IFU

Instructions for Use

HARDY DIAGNOSTICS
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Anti-SARS-CoV-2 Rapid Test

Cat no. RTA0203	50 tests	Anti-SARS-CoV-2 Rapid Test, by Autobio

For prescription use only. For *in vitro* diagnostic use only. For Emergency Authorization Use (EUA) only.

INTENDED USE

The Anti-SARS-CoV-2 Rapid Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human plasma from anticoagulated blood (Heparin/ EDTA/ sodium citrate) or serum. The Anti-SARS-CoV-2 Rapid Test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Anti-SARS-CoV-2 Rapid Test should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, to perform moderate or high complexity tests.

Results are for the detection of SARS -CoV-2 antibodies. IgM and IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for Anti-SARS-CoV-2 Rapid Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using a second, different IgG or IgM assay.

The Anti-SARS-CoV-2 Rapid Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY

Coronaviruses are a large family of single-stranded RNA viruses that infect mammals and birds, causing respiratory infection. SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS coronavirus 2 or SARS-CoV-2) causes an infectious disease named COVID-19 (Coronavirus disease 2019). Those affected may develop a fever, dry cough, fatigue and shortness of breath[2]. The results of this test may vary by apparent disease periods by time after symptom onset. It is not yet known when IgM or IgG antibodies specific to the SARS-CoV-2 virus will become detectable during an infection, or how long antibodies persist following infection. Antibodies are produced gradually by the immune response system after infection. The sensitivity of antibody detection is directly related to the time after infection when blood samples are collected.

MEASUREMENT PRINCIPLE

The Autobio Anti-SARS-CoV-2 Rapid Test is based on a one-step capture method. The Cassette contains membranes which are pre-coated with two mouse anti-human monoclonal antibodies (anti-IgG and anti-IgM) on two separated test lines. SARS-CoV-2 recombinant spike protein antigen reagents which can specifically bind to SARS-CoV-2 antibodies (IgM and/or IgG), are bound to colloidal gold and sprayed on conjugation pads. When the sample is applied to the test wells, antibody and labeled antigen complexes are formed and travel up the strip. The labeled gold colorimetric reagent is used to form a visible red/pink line. The presence of anti-SARS-CoV-2 IgM and/or IgG will be indicated by a visible red/pink test line (T) in the IgM and IgG result windows. Anti-SARS-CoV-2 IgM antibodies are bound on the IgM line, and anti-SARS-CoV-2 IgG antibodies ar[illegible] to the IgG line. Membrane is pre-coated with mouse monoclonal anti-SARS-CoV-2 spike protein [illegible]bodies [illegible]e control (C) line. The control (C) line appears in each result window when sample has flow[illegible] through the st[illegible] The Control Line is used as a procedural control. The control line should always appear [illegible]e test procedure [illegible] performed properly and the reagents are working as intended.

COMPONENTS

1. Sample Diluent

   Sample diluent contains MOPS buffer.

Σ	50tests
Sample Diluent	4.5ml

2. Cassette

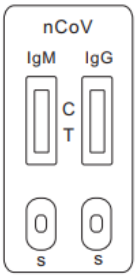
	Σ	
Type		50tes
Cassette	SARS-C (IgM+ Cass	0

   Cassettes are individually sealed in the al[illegible]num foiled [illegible]uch with a desiccant.

   Each Cassette includes two test re[illegible]ns (SARS-CoV-2 IgM and SARS-CoV-2 IgG). (See picture 1)

   Note: The test of SAR[illegible]V-2 IgM [illegible]on the left, and the test of SARS-CoV-2 IgG is on the right. There is no interfere[illegible] between the [illegible]ests [illegible]cause the test membranes are separate.

Picture 1



3. [illegible] copy of instruction for use – included inside each box of cassettes.

STORAGE

1. Store all components at 2-30°C. Do not freeze.
2. The Cassette is stable up to and including the expiration date printed on the outer container. The Cassette should remain in the sealed aluminum foiled pouch until ready for use. It cannot be used beyond the expiration date.
3. Store Sample Diluent at 2-30°C before and after use. It can then be used until the expiration date.

WARNINGS AND PRECAUTIONS

1. For professional use only.
2. Use of this product is limited to laboratories certified under the Clinical Laboratory Improvement Amendment of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests.
3. This test should be performed at 18 to 30°C (64 to 86°F). If stored refrigerated, ensure that the pouched cassette and buffer are brought to operating temperature before performing testing.
4. Follow the instructions for use carefully. Reliability of assay results cannot be guaranteed if there is any deviation from the instructions in this package insert.
5. Professionals must handle the potentially contaminated materials strictly according to local requirements.
6. Do not smoke, drink, eat, or use cosmetics in the working area. Wear Personal Protective Equipment and disposable gloves when working with samples and reagents. Wash hands after operations.
7. Wipe and wash any splashed sample with highly effective disinfectant. Avoid splashing and the formation of aerosols.
8. Use a new clean disposable sample dispensing plastic dropper or tip for every sample to avoid cross contamination.
9. Decontaminate and dispose of all sample, reaction kits, and potentially contaminated materials as if they were infectious waste, in a biohazard waste container.
10. Use the unpacked Cassette as soon as possible to avoid being humidified. The Cassette is sensitive to humidity as well as to heat.
11. Do not use the Cassette beyond the labeled expiry date indicated on the outer container.
12. Do not use the Cassette if the pouch is damaged or the seal is broken.
13. The Cassette cannot be reused.

SPECIMEN COLLECTION AND PREPARATION PROCEDURE

1. Collect specimen in accordance with correct medical practices from the following sources:

   a. <u>Plasma</u>

   Collect the blood into the collection tube (containing anticoagulants such as heparin, EDTA, and sodium citrate) by venipuncture and then centrifuge blood to obtain a plasma sample. Carefully withdraw the plasma into a new pre-labeled tube.

   <u>Serum</u>

   Collect the blood into the collection tube (NOT containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to obtain a serum sample of supernatant. Carefully withdraw the serum into new pre-labeled tube.

2. Sediments and suspended solids in serum or plasma samples may interfere with the test result and should be removed by centrifugation. Ensure that the samples are not contaminated/cloudy prior to use.
3. Incorrect processing of the sample, or sample mixing during transportation, may cause erroneous results.
4. Cap and store the serum or plasma samples at 2-8°C for no more than 24 hours prior to testing. For long-term storage, freeze the serum or plasma samples at -20°C. Avoid multiple freeze-thaw cycles. Mix thawed samples

thoroughly by low speed vortexing or by inverting 10 times. Bring samples to room temperature prior to testing for at least 30 minutes. Visually inspect the samples. If layering or stratification is observed, continue mixing until samples are visibly homogeneous.

5. If proper serum or plasma sample collection and preparation cannot be verified, or particulate matter is observed in the sample, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.

TEST PROCEDURE

Reagent Preparation

1. Bring all reagents, samples and Cassette to room temperature approximately 30 minutes before performing the assay.
2. Remove the Cassette from the aluminum foil pouch and place it on a clean, flat and dry surface.

Cassette Inoculation

1. Identify the Cassette for each sample with the individual's name and/or number.
2. Add 5 µL of the serum or plasma sample into each sample well using a calibrated pipet. Then add 60 µL (2 drops) of the Sample Diluent. For each individual's specimen, use a separate tip and Cassette.
3. Read the test results between 15 and 20 minutes. Do not read the results after 20 minutes.

Note: Sample must be added to both IgG and IgM wells for testing.

INTERPRETATION OF RESULTS

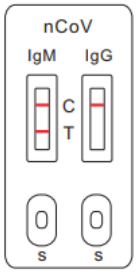
1. **Positive Reactions**

Observe the two colored lines, the control line in the control (C) on both the right and left sides region, and the test line in the Anti-SARS-CoV-2 IgM/IgG test (T) region of the membrane.

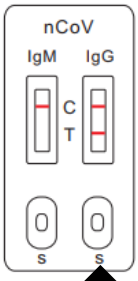
In addition to the presence of both control lines (C), if only the IgM test line (T) appears, the test result indicates the presence of IgM anti-SARS-CoV-2 antibodies.

In addition to the presence of both control lines (C), if only the IgG test line (T) appears, the test result indicates the presence of IgG anti-SARS-CoV-2 antibodies.

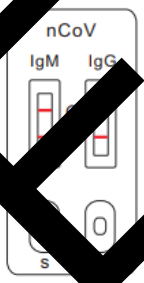
In addition to the presence of both control lines (C), if both IgM and IgG test lines (T) appear, the test result indicates the presence of IgM and IgG anti-SARS-CoV-2 antibodies.



SARS-CoV-2 IgM Positive



SARS-CoV-2 IgG Positive

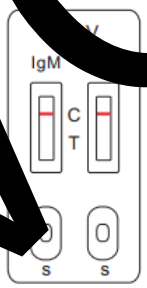


IgG and IgM Positive

Note: The intensity of the color in the test line (T) region will vary. Therefore, any shade of color in the test line (T) region should be considered positive even if faint.

2. **Negative Reaction**

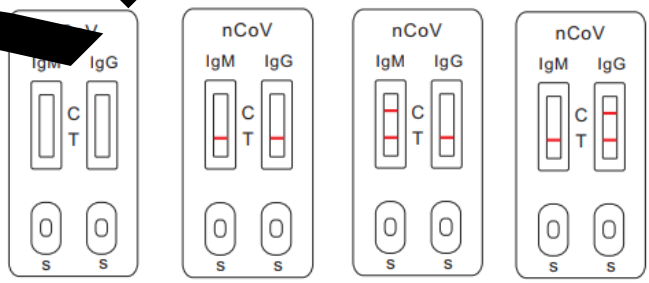
If control lines (C) are present in both result windows and no test lines appear in either IgG or IgM test line regions, the test result is negative for both analytes.



3. **Invalid Reaction**

If control lines (C) do not appear, the test result is invalid regardless of the appearance of the IgM or IgG test line (T).

Some causes of invalid results are: not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the sample be re-tested using a new Cassette.



CONTROL PROCEDURE

An internal procedural control is included in the test. A colored line appearing in the C line is an internal procedural control. It confirms sufficient sample volume, adequate membrane wicking and correct procedural technique.

External positive and negative controls are not supplied with this kit; however, external positive and negative controls should be tested consistent with good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second test of different serology test to confirm an immune response.
2. Do not use with venipuncture whole blood or fingerstick.
3. Reading test results earlier than 15 minutes or later than 20 minutes after the addition of Buffer may yield erroneous results.
4. 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 Anti-SARS-CoV-2 Rapid Test early after infection is unknown. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing methods and clinical findings before a diagnostic determination is made.
5. The test is limited to the qualitative detection of antibodies specific to the SARS-CoV-2 virus. The intensity of the test line does not necessarily correlate to SARS-CoV-2 antibody titer in the specimen.
6. A negative or non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.
7. This test should not be used for screening of donated blood.

CONDITONS OF AUTHORIZATION FOR THE LABORATORY

The Anti-SARS-CoV-2 Rapid Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations-medical-devices-during-covid19#ivd

Authorized laboratories using the Anti-SARS-CoV-2 Rapid Test ("your product" in the conditions below), must adhere to the conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories using your product will include with result reports of your product, all authorized Fact Sheets. Under circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media
2. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
3. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
4. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

5. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Autobio Diagnostics Co. LTD. /Hardy Diagnostics (TechnicalServices@hardydiagnostics.com) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product
7. Authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

   *The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests" as authorized laboratories."

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological and phlebotomy supplies and equipment such as alcohol swabs, centrifuge, sample collection containers, timer, and micropipettes are not provided.

PERFORMANCE CHARACTERISTICS

1. Cross-Reactivity/Analytical Specificity

Cross-reactivity of the Anti-SARS-CoV-2 Rapid Test was evaluated using serum samples containing antibodies to other pathogens. 189 IgM and 189 IgG potential cross-reactant serum samples were tested, no IgM or IgG false positive results were observed with the following potential cross-reactants:

Table 1. Cross-reactivity Results

IgM potential cross-reactant		IgG potential cross-reacta	
Potential cross-reactants	**No. of samples**	**Potential cross-reactants**	**o. of nples**
Influenza A virus (H1N1, H3N2)	18	Influenza A virus (H1N1, H3N2)	
Influenza B virus (Yamagata IgM, Victoria IgM)	18	Influenza B virus (Yamagata IgG, Victoria IgG)	18
Endemic human coronavirus (OC43, 229E))	18	Endemic human coron rus (OC43, 229E))	18
CMV IgM	9	CMV IgG	9
Rubella IgM	9	Rubella IgG	9
Toxo IgM	9	Toxo IgG	
HSV IgM	9	HSV IgG	
Coxsackie virus group B IgM	9	Cox kie viru group B IgG	9
Epstein-Barr virus IgM	9	Epste Barr v G	9
Enterovirus 71 IgM	9	Enterov 71 G	9
Coxsackie virus type A16 IgM	9	C sackie v ype A16 IgG	9
Varicella zoster virus IgM	9	Va a zoste rus IgG	9
Mumps Virus IgM		Mump rus Ig	9
Respiratory syncytial virus IgM	9	Respirato syncytial virus IgG	9
Adenovirus IgM	9	Adenoviru gG	9
Chlamydia pneumoniae IgM		*Chlamydi neumoniae* IgG	9
Mycoplasma pneumoniae IgM		*Mycopla a pneumoniae* IgG	9
Measles virus IgM	9	Meas irus IgG	9

Interference: Poten dogenous terference of the Anti-SARS-CoV-2 Rapid Test was evaluated usi natural ch serum mples. Potential interferents were spiked at different concentr ns into negative sa samples weakly positive for SARS-CoV-2 IgG or IgM a bodies, and samples moderately positive for anti-SARS-CoV-2 IgG or IgM anti dies. Sam s tested with the Anti-SARS-CoV-2 Rapid Test and the highest conce ntion hat did not produce interference were recorded. No IgM or IgG false negative or false p e results we bserved with the following potential interference substances at the d conce tions:

Table 2 Interference Results

Substance	Tested Concentration
HAMA	positive sample
Rheumatoid factor	100 IU/mL
Antinuclear antibody (ANA)	103.748 IU/mL
Anti-mitochondrial antibody (AMA)	80 U/mL
Bilirubin	0.3 mg/mL
Hemoglobin	8 mg/mL
Triglycerides	5mg/mL
α-interferon	2 ng/mL
Zanamivir	142 ng/mL

Ritonavir	53 µg/mL
Tramadol	12 µg/mL
Azithromycin	4 µg/mL
Ceftriaxone	156 µg/mL
Meropenem	10 mg/mL
Levofloxacin	2 mg/mL
Oseltamivir	1275 ng/mL
Mupirocin	10 mg/mL
Benzocaine	1.7 mg/mL
Tobramycin	4 µg/mL
Peramivir	18 µg/mL
Epinephrine	546 pmol/L
Menthol	1.7 mg/mL
Ribavirin	5.4 µg/mL
Lopinavir	2 mg/L

2. Clinical Studies

The clinical performance of the Anti-SARS-CoV-2 Rapid Test was evaluated by testing a total of 717 clinical samples from individual patients: 621 serum samples and [illegible] plasma samples (EDTA, heparin, and citrate). The samples were collected and tested at four sites in [illegible] from January to mid-March 2020.

The Anti-SARS-CoV-2 Rapid Test results for IgM and IgG detection were compared to the results of PCR assays for SARS-CoV-2. Respiratory samples were collected for PCR testing mostly between 1 and 7 days after symptom onset. Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days following PCR sample collection.

Study Results

Across all study sites, serum and plasma samples from a total of 405 patients with positive PCR comparator results and 312 patients with negative PCR comparator results were tested with the Anti-SARS-CoV-2 Rapid Test. Overall study results are shown in Table 3 below. Results stratified by IgM and IgG are shown in Tables 4 and 5. Positive serology results stratified by apparent disease period by day of symptom appearance at the time of blood collection are shown in Tables 6 and 7.

Table 3. Overall Clinical Study Results for all time periods from symptom onset

			PCR Comparator *		Total
			Pos	Neg	
Anti-SARS-CoV-2 Rapid Test	[illegible]	IgG+/IgM+	338	0	338
		IgG-/IgM+	8	1	9
		IgG+/IgM-	11	2	13
	Neg	IgG-/IgM-	48	309	357
Total			405	312	717

*Note: Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

Positive Percent Agreement (PPA)= (IgM positive or IgG positive)/(PCR positive)
PPA: 88.15% (357/405) (95%CI: 84.6% - 90.9%)
Negative Percent Agreement: (NPA) = (IgM negative and IgG negative)/(PCR negative)
NPA: 99.04% (309/312) (95%CI: 97.2% - 99.7%)

Table 4. IgM Results for all time periods from symptom onset

		PCR Comparator*		Total
		Pos	Neg	
Anti-SARS-CoV-2 Rapid Test – IgM Result	Pos	346	1	347
	Neg	59	311	370
Total		405	312	717

*Note: Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

Positive Percent Agreement**:** (PPA)= IgM positive/PCR positive
PPA: 85.43% (346/405), (95%CI: 81.7% - 88.5%)
Negative Percent Agreement**:** (NPA) =IgM negative /PCR negative
NPA: 99.68% (311/312), (95% CI: 98.2% - 99.9%)

Table 5. IgG Results for all time periods from symptom onset

		PCR Comparator*		Total
		Pos	Neg	
Anti-SARS-CoV-2 Rapid Test – IgG Result	Pos	351	0	351
	Neg	54	312	366
Total		405	312	717

*Note: Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

Positive Percent Agreement**:** (PPA)= IgG positive/PCR positive
PPA: 86.17% (349/405), (95%CI: 82.5% - 89.2%)
Negative Percent Agreement**:** (NPA) = IgG negative /PCR negative
NPA: 99.36% (310/312), (95% CI: 97.7% - 99.8%)

Table 6. SARS-CoV-2 IgM Positive Results by time from symptom onset

Infectious period (days)	# PCR positive by time*	# Anti-SARS-CoV-2 Rapid Test positive	PPA	95%CI
≤7	51	19	37.25%	25.3 – 51.0%
8-14	52	38	73.08%	59.8 - 83.2%
≥15	302	289	95.70%	92.8 - 97.5%

*Note: Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

Table 7: SARS-CoV-2 IgG Positive Results by time from symptom onset

Infectious period (days)	# PCR positive at any time*	# Anti-SARS-CoV-2 Rapid Test positive	PPA	95%CI
≤7	51	16	31.37%	20.3 - 45.0%
8-14	52	34	65.38%	51.8 - 76.9%
≥15	302	299	99.01%	97.7 - 99.8%

*Note: Serum and plasma samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

REFERENCES

1. "Naming the coronavirus disease (COVID-19) and the virus that causes it". World Health Organization. Archived from the original on 28 February 2020. Retrieved 28 February 2020.

2. "Coronavirus Disease 2019 (COVID-19) Symptoms". Centers for Disease Control and Prevention, United States. 10 February 2020. Archived from the original on 30 January 2020.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

EC REP

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Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		Do not reuse
CE	CE Mark		