RightSign™ COVID-19 IgG/IgM Rapid Test Cassette
Package Insert

For Emergency Use Authorization only
For prescription use only.
For in vitro diagnostic use only.

【INTENDED USE】

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is a rapid lateral flow chromatographic immunoassay intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in in human venous whole blood (sodium heparin, EDTA, and sodium citrate), serum or plasma (sodium heparin, potassium EDTA and sodium citrate). The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette 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 RightSign™ COVID-19 IgG/IgM Rapid Test Cassette should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C 263a, that meet the requirements to perform moderate or high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. The IgG and IgM 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.

The sensitivity of RightSign™ COVID-19 IgG/IgM Rapid Test Cassette early after infection is unknown. 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 RightSign™ COVID-19 IgG/IgM Rapid Test Cassette 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 second, different IgG or IgM assay.

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is only for use under the Food and Drug Administration's Emergency Use Authorization.

【SUMMARY】

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.
The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is a rapid test that utilizes a combination of SARS-COV-2 antigen coated colored particles for the detection of IgG and IgM antibodies to SARS-COV-2 in human whole blood, serum or plasma.

【PRINCIPLE】

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is a lateral flow immunochromatographic assay for the detection of SARS-CoV-2 antibodies in venous whole blood, serum or plasma. This test uses anti-human IgM antibody (test line IgM), anti-human IgG (test line IgG) and goat anti-mouse IgG (control line C) immobilized on a nitrocellulose strip. The conjugate pad contains recombinant SARS-CoV-2 antigen (Spike protein RBD domain main antigens of SARS-CoV-2) conjugated with colloid gold.

During testing, the specimen binds with SARS-CoV-2 antigen- conjugated gold colloid coated particles in the test cassette. When a specimen followed by assay buffer is added to the sample well, IgM &/or IgG antibodies if present, will bind to COVID-19 conjugates making antigen antibodies complex. This complex migrates through nitrocellulose membrane by capillary action. When the complex meets the line of the corresponding immobilized antibody (anti-human IgM &/or anti-human IgG) the complex is trapped forming a colored line which confirm a reactive test result. Absence of a colored line in the test region indicates a non-reactive test result.

To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

【WARNINGS AND PRECAUTIONS】

- For prescription use only. For in vitro diagnostic use only. Do not use after expiration date.
- This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA to perform moderate or high complexity tests;
- This test has been authorized only for the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- 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 the procedure 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 tests, specimens and potentially contaminated material should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

【STORAGE AND STABILITY】

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

【SPECIMEN COLLECTION AND PREPARATION】

- The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette can be performed using whole blood, serum or plasma.
- Whole blood or plasma could be collected with tube containing Heparin or Citrate.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after specimen collection. 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 3 days. For long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture 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.
- 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 federal regulations for transportation of etiologic agents.

【MATERIALS 】

Materials provided

Kit components	Format 1	Format 2	Format 3
Test cassettes:	20 cassettes	20 cassettes	25 cassettes
Buffer:	20 vials per kit 0.2 ml per vial	1 vial per kit 3 ml per vial	1 vial per kit 3 ml per vial
Droppers or capillaries	20 droppers/capillaries per kit	20 droppers/capillaries per kit	25 droppers/capillaries per kit
Package Insert	1 Package Insert	1 Package Insert	1 Package Insert

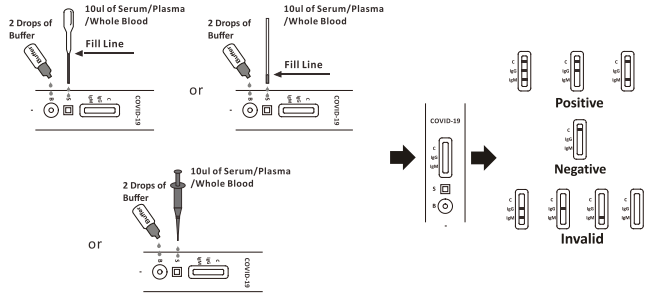
Materials required but not provided

Specimen collection containers	Centrifuge (for plasma only)
Micropipette	Timer

【DIRECTIONS FOR USE】

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

1. Bring the pouch to room temperature before opening. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface.
   - For Serum or Plasma or Whole Blood Specimens:
     - To use a dropper: Hold the dropper vertically, draw the specimen up to the Fill Line (approximately 10µl), and transfer the specimen to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µl) to the buffer well (B) and start the timer. Avoid trapping air bubbles in the specimen well.
     - To use a micropipette: Pipette and dispense 10µl of specimen to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µl) to the buffer well (B) and start the timer.
3. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 20 minutes.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

IgG and IgM POSITIVE: * **Three lines appear**. One colored line should be in the control line region (C), and two-colored lines should appear in IgG test line region and IgM test line region. The color intensities of the lines do not have to match.
IgG POSITIVE: * **Two lines appear**. One colored line should be in the control line region (C), and a colored line appears in IgG test line region. The result is positive for SARS-COV-2 virus specific-IgG.
IgM POSITIVE: * **Two lines appear**. One colored line should be in the control line region (C), and a colored line appears in IgM test line region. The result is positive for SARS-COV-2 virus specific-IgM antibodies.
*NOTE: The intensity of the color in the IgG and/or IgM test line region(s) will vary depending on the concentration of SARS-COV-2 antibodies in the specimen. Therefore, any shade of color in the IgG and/or IgM test line region(s) should be considered positive.
NEGATIVE: **One colored line should be in the control line region (C)**. No line appears in IgG and IgM test line region(s).
INVALID: **Control line fails to appear**. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

【QUALITY CONTROL】

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal valid procedural control, it confirming adequate membrane wicking. 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.

【LIMITATIONS】

For use under an Emergency Use Authorization Only

1. Use of the RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is limited to laboratory personnel who have been trained. Not for home use.
2. The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette is for in vitro diagnostic use only. The test should be used for the detection of SARS-COV-2 antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in SARS-COV-2 antibody concentration can be determined by this qualitative test.

3. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing for the presence of SARS-CoV-2 virus specific antibodies in the serum, plasma or whole blood specimen from individual subjects. For optimal test performance, proper sample collection is critical. Failure to follow the procedure may give inaccurate results.
4. Reading test results earlier than 10 minutes after the addition of Buffer may yield erroneous results. Do not interpret the result after 20 minutes.
5. The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette will only indicate the presence of SARS-COV-2 antibodies in the specimen and should not be used as the sole criteria for the diagnosis of SARS-COV-2.
6. In the early onset of symptom, anti-SARS-COV-2 IgM and IgG antibody concentrations may be below detectable levels.
7. The test may have lower sensitivity for IgG detection in symptomatic individuals prior to 14 days since symptom onset.
8. Results from immunosuppressed patients should be interpreted with caution.
9. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
10. A negative result for individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. 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 RightSign™ COVID-19 IgG/IgM Rapid Test Cassette 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 method(s) and clinical findings before a diagnostic determination is made. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
11. Some specimens containing unusually high titer of rheumatoid factor may affect expected results.
12. 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.
13. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
14. Not for the screening of donated blood.
15. There may be false positive risk with the plasma in EDTA tube after 24hours.
16. The sensitivity of the test is impacted after being open for one hour-the intensity of the T line becomes weak. Testing must be performed within one hour after opening the pouch.

【Conditions of Authorization for the Laboratory】

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

Authorized laboratories using the RightSign™ COVID-19 IgG/IgM Rapid Test Cassette ("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 test result reports, all authorized Fact Sheets. Under exigent 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 Hangzhou Biotest Biotech Co.,Ltd (info.usa@biotests.com.cn) 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, that meet the requirements to perform moderate or high complexity tests" as "authorized laboratories."

【PERFORMANCE CHARACTERISTICS】

POSITIVE AGREEMENT:

Positive agreement was evaluated using specimens collected from symptomatic subjects. All subjects were confirmed positive for SARS-COV-2 by RT-PCR. The positive population consisted of the following subjects.

- Living in Site A during the COVID-19 pandemic.
- Living in Site B-1 during the COVID-19 pandemic.
- Living in Site B-2 during the COVID-19 pandemic

Table1. IgM PPA (Per site and sites combined):

Site	Days post symptom onset	# PCR positive at any time	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
			# positive results	PPA	95%CI
A & B-2 (Serum)	≤7	9	6	66.67%	(35.42% -87.94%)
	8-14	83	77	96.25%	(89.55% - 98.72%)
	≥15	158	150	94.94%	(90.33% - 97.41%)
B-1 (Plasma)	unknown	70	63	90.00%	(80.77% - 95.07%)
Sites combined	-	320	296	92.50%	(89.08% - 94.91%)

Table2. IgG PPA (Per site and sites combined):

Site	Days post symptom onset	# PCR positive at any time	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
			# positive results	PPA	95%CI
A & B-2 (Serum)	≤7	9	6	66.67%	(35.42% - 87.94%)
	8-14	83	76	91.57%	(83.60% - 95.85%)
	≥15	158	152	96.20%	(91.96% - 98.25%)
B-1 (Plasma)	unknown	70	59	84.29%	(74.01% -90.99%)
Sites combined	-	320	293	91.56%	(88.00% - 94.14%)

CI means confidence interval.

NEGATIVE AGREEMENT

Negative agreement was evaluated using 210 samples collected from symptomatic subjects and all were confirmed negative for SARS-COV-2 by RT-PCR. The excluded cases consisted of the following subjects.

- Living in Site A during the COVID-19 pandemic.
- Living in Site B-1 during the COVID-19 pandemic.
- Living in Site C during the COVID-19 pandemic

Table 3. IgM NPA (Per site and sites combined):

Site	# PCR negative	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
		# negative results	NPA	95%CI
A (Serum)	150	146	97.33%	(93.34% - 98.96%)
B -1 (Plasma)	10	10	100.00%	(72.25% -100.00%)
C (Serum)	50	50	100.00%	(92.87% - 100.00%)
Sites combined	210	206	98.10%	(95.12% - 99.26%)

Table 4. IgG NPA (Per site and sites combined):

Site	# PCR Negative	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
		# negative results	NPA	95%CI
A (Serum)	150	149	99.33%	(96.32% - 99.88%)
B -1 (Plasma)	10	10	100.00%	(72.25% -100.00%)
C (Serum)	50	50	100.00%	(92.87% - 100.00%)
Sites combined	210	209	99.52%	(97.38% - 99.99%)

CI means confidence interval.

SEROCONVERSION:

The sensitivity and specificity of the RightSign™ COVID-19 IgG/IgM Rapid Test Cassette was evaluated on samples from individuals residing in Site C for Diagnosis and Treatment of Infectious Disease. The sensitivity was evaluated on 104 samples from 30 hospitalized patients. All the studied cases are confirmed by RT-PCR. Of these objectives, seven subjects were both IgM and IgG positive at the first sample test, twenty subjects seroconverted during observation and three subjects never seroconverted. The sensitivity was 90% (27/30) for the subjects tested.

Table 5. IgM PPA (Site C)

Site	Days post symptom onset	# PCR positive at any time	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
			# positive results	PPA	95%CI
C	≤7	32	10	31.25%	16.12%-50.01%
	8~14	27	14	51.85%	31.95%-71.33%
	≥15	45	43	95.56%	84.85%-99.46%

Table 6. IgG PPA (Site C)

Site	Days post symptom onset	# PCR positive at any time	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
			# positive results	PPA	95%CI
C	≤7	32	5	15.63%	5.28%-32.79%
	8~14	27	12	44.44%	25.48%-64.67%
	≥15	45	41	91.11%	78.78%-97.52%

Table 7: Seroconversion

Case (Patient) ID No.	Sample ID No.	nCoV-2 RT-PCR Results	Days Between Symptoms Onset and Blood Collection	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
				IgM(+)	IgG(+)	IgM(+) and /or IgG(+)
CJG-2000004116	CJG-2000004116-01	+	7	-	-	-
	CJG-2000004116-02	N/A	10	-	-	-
	CJG-2000004116-03	+	18	+	+	+
CSC-2000004214	CSC-2000004214-01	+	10	+	+	+
	CSC-2000004214-02	N/A	13	+	+	+
	CSC-2000004214-03	N/A	18	+	+	+
	CSC-2000004214-04	-	32	+	+	+
CZ-05148433	CZ-05148433-01	+	7	-	-	-
	CZ-05148433-02	-	8	+	+	+
	CZ-05148433-03	+	22	+	+	+
GJ-03013432	GJ-03013432-01	+	1	+	+	+
	GJ-03013432-02	+	2	+	+	+
	GJ-03013432-03	-	9	+	+	+
GXM-05143619	GXM-05143619-01	+	4	-	-	-
	GXM-05143619-02	N/A	6	+	+	+
	GXM-05143619-03	+	9	-	+	+
HHZ-05150218	HHZ-05150218-01	+	11	+	+	+
	HHZ-05150218-02	+	13	+	+	+
	HHZ-05150218-03	+	22	+	+	+
	HHZ-05150218-04	N/A	24	+	+	+
HSJ-03886796	HSJ-03886796-01	+	2	-	-	-
	HSJ-03886796-02	+	5	-	-	-
	HSJ-03886796-03	+	7	+	-	+
	HSJ-03886796-05	N/A	11	+	+	+
	HSJ-03886796-04	+	20	+	+	+
JXJ-2000004055	JXJ-2000004055-01	+	8	-	-	-
	JXJ-2000004055-04	-	14	+	-	+
	JXJ-2000004055-03	+	20	+	+	+
	JXJ-2000004055-02	N/A	24	+	+	+

Case (Patient) ID No.	Sample ID No.	nCoV-2 RT-PCR Results	Days Between Symptoms Onset and Blood Collection	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		
				IgM(+)	IgG(+)	IgM(+) and /or IgG(+)
LE-01613279	LE-01613279-01	-	7	-	-	-
	LE-01613279-02	+	14	-	-	-
	LE-01613279-03	-	33	+	+	+
LH-05079034	LH-05079034-01	+	6	-	-	-
	LH-05079034-02	+	7	+	+	+
	LH-05079034-03	+	20	+	+	+
LMX-05148953	LMX-05148953-06	N/A	7	-	-	-
	LMX-05148953-01	+	8	-	-	-
	LMX-05148953-02	+	10	-	-	-
	LMX-05148953-03	+	11	+	-	+
	LMX-05148953-07	N/A	13	+	+	+
	LMX-05148953-04	N/A	16	+	+	+
	LMX-05148953-05	N/A	24	+	+	+
	LMX-05148953-08	-	33	+	+	+
MRG-2000004008	MRG-2000004008-01	+	4	-	-	-
	MRG-2000004008-02	+	21	+	+	+
	MRG-2000004008-03	N/A	26	+	+	+
MXR-2000004129	MXR-2000004129-01	+	11	+	+	+
	MXR-2000004129-02	N/A	22	+	+	+
	MXR-2000004129-03	+	25	+	+	+
SBZ-2000004184	SBZ-2000004184-01	+	7	-	-	-
	SBZ-2000004184-02	N/A	8	-	-	-
	SBZ-2000004184-03	+	10	-	-	-
SGH-2000004035	SGH-2000004035-01	+	5	-	-	-
	SGH-2000004035-02	+	19	+	+	+
	SGH-2000004035-03	+	24	+	+	+
SWD-2000004137	SWD-2000004137-01	+	15	+	+	+
	SWD-2000004137-02	N/A	19	+	+	+
	SWD-2000004137-03	+	23	+	+	+
	SWD-2000004137-04	-	68	+	+	+
WCD-2000004024	WCD-2000004024-01	+	2	-	-	-
	WCD-2000004024-02	N/A	14	-	-	-
	WCD-2000004024-03	+	22	-	-	-
WCJ-05151120	WCJ-05151120-01	+	6	+	+	+
	WCJ-05151120-02	+	15	+	+	+
	WCJ-05151120-03	N/A	21	+	+	+
	WCJ-05151120-04	+	27	+	+	+
WH-2000004159	WH-2000004159-01	+	7	-	-	-
	WH-2000004159-02	+	10	-	-	-
	WH-2000004159-03	+	14	+	-	+
	WH-2000004159-04	-	29	+	+	+
WJQ-05149865	WJQ-05149865-01, WJQ-05149865-04	+	0	-	-	-
	WJQ-05149865-02	+	1	-	-	-
	WJQ-05149865-03	+	2	+	-	+
	WJQ-05149865-05	N/A	15	+	+	+
WMM-05148912	WMM-05148912-01	+	2	-	-	-
	WMM-05148912-02	+	18	+	+	+
	WMM-05148912-03	+	20	+	+	+
WYC-2000004016	WYC-2000004016-01	+	35	+	-	+
	WYC-2000004016-02	+	41	+	+	+
	WYC-2000004016-03	-	55	+	+	+
XYS-00987017	XYS-00987017-01	+	11	-	-	-
	XYS-00987017-02	+	21	+	+	+
	XYS-00987017-03	+	23	+	+	+
XZC-2000004086	XZC-2000004086-01	+	9	-	-	-
	XZC-2000004086-02	+	25	+	-	+
	XZC-2000004086-03	-	35	-	+	+
YYQ-05148957	YYQ-05148957-01	N/A	3	+	-	+
	YYQ-05148957-02	+	7	+	+	+
	YYQ-05148957-03	+	42	+	+	+
YYX-2000004130	YYX-2000004130-01	+	4	-	-	-
	YYX-2000004130-02	N/A	6	-	-	-
	YYX-2000004130-03	+	15	+	+	+
YZM-2000004131	YZM-2000004131-01	+	4	-	-	-
	YZM-2000004131-02	+	8	+	+	+
	YZM-2000004131-03	N/A	16	+	+	+
ZFS-2000004005	ZFS-2000004005-01	+	13	+	+	+
	ZFS-2000004005-02	+	16	+	+	+
	ZFS-2000004005-03	N/A	17	+	+	+
ZJH-00823994	ZJH-00823994-01	+	3	-	-	-
	ZJH-00823994-02	-	7	-	-	-
	ZJH-00823994-03	N/A	11	-	-	-
ZLQ-05150650	ZLQ-05150650-03	+	7	-	-	-
	ZLQ-05150650-01	+	19	+	-	+

Independent Clinical Agreement Validation

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette was tested on 2020-04-21 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples was confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the RightSign™ COVID-19 IgG/IgM Rapid Test Cassette. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers.

All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, “Negatives” and ii) Ten (10) samples selected from banked serum from HIV+ patients, “HIV+”. Testing was performed by one operator using one lot of the RightSign™ COVID-19 IgG/IgM Rapid Test Cassette. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown below.

Table 8. Summary Results

RightSign™ COVID-19 IgG/IgM Rapid Test Cassette		Comparator Method			Total
		Positive (IgM/IgG) +	Negative (IgM/IgG)-	Negative, HIV+	
Positive	IgM +/ IgG+	28	0	0	28
	IgM+, IgG-	2	0	0	2
	IgM-, IgG+	0	0	0	0
Negative	IgM- / IgG)-	0	70	10	80
Total (n=110)		30	70	10	110

Table 9. Summary Statistics

Measure	Estimate	Confidence Interval
IgM+ Sensitivity (PPA)	(30/30) 100%	(88.7%; 100%)
IgM- Specificity (NPA)	(80/80) 100%	(95.4%; 100%)
IgG+ Sensitivity (PPA)	(28/30) 93.3%	(78.7%; 98.2%)
IgG- Specificity (NPA)	(80/80)100%	(95.4%; 100%)
Combined Sensitivity	(30/30) 100%	(88.7%; 100%)
Combined Specificity	(80/80)100%	(95.4%; 100%)
Combined PPV for prevalence = 5%	100%	(50.5%; 100%)
Combined NPV for prevalence = 5%	100%	(99.4%; 100%)
Cross-reactivity with HIV+	(0/10) 0% not detected	N/A

Cross-reactivity

The RightSign™ COVID-19 IgG/IgM Rapid Test Cassette has been tested with the following potentially cross-reactive substances.

Table 10: Cross-reactive Study Data of RightSign™ COVID-19 IgG/IgM Rapid Test Cassette.

Potentially cross-reactive substances	Number of Samples	RightSign™ COVID-19 IgG/IgM Rapid Test Cassette					
		IgM			IgG		
		NEG	POS	Agreement	NEG	POS	Agreement
Anti-FLU A	24	24	0	100%	24	0	100%
Anti-FLU B	30	30	0	100%	30	0	100%
Anti-Respiratory Syncytial Virus	15	15	0	100%	18	0	100%
Anti-Adenovirus	5	5	0	100%	5	0	100%
Anti-HBsAg	3	3	0	100%	3	0	100%
Anti-Syphilis	3	3	0	100%	3	0	100%
Anti-H. Pylori	3	3	0	100%	3	0	100%
Anti-HIV	6	6	0	100%	6	0	100%
Anti-HCV	6	6	0	100%	6	0	100%
Anti-SARS-COV	1	1	0	100%	0	1	0%
HAMA	13	13	0	100%	13	0	100%
RF	33	31	2	93.90%	33	0	100%
H1N1	3	3	0	100%	3	0	100%
H3N2	3	3	0	100%	3	0	100%
H7N9	3	3	0	100%	3	0	100%
Anti-HBV	6	6	0	100%	6	0	100%
Antinuclear antibody (ANA)	10	10	0	100%	10	0	100%
Anti-Haemophilus influenzae	5	5	0	100%	5	0	100%
Human coronavirus HKU1	2	2	0	100%	2	0	100%
Human coronavirus NL63	1	1	0	100%	1	0	100%
Human coronavirus OC43	2	2	0	100%	2	0	100%
Human coronavirus 229E	2	2	0	100%	2	0	100%
Anti-Rhinovirus	31	31	0	100%	31	0	100%

Anti-SARS-COV and RF show potential risk of cross reactivity with the samples.

Interfering Substances

The following potentially interfering substances were added to COVID-19 negative and spiked positive specimens.

Table 11: Interference Study Data of RightSign™ COVID-19 IgG/IgM Rapid Test Cassette.

Analytes	Concentration	Result			
		Negative Specimen		Spiked with Positive Specimen	
		IgG line	IgM line	IgG line	IgM line
Acetaminophen	20 mg/dL	Negative	Negative	Positive	Positive
Caffeine	20 mg/dL	Negative	Negative	Positive	Positive
Albumin	2 g/dL	Negative	Negative	Positive	Positive
Acetylsalicylic Acid	20 mg/dL	Negative	Negative	Positive	Positive
Gentisic Acid	20 mg/dL	Negative	Negative	Positive	Positive
Ethanol	1%	Negative	Negative	Positive	Positive
Ascorbic Acid	2g/dL	Negative	Negative	Positive	Positive
Creatine	200mg/dl	Negative	Negative	Positive	Positive
Bilirubin	1g/dL	Negative	Negative	Positive	Positive
Hemoglobin	1000mg/dl	Negative	Negative	Positive	Positive
Oxalic Acid	60mg/dL	Negative	Negative	Positive	Positive
Uric acid	20mg/ml	Negative	Negative	Positive	Positive

None of the substances at the concentration tested interfered in the assay.

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Manufactured by: Hangzhou Biotest Biotech Co.,Ltd.
No.17, Futai Road, Zhongtai Street, Yuhang District, Hangzhou, P.R. China

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