PathFlow® SARS-CoV-2 IgG rapid test (EN)

MICROGEN BIOPRODUCTS

Instructions for Use

A rapid test for the qualitative detection of IgG antibodies to SARS-CoV-2 S-RBD and/or N protein in human whole blood, serum, or plasma specimen.
For professional in vitro diagnostic use only.

INTENDED USE

The PathFlow® SARS-CoV-2 IgG rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of IgG antibodies to SARS-CoV-2 spike (S) protein receptor binding domain (RBD) and/or Nucleocapsid protein in human whole blood, serum, or plasma specimens. The PathFlow® SARS-CoV-2 IgG rapid test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2.

SUMMARY

SARS-CoV-2 belongs to the *Coronaviridae* family and is closely related to the SARS coronavirus that appeared in 2002/2003 [1]. The virus was first identified in humans in Wuhan, China, at the end of 2019 and is transmissible from person to person [2]. The main route of transmission is droplet infection, but infections via aerosols and smear have also been described [2-4]. The incubation period is usually three to seven days, up to a maximum of fourteen days [2]. SARS-CoV-2 infected patients are often asymptomatic or experience only mild symptoms such as a dry cough, fever, and shortness of breath [3,4]. Some of the infected persons develop a severe pneumonia, which can lead to death.

PRINCIPLE

The PathFlow® SARS-CoV-2 IgG rapid test is a qualitative membrane-based immunoassay for the detection of IgG antibodies to SARS-CoV-2 in whole blood, serum, or plasma specimens.

Anti-human IgG are coated within the test line region. During testing, the specimen reacts with SARS-CoV-2 antigen-coated particles in the test. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with anti-human IgG present at within the test line region. If the specimen contains IgG antibodies to SARS-CoV-2, a coloured line will appear in test line region as a result of this. If the specimen does not contain IgG antibodies to SARS-CoV-2, a coloured line will fail to appear in the test line region, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains anti-human IgG as the capture reagent, SARS-CoV-2 antigen as the detection reagent. A goat anti-mouse IgG is employed in the control line system.

MATERIALS

Provided: Test cassettes, package insert, extraction buffer, droppers, procedure card, sterile lancets, and alcohol pads.

Not Provided but required: Timer, centrifuge (plasma/serum samples), capillary tubes, pipettes, and specimen collection containers.

WARNINGS AND PRECAUTIONS

- The package insert must be read & understood completely before performing the test. Failure to comply with procedures outlined may yield inaccurate test results.
- For professional *in vitro* diagnostic use only. Do not use after expiration.
- The test cassette should remain in the sealed pouch until use. Do not use if the pouch is damaged.
- Do not eat, drink, or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all 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.
- The used test materials should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.
- Please ensure that an appropriate amount of sample is used for testing. Too much or too little sample size may lead to deviation of results.

STORAGE AND STABILITY

- Store as packaged at room temperature or refrigerated (2-30°C).
- The test is stable until the expiration date printed on cassette pouch.
- The test must remain in the sealed pouch until use.
- **DO NOT FREEZE**
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The PathFlow® SARS-CoV-2 IgG rapid test can be performed using whole blood (from venepuncture or fingerstick), serum or plasma.
- Separate serum or plasma from blood as soon as possible to avoid haemolysis. Use only clear non-haemolysed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days. For long term storage, serum/plasma specimens should be kept below -20°C. Whole blood collected by venepuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature 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 local regulations covering the transportation of etiological agents.
- EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimens.

DIRECTIONS FOR USE

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

Remove the test cassette from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed immediately after opening the foil pouch.

1. Remove the test from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Place the test on a clean and level surface.
3. Add Specimen and Buffer to the test cassette and read results according to the illustrations below.

Note: When adding specimen and buffer, add them to their specified wells only. Do not use the buffer beyond 6 months after opening the vial.

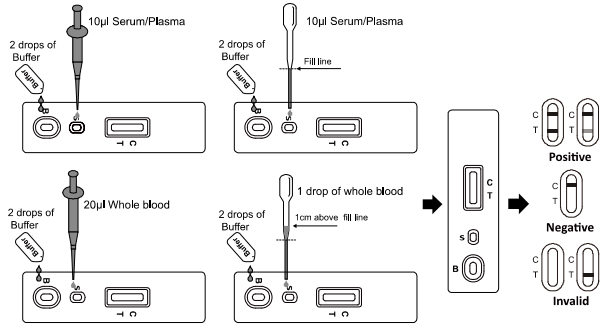
For Venous Whole Blood Specimen, Serum or Plasma Specimen

For Serum or Plasma specimens:

- Use a dropper: Hold the dropper vertically, draw the specimen to the fill line (approximately 10µL), and transfer the specimen to the **Specimen well (S)**, then add **2 drops of buffer** (approximately 80 µL) to **Buffer well (B)**, and start the timer.
- To use a pipette: To transfer **10µL** of specimen to the **Specimen well (S)**, then add **2 drops of buffer** (approximately 80µL) to **Buffer well (B)** and start the timer.

For Venous Whole Blood specimen:

- Use a dropper: Hold the dropper vertically, draw the specimen about **1 cm above the fill line** and transfer **1 full drop** (approx. 20µL) of specimen to the **Specimen well (S)**. Then **add 2 drops of buffer** (approximately 80µL) to **Buffer well (B)** and start the timer.
- Use a pipette: To transfer **20µL** of whole blood to the **Specimen well (S)**, then add **2 drops of buffer** (approximately 80µL) to **Buffer well (B)** and start the timer.



Wait for the coloured line(s) to appear. **Read results at 10 minutes**. Do not interpret the result after 20 minutes.

For Fingerstick Whole Blood specimen:

1. Use alcohol pad to clean the fingertip of the middle finger or ring finger as the puncture site.
2. Carefully rotate and pull off the lancet cap.
3. Push the sterile lancet firmly into the fingertip of the middle finger.
4. Wipe off the first drop of blood. To increase blood flow, use the thumb and forefinger to gently apply pressure around the puncture site.
5. Hold the dropper vertically, draw the blood to **1cm above the fill line** and transfer **1 full drop of blood** (approximately 20µL) **to the specimen well (S)**, then add **2 drops of buffer** (approximately 80 µL) **to the buffer well (B)**, and start the timer.
6. Wait for the coloured line(s) to appear. **Read results at 10 minutes**. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE: *Two distinct coloured lines appear. One coloured line should be present in the control line region (C) and another in the test region (T).

***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentration of IgG antibodies to SARS-CoV-2 present in the specimen.

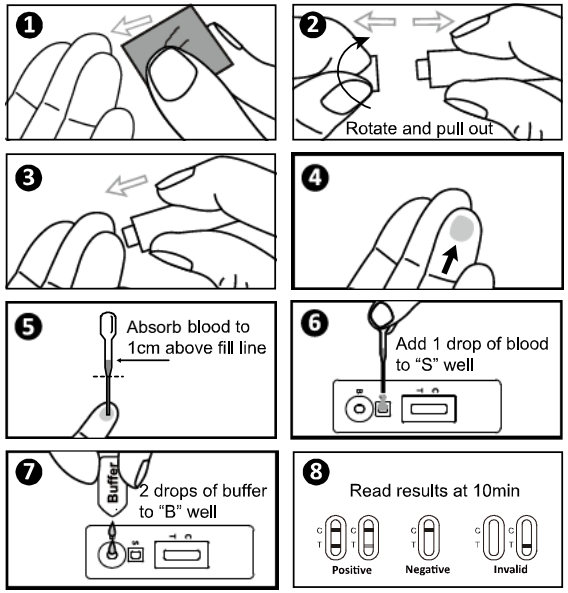
Any shade of colour in the test line region should be considered positive.

NEGATIVE: One coloured line appears in the control line region (C). No line appears in the test line region (T).

INVALID: Colour line (C) fails to appear. Insufficient specimen volume or incorrect procedural technique are the most likely reasons for control line failure.

Review the procedure and repeat the test with a new cassette.

If the problem persists, discontinue using the cassette immediately and contact your local distributor.



QUALITY CONTROL

Internal procedural controls are included in the test. A coloured line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this 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 [5].

LIMITATIONS

1. The test procedure and the interpretation of test result must be followed closely when testing for the presence of SARS-CoV-2 virus specific antibodies. For optimal test performance, proper sample collection is critical. Failure to follow the procedure may give inaccurate results.
2. The PathFlow® SARS-CoV-2 IgG rapid test is for *in vitro* diagnostic use only. Neither the quantitative value nor the rate of increase in the concentration of IgG antibodies to SARS-CoV-2 can be determined by this qualitative test.
3. The PathFlow® SARS-CoV-2 IgG rapid test will only indicate the presence of IgG antibodies to SARS-CoV-2 in the specimen.
4. The results obtained with the test should be considered with other clinical findings from other laboratory tests and evaluations.
5. The haematocrit level of the whole blood can affect the test results. Haematocrit level needs to be between 25% and 65% for accurate results.
6. The test will show negative results under the following conditions: The titre of the novel coronavirus antibodies in the sample is lower than the minimum detection limit of the test, or the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody utilized in the test, or the novel coronavirus antibody has not appeared at the time of sample collection. It is recommended to re-sample the patient a few days later and test again.
7. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
8. Results from immunosuppressed patients should be interpreted with caution.
9. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains or other interference factors.
10. Not for the screening of donated blood.

PERFORMANCE CHARACTERISTICS

The PathFlow® SARS-CoV-2 IgG rapid test was compared with RT-PCR/verification panel/CLIA results; the results are tabulated below.

Clinical study for immune response to previous SARS-CoV-2 infection:

Method		PCR (n=350) & Panel (n=111)		Total Result
PathFlow® SARS-CoV-2 IgG	Results	Positive	Negative	
	Positive	236	2	238
	Negative	6	217	223
Total Result		242	219	461

Relative sensitivity: 98% (95%CI*: 92.0%~98.6%)

Relative specificity: 99% (95%CI*: 96.3%~99.9%)

Accuracy: 98% (95%CI*: 95.6%~99.0%)

Clinical study for immune response to vaccination:

Method		CLIA		Total Result
PathFlow® SARS-CoV-2 IgG	Results	Positive	Negative	
	Positive	22	0	22
	Negative	1	25	26
Total Result		23	25	48

Relative sensitivity: 96% (95%CI*: 78.1%~99.9%)

Relative specificity: 100% (95%CI*: 86.3%~100%)

Accuracy: 98% (95%CI*: 88.9%~99.9%)

INTRA-ASSAY

Within-run precision has been determined by using 3 replicates of two specimens: a negative and an IgG positive. The specimens were correctly identified >99% of the time.

INTER-ASSAY

Between-run precision has been determined by 3 independent assays on the same two specimens: a negative and an IgG positive. Three different lots of the PathFlow® SARS-CoV-2 IgG rapid test have been tested using these over a 3-day period. The specimens were correctly identified >99% of the time.

CROSS-REACTIVITY

The PathFlow® SARS-CoV-2 IgG rapid test has been tested for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, Anti-Measles, HAMA, RF, non-specific IgG, non-specific IgM, anti-EV71, anti-Parainfluenza virus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV, and anti-HCV positive specimens. The results showed no cross-reactivity.

INTERFERING SUBSTANCES

The following compounds have been tested using the PathFlow® SARS-CoV-2 IgG rapid test and no interference was observed.

Triglyceride: 100mg/dL
Bilirubin: 60mg/dL
Ascorbic Acid: 20mg/dL
Total cholesterol: 15mmol/L
Haemoglobin: 1000mg/dL

BIBLIOGRAPHY

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3. Udugama et al., Diagnosing COVID-19: The Disease and Tools for Detection. ACS Nano. 2020;14(4):3822–35.
4. Xiao et al., Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020;92(5):464–7.
5. Westgard JO, Barry PL,Hunt MR, Groth T. A multi-rule Shewhart for quality control in clinical chemistry, Clinical Chemistry 1981;27: 493-501

TABLE OF SYMBOLS

IVD	For *in vitro* diagnostic use only	Σ	Tests per kit	EC REP	Authorized representative
2°C – 30°C	Store between 2-30°C		Use by		Do not reuse
	Do not use if package is damaged	LOT	Lot Number	REF	Catalogue number
	Manufacturer		Consult instructions for use		

This document applies to the following product codes: M598CE.

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