.63% (266)
(% CI)	(82.35-100.00)	(86.77-100.00)	(34.26-49.56)	(47.04-60.75)	(97.93-99.99)
IgG	10.53% (2)	65.38% (17)	96.47% (164)	85.12% (183)	99.63% (266)
(% CI)	(1.30-33.14)	(44.33-82.79)	(92.48-98.69)	(79.64-89.59)	(97.93-99.99)
IgM + IgG	100.00% (19)	100.00% (26)	98.82% (168)	99.07% (213)	99.25% (265)
(% CI)	(82.35-100.00)	(86.77-100.00)	(95.81-99.86)	(96.68-99.89)	(97.32-99.91)

4. Diagnostic Sensitivity and Specificity of 4 specimen types (serum, plasma, venous whole blood and fingerstick) from one clinical center

A total of 405 SARS-COV-2 RT-PCR positive specimens including 120 fingerstick samples, 36 sera, 125 plasmas and 124 venous whole blood and 1299 SARS-COV-2 negative specimens including 222 fingerstick samples, 73 sera, 676 plasmas and 328 venous whole blood samples were collected in one of the clinical centers. For the positive samples, the detection sensitivity of IgM and IgG varies significantly with the timepoint of sample collection in the patient's disease course. The more samples come from patients with post-onset 14 days, the higher the sensitivity of IgG detection. This phenomenon has been well verified in our clinical trials. The sensitivity of IgM and IgG, especially that of IgG in venous whole blood is significantly higher than that in the other sample types, as most venous whole blood samples were collected from convalescent patients (number of samples from onset >14days venous whole blood-101/124, 81.45%; fingerstick-75/120, 62.5%, serum-10(clarified)/36, 27.78%; plasma-91/125, 72.8%)

Table 4 Diagnostic sensitivity and specificity of four specimen types

Specimens type	Positive	Sensitivity		
	Count	IgM (% CI)	IgG (% CI)	Joint (% CI)
Fingerstick	120	87.50% (105)	75.00% (90)	100.00% (120)
		(80.22-92.83)	(66.27-82.45)	(96.97-100.00)
Serum	36	86.11% (31) (70.50-95.33)	80.56% (29) (63.98-91.81)	97.22% (35) (85.47-99.93)
Plasma	125	86.40% (108) (79.12-91.87)	80.80% (101) (72.79-87.29)	92.00% (115) (85.78-96.10)
Venous	124	92.74% (115) (86.67-96.63)	94.35% (117) (88.71-97.70)	99.19% (123) (95.59-99.98)
Total	405	88.64% (359) (85.14-91.56)	83.21% (337) (79.21-86.72)	97.04% (393) (94.88-98.46)
Specimens type	Negative Count	Specificity IgM (% CI)	IgG (% CI)	Joint (% CI)
Fingerstick	222	99.55% (221) (97.52-99.99)	100% (222) (98.35-100.00)	99.55% (221) (97.52-99.99)
Serum	73	100% (73) (95.07-100.00)	100% (73) (95.07-100.00)	100% (73) (95.07-100.00)
Plasma	676	97.93% (662) (96.55-98.86)	99.85% (675) (99.18-100.00)	97.78% (661) (96.37-98.75)
Venous	328	98.78% (324) (96.91-99.67)	99.70% (327) (98.31-99.99)	98.48% 323 (96.48-99.50)
Total	1299	98.54% (1280) (97.73-99.12)	99.85% (1297) (99.44-99.98)	98.38% (1278) (97.54-99.00)

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