553823

ACON® SARS-CoV-2 IgG/IgM Rapid Test

Evaluation Report

June 2020

ACON CE SARS-CoV-2 IgG/IgM Rapid Test Evaluation Report

The *ACON* SARS-CoV-2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human serum, plasma, venous whole blood, or capillary fingertip blood. It 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 how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The SARS-CoV-2 IgG/IgM Rapid Test should not be used to diagnose acute SARS-CoV-2 infection.

1. **Purpose:** To evaluate the performance of the *ACON* CE SARS-CoV-2 IgG/IgM Rapid Test
2. **Material:**

SARS-CoV-2 IgG/IgM Cassettes	COV0040001	COV0040002	COV0040003

3. **Study procedure and results**

3.1 Precision study

Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001, COV0040002, COV0040003
- SARS-CoV-2 IgG/IgM Negative Serum Sample Lot# COV200423N1
- SARS-CoV-2 IgG Low Positive Serum Sample Lot# COV200423IgG L
- SARS-CoV-2 IgM Low Positive Serum Sample Lot# COV200423IgM L
- SARS-CoV-2 IgG High Positive Serum Sample Lot# COV200423IgG H
- SARS-CoV-2 IgM High Positive Serum Sample Lot# COV200423IgM H
- Positive Serum Samples were prepared by adding purified IgG/IgM monoclonal antibodies to spike protein into negative serum.

Procedure:

1. 3 Lots of SARS-CoV-2 IgG/IgM Rapid Test were tested by 3 operators, each operator performed 2 tests on each control for 5 days. Total 90 tests were performed per each control: 2 replicates X 5 days X 3 lots X 3 operators = 90 tests per control.
2. Following product package insert, performed the test and read the result at 15-20 minutes.

Test results:

	SARS-CoV-2 IgG/IgM Negative	SARS-CoV-2 IgG/IgM Low Positive	SARS-CoV-2 IgG/IgM High Positive
Lot 1	- / 30 replicates	+ / 30 replicates	+ / 30 replicates
Lot 2	- / 30 replicates	+ / 30 replicates	+ / 30 replicates
Lot 3	- / 30 replicates	+ / 30 replicates	+ / 30 replicates

Conclusions:

All three lots identified the samples 100% correctly as negative or positive.

3.2 Clinical study – Serum/Plasma/WB

A multi-site clinical study was conducted to evaluate the performance of the SARS-CoV-2 IgG/IgM Rapid Test, and the combined results are shown below.

Site 1 Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001, COV0040002, COV0040003
- Comparison method: RT-PCR (2019 Novel Coronavirus (2019-nCoV) RNA Detection Kit (PCR-Fluorescence Probing), manufactured by DA AN GENE)
- SARS-CoV-2 IgG/IgM Negative serum/plasma samples
- Serum/plasma samples from SARS-CoV-2 infected subjects

Site 1 Procedure:

1. Study was conducted in Hangzhou, China:
   - 300 clinical negative serum/plasma samples were collected before Nov. 2019
   - 112 positive serum/plasma samples were collected from patients infected with SARS-CoV-2 confirmed with RT-PCR method. Positive samples were collected >7 days after initial onset of symptoms
2. Following product package insert, performed the test and read the result at 15-20 minutes.

Site 2 Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV20200504R
- SARS-CoV-2 IgG/IgM Negative serum/plasma samples

Site 2 Procedure:

1. Study was conducted in San Diego, CA:
   - 368 clinical negative serum/plasma samples were collected before Nov. 2019
2. Following product package insert, performed the test and read the result at 15-20 minutes.

Site 3 Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV20200424R
- SARS-CoV-2 IgG/IgM Negative serum/plasma samples

Site 3 Procedure:

1. Study was conducted in San Diego, CA:
   - 100 clinical negative serum/plasma samples were collected before Nov. 2019
2. Following product package insert, performed the test and read the result at 15-20 minutes.

Site 4 Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV20200424R
- Capillary whole blood samples from patients with confirmatory PCR results

- Comparison method: All patients were confirmed as Positive or Negative with PCR (Quest Diagnostics, LabCorp, Bioreference Laboratories)

Site 4 Procedure:

1. Study was conducted in New York, NY:
   - 30 fingerstick capillary whole blood samples were collected ≥14 days after initial onset of symptoms from patients confirmed as PCR positive
   - 30 fingerstick blood samples were collected from patients confirmed as PCR negative, with 20 asymptomatic patients and 10 patients that were 0 – 77 days after initial onset of symptoms
2. Following product package insert, performed the test and read the result at 15-20 minutes.

Combined test results:

Result		PCR Comparator		
		Positive	Negative	Total
ACON Test Results	IgM+/IgG+	100	0	100
	IgM-/IgG+	39	9**	48
	IgM+/IgG-	1	3	4
	Negative (IgM-/IgG-)	2*	786	788
	Total	142	798	940

* 1 asymptomatic subject tested 14 days after Positive PCR test

** 3 subjects with symptoms consistent with COVID-19 and contact history with COVID-19 patients tested Negative by PCR

Conclusions:

The sensitivity, specificity, and accuracy are all greater than 98%.

	Results	95% Confidence Interval
Sensitivity	98.6% (140 / 142)	95.0% - 99.8%
Specificity	98.5% (786 / 798)	97.5% - 99.2%
Accuracy	98.5% (926 / 940)	97.5% - 99.2%

3.3 Interference

Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040002
- SARS-CoV-2 IgG/IgM Negative Serum Sample Lot# COV200423N1
- SARS-CoV-2 IgG Low Positive Serum Sample Lot# COV200423IgG L
- SARS-CoV-2 IgM Low Positive Serum Sample Lot# COV200423IgM L

No.	Potential Interfering Substances	Concentration Tested
1	Creatinine hydrochloride	5 mg/dl
2	Urea	257 mg/dl
3	Hemoglobin	9 g/L
4	Glucose	1000 mg/dl
5	Glybenclamide	1 mg/dl
6	Dopamine	0.09 mg/dl
7	Ibuprofen	50 mg/dl
8	Sulfamethoxazole	40 mg/dl
9	Bezafibrate	10 mg/dl
10	Acetylsalicylic acid	65 mg/dl
11	Sodium Pyroracemic	2.7 mg/dl
12	Lactate dehydrogenase	2000 mg/dl
13	Methydopa	250 U/L
14	Indomethacin	1.5 mg/dl
15	Nicotinic acid	3.6 mg/dl
16	Dextran	0.1 mg/dl
17	Quinidine hydrochloride monohydrate	6 g/dl
18	Probenecid	1.2 mg/dl
19	Acetaminophen	60 mg/dl
20	Ascorbic acid	20 mg/dl
21	Glycerinum	30 mg/dl
22	Oxalic acid	6.4 mg/dl
23	Furosemide	1 mg/dl
24	Albumin Human	6 mg/dl
25	Bilirubin	37.5 mg/dl (342 µmol/L)
26	Uric acid	25 mg/dl
27	Biotin	1200 ng/ml
28	Triglyceride	1329 mg/dL (15 mmol/L)
29	Rheumatoid factors	80 IU/mL
30	Antinuclear antibody (ANA)	titer: 1:240
31	Anti-mitochondrial antibody (AMA)	80 U/mL
32	Mouse IgG	1000 µg/mL

Procedure:

1. Potential interference substances were spiked individually, at the concentrations indicated, into SARS-CoV-2 IgG/IgM Negative Serum Sample and SARS-CoV-2 IgG Low Positive Serum Sample and SARS-CoV-2 IgM Low Positive Serum Sample.

2. Tested the samples with analytes in replicates of three. Tested the samples without interference analytes as control.
3. Record the color grade at 15-20 mins.

Test results:

The test results of all Interference Analyte added samples is uniform and the color difference is less than two color grades when compared to the control condition. All positive samples give positive results, and all negative samples give negative results.

Conclusions:

These substances will not interfere with the result of the SARS-CoV-2 IgG/IgM Rapid Test.

3.4 Cross reactivity

Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001
- Comparison test used to confirm SARS-CoV-2 IgG/IgM negative if sample collected after Nov, 2019

Procedure:

1. Serum/plasma specimens were collected from patients infected with HIV, Hepatitis B virus, Hepatitis C virus, Treponema pallidum, EB virus, Mycoplasma pneumoniae, Varicella-zoster virus, Influenza A/B, Chlamydiae pneumonia, Legionella pneumophila, Adenovirus, Measles virus, Cytomegalovirus, Herpes simplex virus-1/2, Respiratory syncytial virus, rhinovirus, Haemophilus influenza, other coronaviruses (229E, NL63, OC43, HKU1), and collected specimens with Rheumatoid factor positive and ANA positive. These samples were all collected before Nov. 2019 or were confirmed SARS-CoV-2 IgG/IgM negative with a comparison test. A minimum of 5 individual samples should be tested for each disease/infectious agent listed above.
2. Performed the test with ACON SARS-CoV-2 IgG/IgM Rapid Test once at each sample. Recorded the results at read time.

Test results:

Potential cross samples	Result
Influenza A IgM	- / 5 samples
Influenza A IgG	- / 5 samples
Influenza B IgM	- / 5 samples
Influenza B IgG	- / 5 samples
HCV IgM	- / 5 samples
HCV IgG	- / 5 samples
HBV IgM	- / 5 samples
HBV IgG	- / 5 samples
Respiratory syncytial virus IgM	- / 5 samples
Respiratory syncytial virus IgG	- / 5 samples
Rhinovirus IgM	- / 5 samples
Rhinovirus IgG	- / 5 samples
Haemophilus influenzae IgM	- / 5 samples
Haemophilus influenzae IgG	- / 5 samples
Coronavirus-229E	- / 5 samples
Coronavirus-NL63	- / 5 samples
Coronavirus-OC43	- / 5 samples
Coronavirus-HKU1	- / 5 samples
HIV	- / 5 samples
Treponema pallidum	- / 5 samples
EB virus	- / 5 samples
Mycoplasma pneumoniae	- / 5 samples
Varicella-zoster virus	- / 5 samples
Chlamydiae pneumonia	- / 5 samples
Legionella pneumophila	- / 5 samples
Adenovirus	- / 5 samples
Measles virus	- / 5 samples
Cytomegalovirus	- / 5 samples
Herpes simplex virus - 1/2	- / 5 samples
ANA	- / 5 samples
Rheumatoid factor	- / 5 samples

Conclusions:

The SARS-COV-2 Rapid Test is specific to SARS-COV-2 IgG and IgM. No cross reactivity was observed with specimens from patients infected with HIV, Hepatitis B virus, Hepatitis C virus, Treponema pallidum, EB virus, Mycoplasma pneumoniae, Varicella-zoster virus, Influenza A/B, Rhinovirus, Haemophilus influenza, other coronaviruses (229E, NL63, OC43, HKU1), Chlamydiae pneumonia, Legionella pneumophila, Adenovirus, Measles virus, Cytomegalovirus, Herpes simplex virus-1/2 or Respiratory syncytial virus. And there is no cross reactivity with Rheumatoid factor positive and ANA positive samples.

3.5 Hook effect

Material:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001
- SARS-CoV-2 IgG/IgM Negative Serum Sample Lot# 20200501-1
- SARS-CoV-2 IgG High Positive Serum Sample Lot# 20200413-1
- SARS-CoV-2 IgM High Positive Serum Sample Lot# 20200413-2

Procedure:

1. Dilute SARS-CoV-2 IgG high positive serum sample and SARS-CoV-2 IgM high positive serum sample with SARS-CoV-2 IgG/IgM negative serum sample with the ratio of 1,1:2, 1:5, 1:10, 1:20, 1:50 and 1:100.
2. Following product package insert, performed the test with each sample in three replicates and read the result at read time. The line intensity was recorded according to the ACON color chart.

Test results:

Dilution Rate		Test result of IgG		
1	IgG	+	+	+
1:2	IgG	+	+	+
1:5	IgG	+	+	+
1:10	IgG	+	+	+
1:20	IgG	+	+	+
1:50	IgG	+	+	+
1:100	IgG	+	+	+

Dilution Rate		Test result of IgM		
1	IgM	+	+	+
1:2	IgM	+	+	+
1:5	IgM	+	+	+
1:10	IgM	+	+	+
1:20	IgM	+	+	+
1:50	IgM	+	+	+
1:100	IgM	+	+	+

Conclusions:

Intensity of test lines for IgG and IgM show no sign of hook effect.

3.6 Whole blood evaluation

3.6.1 Hematocrit

Materials:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001
- SARS-CoV-2 IgG High Positive Serum Sample Lot# COV200423IgG H
- SARS-CoV-2 IgM High Positive Serum Sample Lot# COV200423IgM H
- Comparison test: 2019-Ncov IgM/ IgG Rapid Test from Nanjing Vazyme Medical Technology Co., Ltd.

Procedure:

1. Collected 10 ml venous blood with O type from a donor. Tested the sample with comparison test to confirm it is SARS-CoV-2 IgG/IgM negative.
2. Split this sample into 2 containers. Sample in one container was adjusted to HCT to 30%, 40%, 50% and 60% separately by adding or taking out plasma.
3. Sample in another container, replaced the plasma with SARS-CoV-2 IgG/IgM Positive Sample. And then they were adjusted to HCT 30%, 40%, 50% and 60% respectively by adding plasma.
4. Following product package insert, performed the test with each sample in duplicates with one lot of ACON SARS-CoV-2 IgG/IgM Rapid Test and read the results at read time.
5. Recorded the line intensity according to the ACON color chart.

Test results:

HCT	Negative		Positive	
	IgG	IgM	IgG	IgM
30%	- / 2 replicates	- / 2 replicates	+ / 2 replicates	+ / 2 replicates
40%	- / 2 replicates	- / 2 replicates	+ / 2 replicates	+ / 2 replicates
50%	- / 2 replicates	- / 2 replicates	+ / 2 replicates	+ / 2 replicates
60%	- / 2 replicates	- / 2 replicates	+ / 2 replicates	+ / 2 replicates

Conclusion:

There is no difference from 30% to 60% HCT.

3.6.2 Sample Type/Matrix Equivalency

Materials:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001
- SARS-CoV-2 IgG High Positive Serum Sample Lot# 20200502-1
- SARS-CoV-2 IgM High Positive Serum Sample Lot# 20200502-2
- Comparison test: 2019-Ncov IgM/ IgG Rapid Test from Nanjing Vazyme Medical Technology Co., Ltd.

Procedure:

1. Collected SARS-CoV-2 IgG/IgM negative samples from 5 donors. Each donor donated 6 tubes of whole blood and 1 tube of serum. Among 6 tubes of whole blood, there were 2 tubes with EDTA anticoagulant, 2 tubes with Heparin sodium anticoagulant, and 2 tubes with Sodium citrate anticoagulant.
2. One tube of whole blood with each anticoagulant was centrifuged to get plasma.
3. Tested each sample with comparison test to confirm it was SARS-CoV-2 IgG/IgM negative.
4. Tested each sample with ACON SARS-CoV-2 IgG/IgM Rapid Test in replicates of two.
5. Split each tube of serum, whole blood, and plasma into three containers. Spiked with the same concentration of SARS-CoV-2 IgG and IgM into one container of each sample. Total three concentrations (negative, low positive, and high positive) were spiked for each sample.
6. Tested each spiked sample with ACON SARS-CoV-2 IgG/IgM Rapid Test in replicates of two.
7. A total of 40 results per sample type were tested, including of negative, high negative, low positive and high positive.
8. Calculate agreement between the test result and the expected result.

Test results:

Positive percent agreement for each sample type comparing with serum is 100%, negative percent agreement for each sample type comparing with serum is 100%.The intensity of IgG/IgM test result for each sample type is no more than 2 color grades comparing with serum at the same condition.

Conclusion:

EDTA, heparin sodium and sodium citrate anticoagulants have no difference. Plasma and whole blood have no difference with serum.

3.6.3 Venous and Capillary Whole Blood Equivalency

Materials:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040001
- SARS-CoV-2 IgG High Positive Serum Sample Lot# 20200502-1
- SARS-CoV-2 IgM High Positive Serum Sample Lot# 20200502-2
- Comparison test: 2019-Ncov IgM/ IgG Rapid Test from Nanjing Vazyme Medical Technology Co., Ltd.

Procedure:

1. Collected 10 SARS-CoV-2 IgG/IgM negative venous whole blood samples and 10 capillary whole blood samples with EDTA anticoagulant from same 10 donors. Tested each sample with comparison test to confirm it was SARS-CoV-2 IgG/IgM negative.
2. Split each blood sample into two containers. Spiked with the same concentration of SARS-CoV-2 IgG and IgM into one container of each venous and capillary blood sample from same donor. Samples from different donors were spiked with different concentrations of SARS-CoV-2 IgG and IgM.
3. Following the product package insert, tested each sample with ACON SARS-CoV-2 IgG/IgM Rapid Test in replicates of two. Results were read at 15-20 minutes.
4. Recorded the test line intensity according to the ACON color chart.

Test results:

Sample #	Venous whole blood				Sample #	Capillary whole blood			
	Negative		Mimic positive			Negative		Mimic positive	
	IgG	IgM	IgG	IgM		IgG	IgM	IgG	IgM
1	-	-	+	+	1	-	-	+	+
2	-	-	+	+	2	-	-	+	+
3	-	-	+	+	3	-	-	+	+
4	-	-	+	+	4	-	-	+	+
5	-	-	+	+	5	-	-	+	+
6	-	-	+	+	6	-	-	+	+
7	-	-	+	+	7	-	-	+	+
8	-	-	+	+	8	-	-	+	+
9	-	-	+	+	9	-	-	+	+
10	-	-	+	+	10	-	-	+	+

Conclusion:

There is no difference between venous whole blood and capillary whole blood.

3.7 Comparison study

Materials:

- SARS-CoV-2 IgG/IgM Rapid Test, Lot# COV0040002
- Comparison test: 2019-Ncov IgM/ IgG Rapid Test from Nanjing Vazyme Medical Technology Co., Ltd.
- SARS-CoV-2 IgG/IgM negative whole blood sample Lot#20200430
- SARS-CoV-2 IgG/IgM Negative Serum Sample Lot# COV200423N1
- SARS-CoV-2 IgG Low Positive Serum Sample Lot# COV200423IgG L
- SARS-CoV-2 IgM Low Positive Serum Sample Lot# COV200423IgM L
- SARS-CoV-2 IgG High Positive Serum Sample Lot# COV200423IgG H
- SARS-CoV-2 IgM High Positive Serum Sample Lot# COV200423IgM H

Procedure:

1. Each sample was tested with ACON SARS-CoV-2 IgG/IgM Rapid Test in three replicates.
2. Each sample was tested with the comparison product according to the package insert.
3. Recorded the test line intensity according to the ACON color chart.

Results:

Product	Item	Negative serum	Negative Whole blood	Low Positive			High Positive		
ACON	IgG	- / *3	- / *3	+	+	+	+	+	+
	IgM	- / *3	- / *3	+	+	+	+	+	+
Comparison product	IgG	- / *3	- / *3	+	+	+	+	+	+
	IgM	- / *3	- / *3	+	+	+	+	+	+

Conclusion:

There is no difference with the comparison product.

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