COVID-19 ANTIBODY TEST

Screens COVID-19 IgG and IgM antibodies

Intended use

The Rapid COVID-19 IgM/IgG Antibody Screen Test is a rapid visual qualitative detection of COVID-19 IgM/IgG antibodies in fingerstick whole blood, sera and plasma as an aid in the diagnosis of SARS-CoV-2 infection. The tests are available in Cassette format. It is intended for prescription use only (for laboratories or healthcare workers at the point-of-care).

Summary

SARS-CoV-2 is a novel beta coronavirus that causes coronavirus disease 2019 (COVID-19). It belongs to the family of Coronaviruses,named for the crown-like spikes on their surface. There are four (4) main sub-groupings of coronaviruses, alpha, beta, gamma, and delta. Common human coronaviruses are 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) and HKU1 (beta coronavirus).
These viruses typically cause mild to moderate upper-respiratory tract illnesses, like the common cold. Other human coronaviruses such as MERS-CoV and SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome or SARS) have caused more severe respiratory illness with higher rates of morbidity and mortality. SARS-CoV-2 caused an outbreak in December 2019 in Wuhan City, Hubei Province, China and has spread globally since.

Patients with COVID-19 report mild to severe respiratory illness with symptoms of fever, cough and shortness of breath.

After the infection, the body develops IgM antibodies that mainly exist in the blood from a few hours to several days. IgM antibodies appear (Symptomatic 3-5 days, asymptomatic 7 days) and gradually disappear earlier than IgG antibodies, while IgG antibodies are relatively longstanding.

The Rapid COVID-19 IgM/IgG Antibody Screen Test utilizes indirect immunoassay to detect COVID-19 IgM/IgG antibodies qualitatively and selectively in serum, plasma or whole blood.

Principle

The Rapid COVID-19 IgM/IgG Antibody Screen Test is a qualitative membrane strip-based immunoassay for the detection of COVID-19 IgM/IgG antibody in serum, plasma or whole blood. Anti-human IgM or anti-human IgG antibody is immobilized in the test line region of the test. After a serum, plasma, or whole blood specimen is added in the specimen well, it reacts with COVID-19 antigen coated particles that have been applied to the specimen pad. This mixture migrates chromatographically along the test strip and interacts with the immobilized anti-human IgM or IgG. If the specimen contains COVID-19 IgM/IgG antibodies, a colored band will appear in the test line region indicating a positive result. If the specimen does not contain COVID-19 IgM or IgG antibodies, no colored band would appear in this region indicating a negative result. To serve as a procedural control, a colored band will appear at the control line region as indication that a sufficient volume of specimen has been added and membrane wicking has occurred.
REAGENTS AND MATERIALS SUPPLIED

Reagents and materials supplied

One kit contains:
- 25 test devices
- 25 transfer pipets
- One package insert
- Buffer

Materials required but not supplied

- Specimen collection containers
- Timer
- Centrifuge (for plasma only)
- Lancets / antiseptic wipes (for fingerprick whole blood)

Storage and Stability

Store test kit at room temperature (4-30°C or 39-86°F) in the sealed pouch. Kit contents are stable until the expiration date.
DO NOT FREEZE.

Precautions

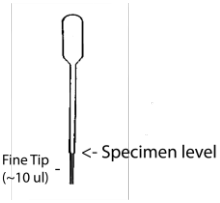
- FOR IN VITRO DIAGNOSTIC USE ONLY。
- Do not use it after the expiration date.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the entire procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature may adversely affect results.

Specimen collection and storage

- The Rapid COVID-19 IgM/IgG Antibody Screen Test can be performed with fingerstick whole blood, serum and plasma.
- Follow standard laboratory procedures to obtain specimen.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used. Lipemic, icteric, or hemolyzed specimens may give inconsistent test results. Specimens of containing precipitate should be clarified prior to testing.
- Testing should be performed immediately after the specimens are collected. Do not leave the specimens at 4-30°C for prolonged periods. Fingerstick whole blood should be tested immediately. Whole blood may be stored at 2-8°C for up to 3 days, serum and plasma may be stored at 2-8°C for up to 14 days. For long-term storage, serum and plasma specimens should be kept below -20°C.
- Bring specimens to room temperature (4-30°C) prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with national regulations covering the transportation of etiologic agents.

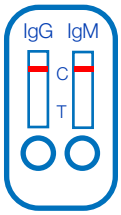
Test Procedure

Allow the test device, buffer and specimen to equilibrate to room temperature (4-30°C) prior to testing.
- Review specimen collection instructions.
- Remove the test cassettes from its protective pouch (bring the sealed pouch to room temperature before opening to avoid condensation of moisture on the membrane). Label the cassettes with patient or control identifications.
- Add the specimen to each sample well:
- Transfer Pipette: Hold the provided transfer pipet in vertical position and draw a volume (~10 ul) to the specimen level (length of the fine tip)
- Transfer 1 drop (10 µl) of fingerstick blood or serum or plasma sample into each sample well.



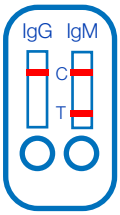
- Immediately add 2 drops (~100 µl total) of Buffer to each well.
- Start the timer.
- Wait for the red line(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 15 minutes.

Interpretation of results



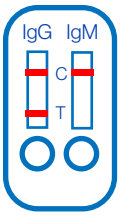
Negative

The control line appears in the window, but the test line is not visible.

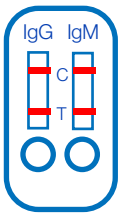


Positive

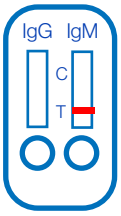
The colored Control bands present and any of a colored band in test windows is visible, It is positive (The IgM test is positive). **The intensity of the test line may be less than that of the control line, which still means positive result.**



The sample device demonstrates that IgG test is positive, and the IgM test shows negative result.



The sample device demonstrates that both IgG and IgM tests are positive.



Invalid

The test is invalid if no control line is visible at five minutes. The test failed, or the test procedure was not followed properly. Verify the test procedure and repeat the test with a new test device.

NOTE: The intensity of the colored lines (bands) in the test line region (T) will vary depending on the concentration of COVID-19 IgM/IgG antibodies present in the specimen. Therefore, any shade

Limitations

•The Rapid COVID-19 IgM/IgG Antibody Screen Test is for in vitro diagnostic and professional use only.
•Neither the quantitative value nor the rate of increase in COVID-19 IgM/IgG antibodies can be determined by this qualitative test.
•The Rapid COVID-19 IgM/IgG Antibody Screen Test only indicates presence of COVID-19 IgM/IgG antibodies in the specimen or not. It should not be used as the sole criteria for the diagnosis of COVID-19 infection.
•A diagnostic decision must be made together with other available clinical information and results from laboratory tests to the physician.
•A negative result does not exclude the possibility of COVID-19 infection, particularly in those who have been in contact with the virus. Repeat testing should be performed, after three (3) to five (5) days for symptomatic individual. If the test result is still negative and clinical symptoms are persisting, a further diagnostic testing with other clinical diagnostic tests are recommended.

•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.
•This test has not been reviewed by the FDA. The test is submitted for FDA EUA approval.
•Not for the screening of donated blood.

Quality Control

An internal procedural control is built in the test. A colored band appearing in the control region is considered as an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standard solutions are not provided in the kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Performance characteristics

Accuracy (Clinical Agreement Study):

The Rapid COVID-19 IgM/IgG antibody Screen test was performed in comparison with RT-PCR assays and clinical diagnostics. The specimen matrix of the clinical specimens is plasma. For IgM antibody test, 126 clinical confirmed samples (77 positive samples and 49 negative samples) were tested. For COVID-19 IgG antibody test, forty-nine (49) negative specimens and forty-three (43) clinical confirmed positive samples (by both RT-PCR assays and clinical diagnostic methods) that were collected from the patients in convalescence period were tested in blind study by laboratory professionals. The results were summarized in tables below:

IgM Antibody Test

Items		Comparator Method (RT-PCR)	
		Positive	Negative
COVID-19 IgM Antibody Test	Positive	72	0
	Negative	5	49
Total		77	49

Sensitivity or PPA = 93.51 % Specificity or NPA =100 %

IgG Antibody Test

Items		Comparator Method (RT-PCR and Clinical Diagnostics)	
		Positive	Negative
COVID-19 IgG Antibody Test	Positive	41	0
	Negative	2	49
Total		43	49

Sensitivity or PPA = 95.35 % Specificity or NPA =100 %

Sensitivity:

The test samples were prepared using confirmed COVID-19 IgM/IgG negative serum sequence dilute the confirmed COVID-19 IgM/IgG positive pooled serum specimens from 1:2 to 1:256, respectively. The test results are summarized in table below:

IgM Test:

Sample #	Dilution							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
# 1	+ 20/20	+ 20/20	+ 19/20	+ 18/20	+ 18/20	+ 18/20	- 18/20	- 20/20
# 2	+ 20/20	+ 20/20	+ 19/20	+ 19/20	+ 18/20	+ 18/20	- 19/20	- 20/20
# 3	+ 20/20	+ 20/20	+ 18/20	+ 19/20	+ 19/20	+ 18/20	- 19/20	- 20/20

IgG Test:

Sample #	Dilution							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
# 1	+ 20/20	+ 20/20	+ 18/20	+ 20/20	+ 18/20	+ 19/20	- 18/20	- 20/20
# 2	+ 20/20	+ 20/20	+ 19/20	+ 19/20	+ 18/20	+ 19/20	- 18/20	- 20/20
# 3	+ 20/20	+ 20/20	+ 19/20	+ 20/20	+ 19/20	+ 18/20	- 19/20	- 20/20

Conclusion:

The lowest of detection limit of Rapid COVID-19 IgM Antibody Screen Test is 1:64 dilution for positive serum specimen. The IgG antibody detection is 98.3% at 1:64 dilution.

Specificity

Cross-Reactivity:

The Rapid COVID-19 IgM/IgG Antibody Screen Test is specifically to detect COVID-19 IgM / IgG antibodies. It does not cross-react with any of the following disease or infectious agents:

Anti-influenza A (IgM and IgG)
Anti-influenza B (IgM and IgG)
Anti-HCV (IgM and IgG)
Anti-HBV (IgM and IgG)
ANA (IgM and IgG)
Anti-respiratory syncytial virus (IgG and IgM)
Anti-Haemophilus influenzae (IgG and IgM)
RF (*Rheumatoid factor*) (IgG and IgM)
H1N1
H3N2
H5N1
H7N9
Influenza B Yamagata
Influenza B Victoria
RSV
Rhinovirus
Adenovirus
EBv
Measles virus
HCMV
Rv
Norovirus
Mumps virus
Varicella zoster virus
HKU1
OC43
NL63
229E
Mycoplasma pneumoniae

Interference:

The study was performed by spiking each of the following substance into the negative controls and tested with Rapid COVID-19 IgM/IgG Antibody Screen Test in duplicate each sample.

Compounds	Concentration
Bilirubin	200 mg/L
Cholesterol	2500 mg/L
Triglyceride	2500 mg/L
Hemoglobin	25g/L
Human Hemoglobin	30 mg/g
Human Blood	200 µL/g
Interferon-alpha	2 mg/g
Zanamivir	2 mg/g
Ribavirin	2 mg/g
Oseltamivir	2mg/g
Peramivir	2mg/g
Lopinavir	2mg/g
Ritonvir	2mg/g
Arbidol	2mg/g
Barium Sulphate	20mg/g
Bismuth (III) Subsalicylate	0.7mg/g
Calcium Carbonate	20 mg/g
Cimetidine	2 mg/g
Mucin	14 mg/g
Omeprazole	2 mg/g
Palmitic Acid	16 mg/g
Ranitidine Hydrochloride	2 mg/g
Stearic Acid	16 mg/g

Precision (Reproducibility):

This precision is determined by using 10 replicates each of the three (3) lot in three (3) clinical sites by three (3) trained professionals. The results are summarized in tables below:

IgM test

	LOT / Results	R202002604 Operator 1	R202002605 Operator 2	R202002606 Operator 3
Negative control		n=10		
R1	# Positive	0	0	0
	# Negative	10	10	10

	LOT / Results	R202002604 Operator 1	R202002605 Operator 2	R202002606 Operator 3
Positive control		n=10		
R2	# Positive	10	10	10
	# Negative	0	0	0

	LOT / Results	R202002604 Operator 1	R202002605O Operator 2	R202002606 Operator 3
Positive control		n=10		
R3	# Positive	10	10	10
	# Negative	0	0	0

IgG Test

	LOT / Results	R202002604 Operator 1	R202002605 Operator 2	R202002606 Operator 3
$R1_2$		n=10		
	# Positive	0	0	0
	# Negative	10	10	10

	LOT / Results	R202002604 Operator 1	R202002605 Operator 2	R202002606 Operator 3
$R2_2$		n=10		
	# Positive	10	10	10
	# Negative	0	0	0

	LOT / Results	R202002604 Operator 1	R202002605 Operator 2	R202002606 Operator 3
$R3_2$		n=10		
	# Positive	10	10	10
	#Negative	0	0	0

The results demonstrate the consistency of lot-to-lot and operator-to-operator runs.

Reference

1. Drosten, C., S. Gunthe, W. Preiser, et al. 2003. Identification of a novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. 348:1967–1976
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3. Kazutaka Katoh, John Rozewicki, Kazunori D Yamada, MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization, Briefings in Bioinformatics, Volume 20, Issue 4, July 2019, Pages 1160–1166
4. Chakraborty, Supriyo, Nag, Debojyoti, Mazumder, Tarikul Huda, Uddin, Arif, Codon usage pattern and prediction of gene expression level in Bungarus species, Gene (2016), doi:10.1016/j. gene. 2016.11. 023

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