AMP Rapid Test SARS-CoV-2 GM Cassette

Technical Documentation

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1. Product Description

1.1 Order Information

AMP Rapid Test SARS-CoV-2 GM Cassette is available in the following commercial units:

Part Number	Product Denomination	Sample Type	Contents
RT2941	AMP Rapid Test SARS-CoV-2 GM	WB, Serum, Plasma	10 Tests
RT2942	AMP Rapid Test SARS-CoV-2 GM	WB, Serum, Plasma	25 Tests
RT2945	AMP Rapid Test SARS-CoV-2 GM	WB, Serum, Plasma	50 Tests

1.2 Kit Composition

Each of the aforementioned commercial kits of AMP Rapid Test SARS-CoV-2 IgG/IgM Cassette consists of:

- Kit box (made of cardboard)
- Kit label

  Label contents:

  - Part number
  - Product Denomination
  - Kit contents
  - Storage conditions
  - Lot number
  - Expiry date
  - CE mark
  - IVD symbol
  - Reference to Instructions for Use
- Respective number of test cassettes, each separately packed in sealed foil pouch

  The foil pouch also contains dessicant to ensure dry storage of the test cassette
- Buffer
- Disposable pipette
- Instructions for use
- Kit seal

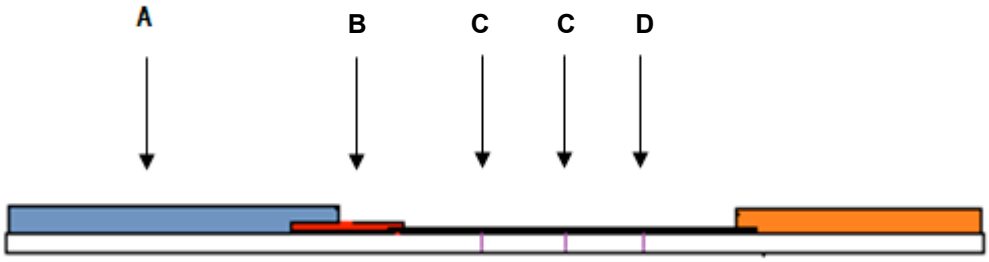
2. Test Description

2.1 Test Principle

AMP Rapid Test SARS-CoV-2 IgG/IgM is a rapid chromatographic immunoassay for qualitative detection of IgG and IgM antibodies to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in human whole blood, serum and plasma as an aid in diagnosis of Coronavirus (COVID-19) infection. It utilizes a combination of recombinant SARS-CoV-2 antigens conjugated with colloid gold, anti-human IgG, anti-human IgM, rabbit IgG gold conjugate and goat anti-rabbit IgG to selectively detect IgG and IgM antibodies to SARS-CoV-2 in whole blood, serum or plasma.

The test is performed by applying the whole blood, serum or plasma to the sample well of the cassette and observing the formation of colored lines.

If present in the sample, IgG and IgM antibodies to SARS-CoV-2 react with SARS-CoV-2 conjugate and are captured by anti-human IgG or IgM particles with the formation of a colored line in the IgG/IgM Test (T) region. The presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of sample has been added and membrane wicking has occurred.



The sample (A) migrates via capillary action along the membrane to react with the gold conjugate (B). IgG and IgM antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 conjugate, forming a colored antibody-antigen complex. Anti-human IgM and IgG immobilized in the test zones capture the test region (C). The formation of visible colored lines in the test regions indicates a positive result (C). The absence of a colored line in the test region indicates a negative result. In the control region of the membrane, immobilized goat anti-rabbit IgG captures colored conjugate regardless of the sample composition. The visible colored line (D) developed in the control region confirms correct performance of the test.

For convenient, reliable and safe performance of the test the test strip is mounted inside the cassette housing. This enables convenient application of the correct sample volume to the sample well. Test and Control line will appear in the results window of the cassette and are marked accordingly on the cassette for easy identification.

2.2 Test Composition

The test strip contains:

- Goat anti-rabbit IgG
- Anti-human IgG (Capture)
- Recombinant SARS-CoV-2 antigen (Detection)
- Sample pad
- Rabbit IgG gold conjugate
- Anti-human IgM (Capture)
- NC membrane
- Absorbant pad

- Label pad
- Buffer
- Plastic card

The test strip is mounted inside the plastic cassette and the cassette is packed together with a single use pipette and desiccant in a tamper-proof foil pouch.

2.3 Test Procedure

2.3.1 Sample Collection and Storage

Whole blood collected either from finger stick or by venipuncture, as well as serum and plasma can be used to perform the test.

Finger stick:

1. Clean puncture site with alcohol swab and allow to dry.
2. Massage hand without touching the puncture site to stimulate perfusion.
3. Puncture site with sterile lancet and wipe off first sign of blood.
4. Gently rub hand from wrist via palm to finger to form a round drop of blood over puncture site.

Whole blood sample collected by finger stick is to be tested immediately.

Venipuncture:

Collecting whole blood use EDTA, heparin or sodium citrate as anticoagulant. Separate serum or plasma as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed sample.

Perform test as soon as possible. Do not leave sample at room temperature for a prolonged period of time.

Whole blood: may be stored at 2-8°C for up to 2 days

Do not freeze whole blood samples

Serum or plasma: may be stored at 2-8°C for up to 3 days

For long term storage keep sample below -20°C

Bring sample to room temperature prior to testing. Frozen samples must be completely thawed and mixed well prior to testing. Samples should not be frozen and thawed repeatedly.

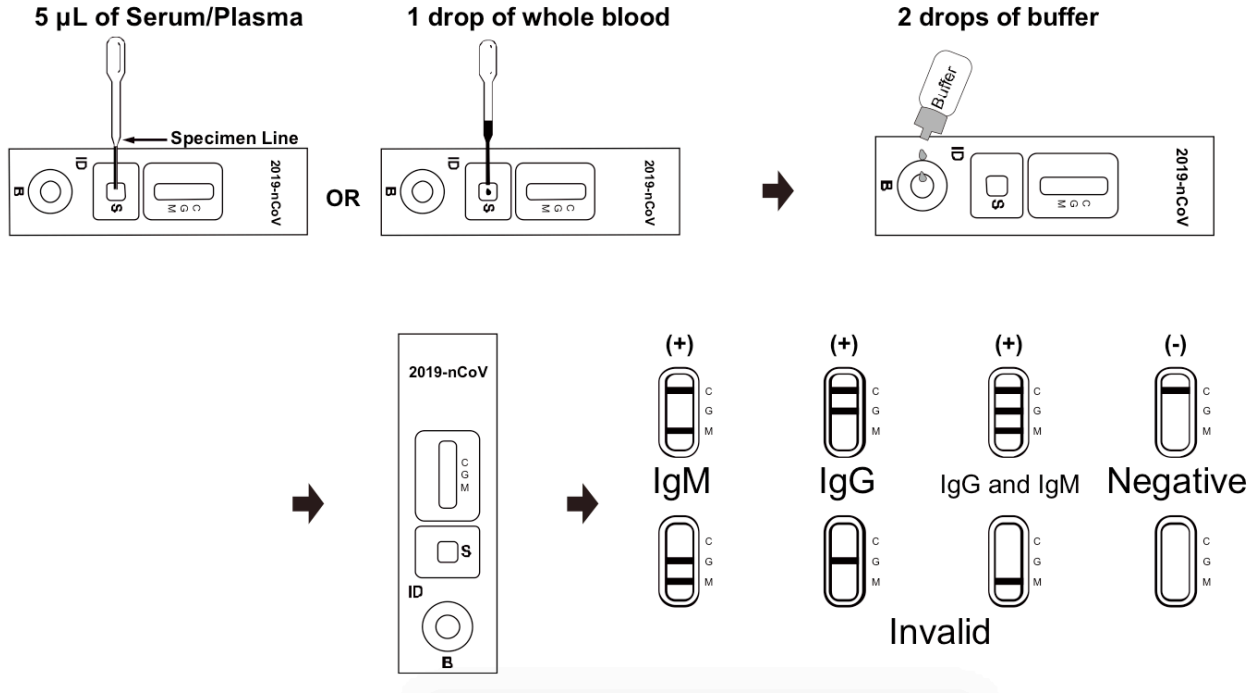
2.3.2 Test Procedure

Test cassette, buffer and sample must be at room temperature (15-30°C) prior to testing.

1. Remove test cassette from the foil pouch and place it on a flat and clean surface.
   For best results, the assay should be performed within one hour.
2. *Serum or plasma:*
   Hold disposable pipette vertically and draw sample up to the sample line (**5 µL**), transfer to the sample well (S) of the cassette and **add 2 drops of buffer** (appr. 80 µL) to the buffer well (B) immediately. Avoid air bubbles.

   Venipuncture whole blood:
   Hold disposable pipette vertically and draw sample about 1 cm above the fill line (**appr. 10 µL**), transfer to the sample well (S) of the cassette and **add 2 drops of buffer** (appr. 80 µL) to the buffer well (B) immediately. Avoid air bubbles.

3. Wait for the colored lines to appear and read the test result after **10 minutes**.
   IMPORTANT: **Do not read the result after 15 minutes.**



2.3.3 Interpretation of Test Results

IgG Positive (+)

Two colored lines appear on the membrane. One line appears in the Control (C) and another in the IgG Test (T) region.

IgM Positive (+)

Two colored lines appear on the membrane. One line appears in the Control (C) and another in the IgM Test (T) region

IgG and IgM Positive (+)

Three colored lines appear on the membrane. One line appears in the Control (C) and the other two lines appear in the IgG and IgM Test (T) region.

Note: Color intensity of the line appearing in the Test (T) region (IgM and IgG) may vary depending on the concentration of SARS-CoV-2 antibodies in the sample. Therefore, any shade of color in the Test (T) region is to be considered as a positive result.

Negative (-)

Only one colored line appears in the Control (C) region. No colored line appears in the Test (T) region.

Invalid

If a color line is visible only in the Test (T) region or no color line is visible at all the test is invalid and needs to be repeated with a new test cassette.

Note: Insufficient sample volume, incorrect procedure or expired test are most common reasons of invalid results.

2.3.4 Quality Control

Although the test itself includes an internal procedural control use of external controls is highly recommended as part of Good Laboratory Practice to confirm and verify the test procedure and proper performance of the test. Controls are to be tested following the same procedure as applied for patient samples. Positive and negative controls shall give the expected results.

2.3.5 Limitations

This test is for professional *in vitro* diagnostic use and is to be used for qualitative detection of IgG/IgM antibodies to SARS-CoV-2 in human whole blood, serum or plasma samples only.

Fresh samples are to be used whenever possible. Frozen and thawed samples contain particles that can block the membrane. This slows the flow of reagents and can lead to high background color, making the interpretation of results difficult.

Optimal assay performance requires strict adherence to the assay procedure. Deviations may lead to aberrant results.

A negative result indicates absence of detectable anti-SARS-CoV-2 antibodies. However, a negative result does not preclude the possibility of exposure to or infection with SARS-CoV-2.

A negative result can occur if the quantity of anti-SARS-CoV-2 antibodies present in the sample is below the detection limits of the assay, or antibodies are not present during the stage of disease in which a sample is collected.

Some samples containing unusually high titer of heterophile antibodies or rheumatoid factor may affect the results.

As for all diagnostic tests, results must be interpreted by a physician only after all clinical and laboratory findings have been evaluated.

3. Manufacturing Procedure

1) Coat label pad with gold conjugated recombinant SARS-CoV-2 antigen and rabbit IgG gold conjugate.
2) Use spayer to dispense anti-human IgG/IgM and goat anti-rabbit IgG onto the membrane.
3) Assemble test by applying membrane, label pad, absorbent pad, sample pad and antigen pad for test identification to the plastic card in the correct position.
4) Use cutter to cut the plastic card into strips.
5) Place the strip in the foreseen position in the lower part of the test cassette and mount the upper part of the test cassette.
6) Pack cassette, single use pipette and desiccant into the foil pouch and seal the pouch.
7) Test the adequate number of cassettes as defined in the QC protocol for release of the production batch.

4. Performance Data

4.1 Cross Reactivity

Cross reactivity has been tested with HAV, HCV, HBsAg, HIV, RF, Syphilis, H. pylori, Dengue positive samples. Samples were tested in three replicates using tests from three different lots and reading results after 10 minutes.

Sample	Lot no. 20010001			Lot no. 20010002			Lot no. 20010003		
HAV	-	-	-	-	-	-	-	-	-
HCV	-	-	-	-	-	-	-	-	-
HBsAg	-	-	-	-	-	-	-	-	-
HIV	-	-	-	-	-	-	-	-	-
RF	-	-	-	-	-	-	-	-	-
Syphilis	-	-	-	-	-	-	-	-	-
H. pylori	-	-	-	-	-	-	-	-	-
Dengue	-	-	-	-	-	-	-	-	-

Conclusion: There is no cross-reaction with the substance above at 10 minutes.

4.2 Reproducibility

Negative and positive serum and plasma samples were run on 5 consecutive days using the same lot of reagents and test cassettes. Results were read after 10 min.

Day	Negative		IgG Positive		IgM Positive	
	Serum	Plasma	Serum	Plasma	Serum	Plasma
1	-	-	+	+	+	+
2	-	-	+	+	+	+
3	-	-	+	+	+	+
4	-	-	+	+	+	+
5	-	-	+	+	+	+
6	-	-	+	+	+	+
7	-	-	+	+	+	+
8	-	-	+	+	+	+
9	-	-	+	+	+	+
10	-	-	+	+	+	+

Conclusion: Test results confirm that the performance is consistent over ten days' period.

4.3 Interference Study

Analytes were spiked individually, at the concentrations indicated, into negative plasma and serum and positive IgG and IgM plasma and serum samples. The samples were tested in triplicate with tests from 3 different lots. Test results were read after 10 minutes.

IgG Negative Sample		Lot no. 20010001						Lot no. 20010002						Lot no. 20010003					
Analyte	Concentration	Serum			Plasma			Serum			Plasma			Serum			Plasma		
Ascorbic Acid	2000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemoglobin	1000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gentisic Acid	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxalic Acid	60 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bilirubin	1000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetoaminophen	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	200 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Albumin	2000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caffeine	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetylsalicilic Acid	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IgG Positive Sample		Lot no. 20010001						Lot no. 20010002						Lot no. 20010003					
Analyte	Concentration	Serum			Plasma			Serum			Plasma			Serum			Plasma		
Ascorbic Acid	2000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemoglobin	1000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gentisic Acid	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxalic Acid	60 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilirubin	1000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetoaminophen	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Creatinine	200 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Albumin	2000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caffeine	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetylsalicilic Acid	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

IgM Negative Sample		Lot no. 20010001						Lot no. 20010002						Lot no. 20010003					
Analyte	Concentration	Serum			Plasma			Serum			Plasma			Serum			Plasma		
Ascorbic Acid	2000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemoglobin	1000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gentisic Acid	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxalic Acid	60 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bilirubin	1000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetoaminophen	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	200 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IgM Negative Sample		Lot no. 20010001						Lot no. 20010002						Lot no. 20010003					
Analyte	Concentration	Serum			Plasma			Serum			Plasma			Serum			Plasma		
Albumin	2000 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caffeine	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetylsalicilic Acid	20 mg/dl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IgM Positive Sample		Lot no. 20010001						Lot no. 20010002						Lot no. 20010003					
Analyte	Concentration	Serum			Plasma			Serum			Plasma			Serum			Plasma		
Ascorbic Acid	2000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemoglobin	1000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gentisic Acid	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxalic Acid	60 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilirubin	1000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetoaminophen	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Creatinine	200 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Albumin	2000 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caffeine	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetylsalicilic Acid	20 mg/dl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Conclusion: No substances showed any interference with the test..

5. Clinical Studies

5.1 Sensitivity and Specificity

AMP Rapid Test SARS-CoV-2 IgG/IgM was compared with results determined with RT-PCR and confirmed by computer tomography. Results were read at 10 minutes. Sensitivity, specificity and correlation have been found to be as following:

IgM		**RT-PCR**		**Total Results**
AMP Rapid Test SARS-CoV-2 IgG/IgM	Results	Positive	Negative	
	Positive	132	1	133
	Negative	6	37	43
Total Results		138	38	176

Sensitivity: 95.7%
Specificity: 97.3%

IgG		RT-PCR		Total Results
AMP Rapid Test SARS-CoV-2 IgG/IgM	Results	Positive	Negative	
	Positive	44	1	45
	Negative	4	27	31
Total results		48	28	76

Sensitivity: 91.7%
Specificity: 96.4%

Conclusion: Compared with samples confirmed by RT-PCR and computer tomography, AMP Rapid Test SARS-CoV-2 IgG/IgM showed a sensitivity 95.7% and a specificity of 97.3% for IgM and a sensitivity of 91.7% and a specificity of 96,4% for IgG.

5.2 Precision

A study was conducted at three different hospitals by untrained operators using three different lots of AMP Rapid Test SARS-CoV-2 IgG/IgM to demonstrate the within run, between run and between operator precision. Identical sets of samples, containing negative and positive samples were provided to each site.

Lot 20020012	Samples	Site A		Site B		Site C	
		-	+	-	+	-	+
Negative	10	10	0	10	0	10	0
Positive	10	0	10	0	10	0	10

Conclusion: The study confirmed a high precision of AMP Rapid Test SARS-CoV-2 IgG/IgM.

6. Stability Studies

6.1 Accelerated Stability

To evaluate the product shelf life of AMP Rapid Test SARS-CoV-2 IgG/IgM an accelerated stability test was performed. Tests from three different lots have been placed in incubators with a calibrated temperature of 55°C. Relative humidity (RH) inside the incubators was controlled to be 60%.

Tests in 3 replicates have been performed for each lot using negative and SARS-CoV-2 positive samples after 0, 7, 14, 21, 28, 35 and 42 days for the tests kept at 55°C.

Results were as following:

55°C Day	Samples	Lot no. 20010005			Lot no. 20010006			Lot no. 20010007		
0	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
14	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
28	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
35	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
42	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+

Conclusion: AMP Rapid Test SARS-CoV-2 IgG/IgM is stable for 42 days at 55°C. Plotting these stability data on an Arrhenius Plot confirms that the shelf life of AMP Rapid Test is at least 24 months from the date of production.

Accelerated stability study at 45°C is actually ongoing. Data available so far are confirming the data obtained in the study at 55°C.

6.2 Real Time Stability

Real time stability studies have been started and are actually ongoing.

6.3 Transport Stability

Transport stability studies have been started and are actually ongoing.