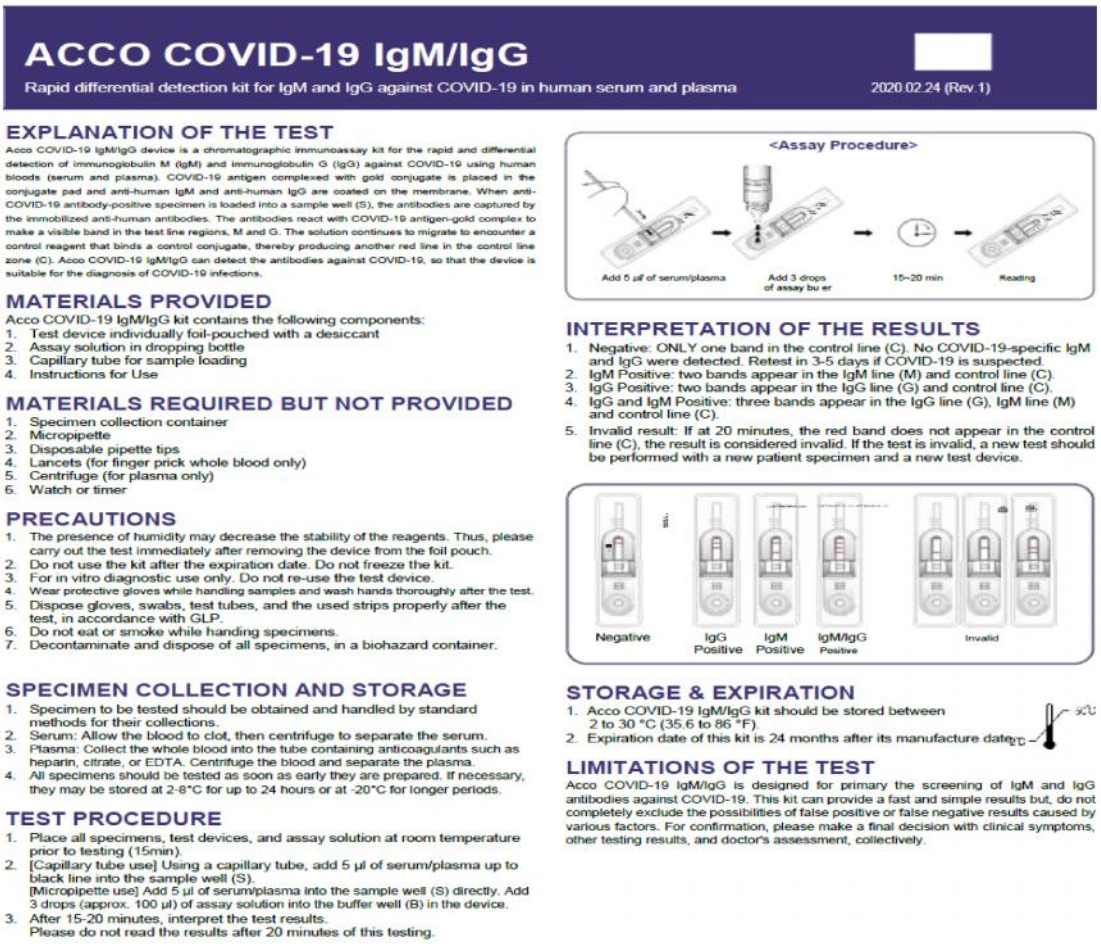
The procedures to test the kits



ACCO COVID-19 IgM/IgG

Rapid differential detection kit for IgM and IgG against COVID-19 in human serum and plasma

2020.02.24 (Rev.1)

EXPLANATION OF THE TEST

Acco COVID-19 IgM/IgG device is a chromatographic immunoassay kit for the rapid and differential detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) against COVID-19 using human bloods (serum and plasma). COVID-19 antigen complexed with gold conjugate is placed in the conjugate pad and anti-human IgM and anti-human IgG are coated on the membrane. When anti-COVID-19 antibody-positive specimen is loaded into a sample well (S), the antibodies are captured by the immobilized anti-human antibodies. The antibodies react with COVID-19 antigen-gold complex to make a visible band in the test line regions, M and G. The solution continues to migrate to encounter a control reagent that binds a control conjugate, thereby producing another red line in the control line zone (C). Acco COVID-19 IgM/IgG can detect the antibodies against COVID-19, so that the device is suitable for the diagnosis of COVID-19 infections.

MATERIALS PROVIDED

Acco COVID-19 IgM/IgG kit contains the following components:

1. Test device individually foil-pouched with a desiccant
2. Assay solution in dropping bottle
3. Capillary tube for sample loading
4. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Specimen collection container
2. Micropipette
3. Disposable pipette tips
4. Lancets (for finger prick whole blood only)
5. Centrifuge (for plasma only)
6. Watch or timer

PRECAUTIONS

1. The presence of humidity may decrease the stability of the reagents. Thus, please carry out the test immediately after removing the device from the foil pouch.
2. Do not use the kit after the expiration date. Do not freeze the kit.
3. For in vitro diagnostic use only. Do not re-use the test device.
4. Wear protective gloves while handling samples and wash hands thoroughly after the test.
5. Dispose gloves, swabs, test tubes, and the used strips properly after the test, in accordance with GLP.
6. Do not eat or smoke while handing specimens.
7. Decontaminate and dispose of all specimens, in a biohazard container.

SPECIMEN COLLECTION AND STORAGE

1. Specimen to be tested should be obtained and handled by standard methods for their collections.
2. Serum: Allow the blood to clot, then centrifuge to separate the serum.
3. Plasma: Collect the whole blood into the tube containing anticoagulants such as heparin, citrate, or EDTA. Centrifuge the blood and separate the plasma.
4. All specimens should be tested as soon as early they are prepared. If necessary, they may be stored at 2-8°C for up to 24 hours or at -20°C for longer periods.

TEST PROCEDURE

1. Place all specimens, test devices, and assay solution at room temperature prior to testing (15min).
2. [Capillary tube use] Using a capillary tube, add 5 µl of serum/plasma up to black line into the sample well (S).
   [Micropipette use] Add 5 µl of serum/plasma into the sample well (S) directly. Add 3 drops (approx. 100 µl) of assay solution into the buffer well (B) in the device.
3. After 15-20 minutes, interpret the test results.
   Please do not read the results after 20 minutes of this testing.

<Assay Procedure>

INTERPRETATION OF THE RESULTS

1. Negative: ONLY one band in the control line (C). No COVID-19-specific IgM and IgG were detected. Retest in 3-5 days if COVID-19 is suspected.
2. IgM Positive: two bands appear in the IgM line (M) and control line (C).
3. IgG Positive: two bands appear in the IgG line (G) and control line (C).
4. IgG and IgM Positive: three bands appear in the IgG line (G), IgM line (M) and control line (C).
5. Invalid result: If at 20 minutes, the red band does not appear in the control line (C), the result is considered invalid. If the test is invalid, a new test should be performed with a new patient specimen and a new test device.

STORAGE & EXPIRATION

1. Acco COVID-19 IgM/IgG kit should be stored between 2 to 30 °C (35.6 to 86 °F).
2. Expiration date of this kit is 24 months after its manufacture date.

LIMITATIONS OF THE TEST

Acco COVID-19 IgM/IgG is designed for primary the screening of IgM and IgG antibodies against COVID-19. This kit can provide a fast and simple results but, do not completely exclude the possibilities of false positive or false negative results caused by various factors. For confirmation, please make a final decision with clinical symptoms, other testing results, and doctor's assessment, collectively.

Name	Composition	Quantity	CAS number
Test Strip	Mouse-gold conjugate	QS	N/A
	Anti-human IgM-gold conjugate	QS	N/A
	Anti-human IgG-gold conjugate	QS	N/A
	Anti-mouse IgG	QS	N/A
	Recombinant COVID-19 NP	QS	N/A
	Absorbance pad	QS	N/A
	Conjugation pad	QS	N/A
	Nitrocellulose membrane	QS	N/A
Test Device	Poly Ethylene	N/A	N/A
Others	Silica gel	N/A	N/A

Classification of the product

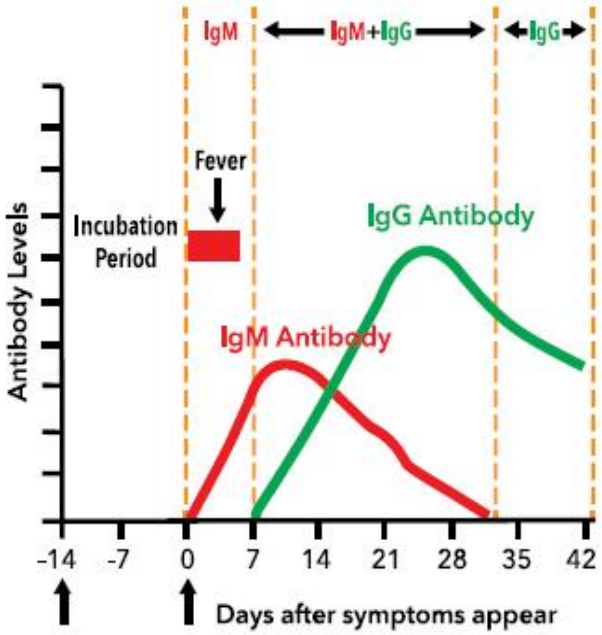
EDMA code: 15 04 80 90 00 Other Viral Antigen/Antibody Detection

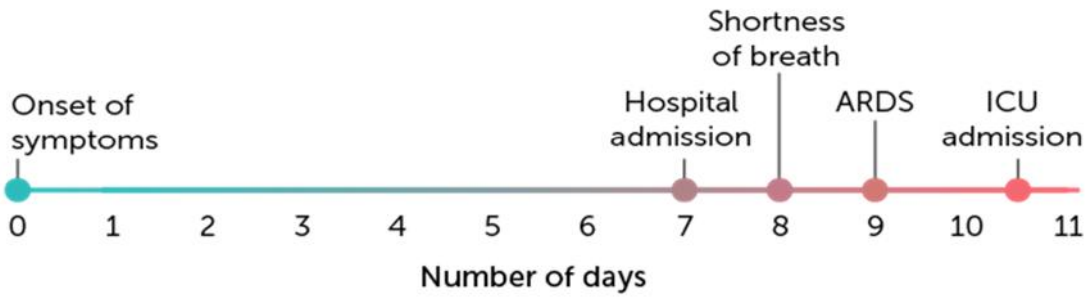
IVDD Classification: Others

(Neither Listed in Annex II of IVDD, nor self-testing device)

COVID -19 diagnosis in 10-15 minutes

- It is widely accepted that IgM provides the first line of defense during viral infections, followed by the generation of adaptive, high affinity IgG responses for long term immunity and immunological memory.
- Therefore testing of COVID-19 IgM and IgG antibodies is an effective method for the rapid diagnosis of COVID-19 infection.
- Furthermore, detection of COVID-19 IgM antibodies tends to indicate a recent exposure to COVID-19, whereas detection of COVID-19 IgG antibodies indicates a later stage of infection.
- Thus, this combined antibody test could also provide information on the stage of infection.



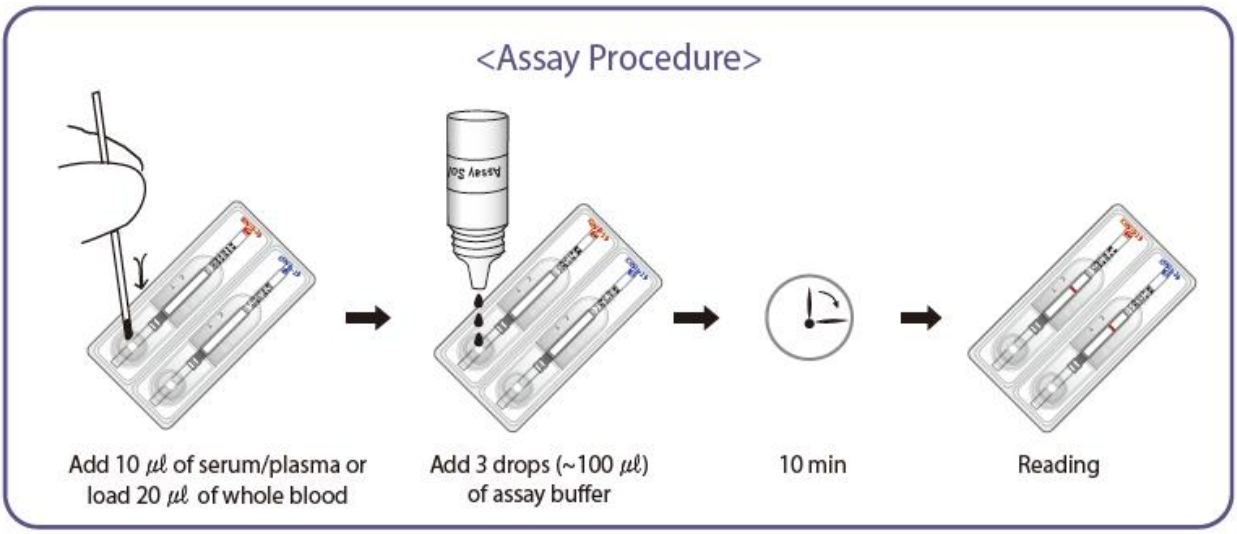


Specimen collection, storage and precaution

(1) Specimen to be tested should be obtained and handled by standard methods for their collections.
(2) Serum: Allow the blood to clot, then centrifuge to separate the serum.
(3) Plasma: Collect the whole blood into the tube containing anticoagulants such as heparin, citrate, or EDTA. Centrifuge the blood and separate the plasma.
(4) Whole blood: whole blood should be collected over heparin, citrate, or EDTA. Mix the blood by inversion and use it to the test. If fingertip blood is used to the test, prick the finger and collect the blood by a capillary tube. And then, load the blood onto the sample well (S) of the test device.
(5) All specimens should be tested as soon as early they are prepared. If necessary, they may be stored at 2-8°C for up to 24 hours or at -20°C for longer periods.

Test procedure

(1) Place all specimens, test devices, and assay solution at room temperature prior to testing (15min).
(2) [Capillary tube use] Using a capillary tube, add 10 µl of serum/plasma or load 20 µl of whole blood up to black line into the sample well (S).
[Micropipette use] Add 10 µl of serum/plasma or load 20 µl of whole blood into the sample well (S) directly. Add 3 drops (approx. 100 µl) of assay solution into the buffer well (S) in the device.
(3) After 10 minutes, interpret the test results.
Please do not read the results after 10 minutes of this testing.



Reading and interpretation of results

[Qualitative reading]

(1) Negative: ONLY one band in the control line (C). No COVID-19-specific IgM and IgG were detected. Re-test in 3-5 days if COVID-19 is suspected.

(2) IgM Positive: two bands appear in the test line (T) and control line (C) in the left side of device.

(3) IgG Positive: two bands appear in the test line (T) and control line (C) in the right side of device.

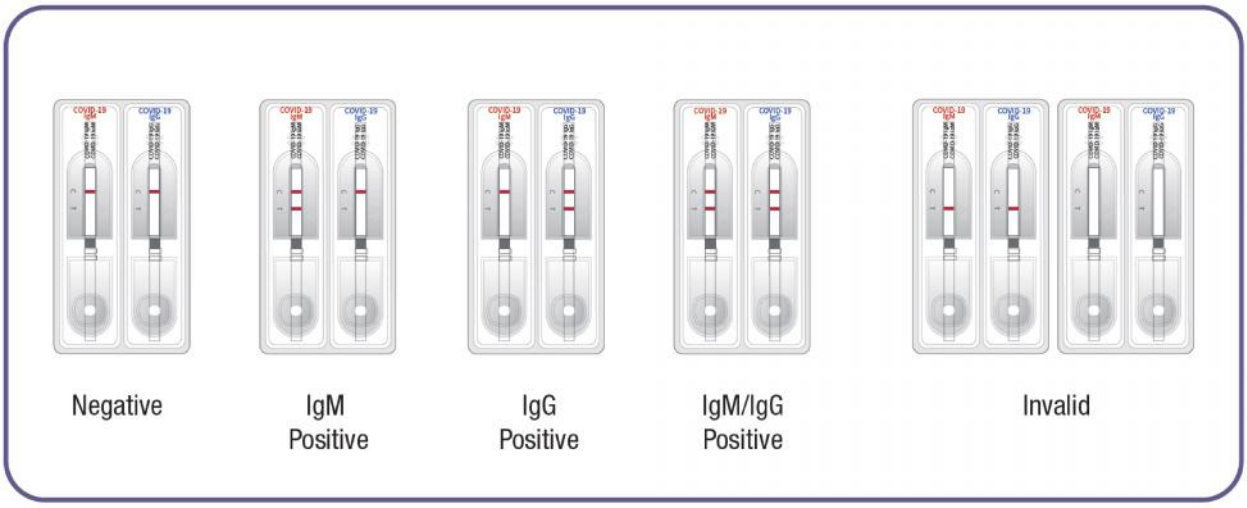
(4) IgG and IgM Positive: each two bands appear in the test line (T) and control line (C) in both side of device.

(5) 5. Invalid result: If at 20 minutes, the red band does not appear in the control line (C), the result is considered invalid. If the test is invalid, a new test should be performed with a new patient specimen and a new test device.

[Use quantitative Analyzer]

(1) Using Confiscope G20 is optional.

(2) Please refer to the instructions for use in analyzer package.



Storage and expiration

(1) should be stored between 2 to 30°C (35.6 to 86°F).

(2) Expiration date of this kit is 24 months after its manufacture date.

Limitations of the method

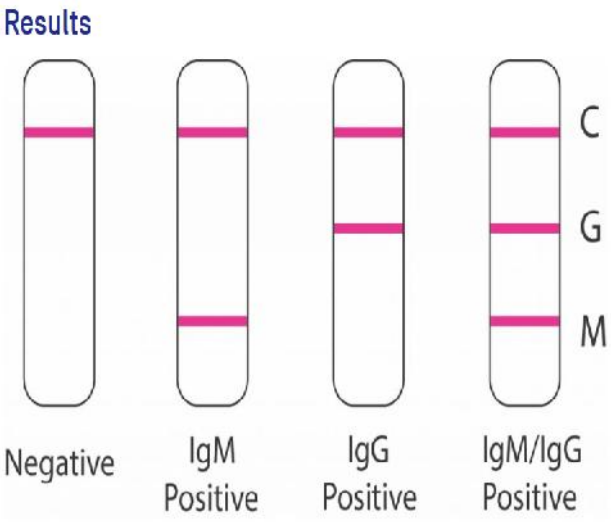
Acco COVID-19 IgM/IgG is designed for primary the screening of IgM and IgG antibodies against COVID-19. This kit can provide a fast and simple results but, do not completely exclude the possibilities of false positive or false negative results caused by various factors. For confirmation, please make a final decision with clinical symptoms, other testing results, and doctor's assessment, collectively.

Precautions

1. For *in vitro* diagnostic use only. Do not use after expiry date.
2. Do not eat, drink or smoke in the area where the specimens or kits are handled.
3. Do not use test if pouch is damaged.
4. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
5. The used test should be discarded according to local regulations.
6. Keep out of the reach of children.

Display- Results/Expected Values :

immunochromatography based



A total of three detection lines are possible, with the control (C) line appearing when sample has been flowed through the cassette.

1) **Negative Result** : If only the quality control line (C) appears and the detection lines G and M are not visible, then no novel coronavirus antibody has been detected . Result is **negative**.

2 **) Positive Result**, M only : If both the quality control line (C) and the detection line M appears, then the novel coronavirus IgM antibody has been detected. Result is **positive for the IgM antibody**.

3 **) Positive Result**, G only: If both the quality control line (C) and the detection line G appears, then the novel coronavirus IgG antibody has been detected. Result is **positive for the IgG antibody.**

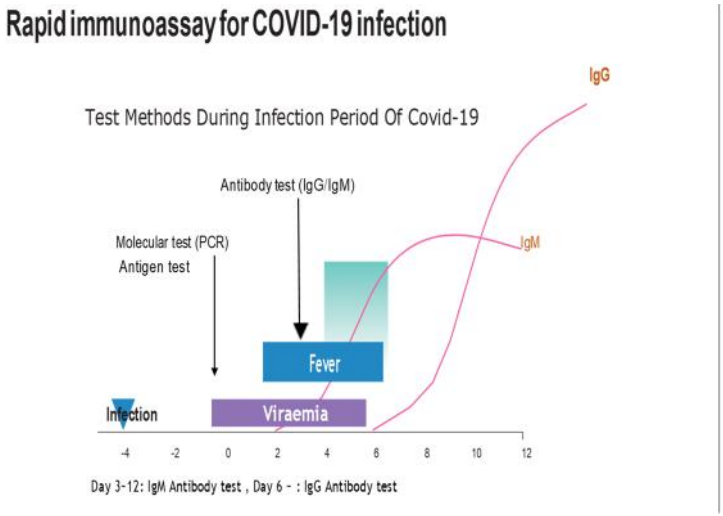
4) **Positive Result**, G and M:• If the quality control line (C) and both detection lines G and M appear, then the novel coronavirus IgG and IgM antibodies have been detected. Result is **positive for both the IgG and IgM antibodies.**

Diagnostic Accuracy of Acco COVID-19 IgM/IgG

	Molecular Testing (RT-PCR)	Acco COVID-19 IgG/IgM Rapid Test
Principle	Nucleic acid test of COVID-19	Antibody (IgM & IgG) detection in the blood
Accuracy in the fields	-China: 30 ~ 50% (Jungangilbo.2020.02.13) -Depending on the swab positioning of specimen and yield of gene extraction	-Before Day 5: very low -After Day 5: 50~81% for IgM, 81~100% for IgG
Test time	>6 hours	10 minutes
Test cost	Very expensive	Economic
Users	Skilled & trained	Normal
Specimen	Throat, anal, nasopharyngeal, sputum	Whole blood, serum, plasma
Test capacity	Limited	Possible to bulk testing
Adv/disadvantages	• Good accurate at early stage. Difficult to detect at latent or asymptomatic period. • Appropriate for early stage with limited cases of patients	• Possible to detect at latent or asymptomatic period. Inaccurate at from Day 0 to Day 5 after infection • Appropriate for 5 day-after with bulk cases of patients

Comparison With Molecular Testing

Parameters	Performance (Ongoing)	Comments
Analytical sensitivity	1.84 s/CO for IgM 1.57 s/CO for IgG	w. ELISA
Sensitivity	Day 3 after symptom: IgM- 30%, IgG- 0% After Day 7 from symptom: IgM- 80%, IgG- >95%	w. limited cases
Specificity	IgM 98% (118/120), IgG: 99% (119/120)	



Interpretation Method

Molecular test	Antibody test		Interpretation
	IgM	IgG	
Positive	Negative	Negative	Acute infection (D1 ~ D3)
Positive	Positive	Negative	Acute infection (D3 ~ D8)
Positive	Positive	Positive	Infected (D8 ~ D15)
Positive	Negative	Positive	Infected (>D15) or secondary infected
Negative	Positive	Negative	Early stage of infection. Need the additional molecular test
Negative	Positive	Positive	Infection (D8~D15), Need the additional molecular test
Negative	Negative	Positive	Passed infection or (if IgG negative change to IgG positive) infected
Negative	Negative	Negative	Not infected