Infection

ichroma™ COVID-19 Ab

INTENDED USE

ichroma™ COVID-19 Ab is a fluorescence immunoassay (FIA) for the qualitative determination of IgG/IgM antibodies against of novel corona virus (SARS-CoV-2) in human whole blood/serum/plasma. It is helpful as an aid in the screening of early mild, asymptomatic or acute patients for identification of 'Novel Coronavirus (eg. SARS-CoV-2)' infection.

For *in vitro* diagnostic use only.

INTRODUCTION

The third zoonotic human coronavirus (CoV) of the century emerged in December 2019, with a cluster of patients connected to Wuhan, Hubei Province, China. This virus, the newly identified coronavirus SARS-CoV-2, could cause risky pneumonia so that prevention and control of the infection has become highly required. The SARS-CoV-2 is a member of the Betacoronavirus Genus, that also includes Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV). Since it is identified that symptoms become rapidly severe without a proper treatment after onset of illness, early diagnosis of the virus infection is quite crucial. Currently, the spread of the viral transmission become fast so that the prevention of local transmission requires a point-of care test (POCT), which shows quick outcome within 20 minutes.

ichroma™ COVID-19 Ab test is an *in vitro* diagnostic medical device that helps you to diagnose Novel Coronavirus infections quickly and accurately by measuring the IgG or IgM antibody for the SARS-CoV-2.

* The benefits of using this product are;

1) To prevent the spread (secondary infection) and recovery of CoV infections, the most important serological test results, determined between the first two weeks after infection, can increase the confidence of confirmatory testing with RT-PCR.
2) Periodic serological tests after an infection is confirmed can help determine when to end treatment by analyzing the formation of protective antibodies through seroconversion and recovery of infection through treatment.

PRINCIPLE

This test uses a sandwich immunodetection method.

The detector antigens in buffer binds to antibodies in the sample, forming antibody-antigen complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-anti-human IgG & anti-human IgM on a test strip.

More antibodies in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antigens, which is processed by the instrument for ichroma™ tests to show the COVID IgG & IgM 'Positive' / 'Negative' / 'Indeterminate' in the sample.

COMPONENTS

ichroma™ COVID-19 Ab consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has anti-human IgM at the test line 1, anti-human IgG at the test line 2 and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has a granule containing antigen-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in Tris-HCl. All detector tubes are packed in a pouch.
- The detector diluent contains salt, detergent and sodium azide as a preservative in Tris-HCl and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow the cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The ichroma™ instruments may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with the relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- **ichroma™ COVID-19 Ab** will provide accurate and reliable

results subject to the below conditions.
- **ichroma™ COVID-19 Ab** should be used only in conjunction with the instrument for ichroma™ tests.
- Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium EDTA, K_2 EDTA, Sodium Heparin, Lithium heparin, Sodium citrate

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 - 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
		12 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-114

Components of **ichroma™ COVID-19 Ab**
- Cartridge Box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ COVID-19 Ab.**

Please contact our sales division for more information.
- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
  - **ichroma™-50** REF FPRR022
  - **ichroma™-50 PLUS** REF FPRR036
- **Boditech COVID-19 Ab Control** REF CFPO-292

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ COVID-19 Ab** is human whole blood/serum/plasma.
- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- If testing will be delayed more than 24 hours, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ COVID-19 Ab**: Sealed cartridges, detector tubes, a detector diluent, ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube~~s~~, detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Avoid directly windy place. The air flow can affect the flow of samples.
- Turn on the instrument for ichroma™ test.

※ **Please refer to the instrument for ichroma™ tests operation manual for the complete information and operating instructions.**

TEST PROCEDURE

- **ichroma™ II, ichroma™ M2**

Multi test mode / Read now mode

1) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
2) Take 10 µL of sample (whole blood/serum/plasma/ control) using a pipette and dispense it to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Leave the cartridge at room temperature for 10 minutes.
   ⚠ *Scan the sample loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before

pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
7) Tap the "Start" button on the instrument for ichroma™ test to start the scanning process.
(ichroma™ M2 is tested automatically after inserting.)
8) The instrument for ichroma™ tests will start scanning the sample loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode / Walk away mode

1) The test procedure is same with 'Multi test mode 1) – 4)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Tap the "Start" button on the instrument for ichroma™ test.
(ichroma™ M2 is tested automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 10 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

- **ichroma™ III**

1) The test procedure is same with the 'Single test mode'.

- **ichroma™-50, ichroma™-50 PLUS**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tubes in place.
3) Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4) Insert the cartridge magazine with the cartridges into the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of the detector tube by tapping.
8) Set the number of pipette tips by tapping.
9) Tap the 'Start' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays 'Positive' / 'Negative' /'Indeterminate' with ancillary value, cut-off index (COI).

Cut-off index	Result	Note
< 0.9	Negative for IgG	No need to retest
0.9 ≤ COI< 1.1	Indeterminate	Need to retest
≥1.1	Positive for IgG	Need to confirmation test

Cut-off index	Result	Note
< 0.9	Negative for IgM	No need to retest
0.9 ≤ COI< 1.1	Indeterminate	Need to retest
≥1.1	Positive for IgM	Need to confirmation test

- If the test result is "Negative" even though the patient has significant infectious symptoms, it should be recommended to conduct additional test including PCR or culture test.
- The accurate determination of test result as "Positive" should be confirmed by additional clinical evaluation.
- "Negative" result should be considered with possibilities of other infections. Positive result should be considered with additional infections by another pathogenic bacterium.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ COVID-19 Ab**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
(Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Sensitivity**

- **Cut-off**

The **ichroma™ COVID-19 Ab** test result indicates 'positive' or 'negative' of a sample defined by the algorithm of ichroma™ reader based on COI (cut-off index).

Cut-off index (COI)	Result
< 0.9	Negative for IgG / IgM
0.9 ≤ COI< 1.1	Indeterminate
≥1.1	Positive for IgG / IgM

- **Analytical specificity**

- **Cross-reactivity**

Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ COVID-19 Ab** test results did not show any significant cross-reactivity with these biomolecules.

No.	Cross-reactants	Sample type
1	Cytomegalovirus(CMV)	Positive serum
2	Epstein-Barr virus(EBV)	Positive serum
3	Hepatitis A virus(HAV)	Positive serum
4	Hepatitis C virus(HCV)	Positive serum
5	Hepatitis B virus(HBV)	Positive serum
6	Herpes simplex virus(HSV)	Positive serum
7	Rubella virus	Positive serum
8	Varicella-zoster virus(VZV)	Positive serum
9	Treponema pallidum	Positive serum
10	Anti Nuclear Antibody(ANA)	Positive serum
11	Rheumatoid factor(RF)	Positive serum
12	Early stage of pregnancy	Pregnant women sample

13	Middle stage of pregnancy	Pregnant women sample
14	Hepatitis B antibody (anti-HBs)	Hepatitis B (HBsAg) Ab positive sample
15	Influenza A	Positive serum
16	Influenza B	Positive serum
17	RSV	Positive serum
18	Mycoplasma pneumoniae	Positive serum

- **Interference**

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ COVID-19 Ab** test results did not show any significant interference with these materials.

No.	Interferents	Concentration
1	Li-Heparin	100,000 U/L
2	Na-Heparin	100,000 U/L
3	Na-EDTA	1.6 mg/mL (4 μM)
4	K_2-EDTA	1.6 mg/mL (4 μM)
5	Sodium citrate	25 mg/mL (0.085 μM)
6	Hemoglobin	2 mg/ml
7	BSA	60 mg/ml
8	Bilirubin	0.24 mg/mL (400 μM)
9	Triglycerides	1.5 mg/ml
10	Cholesterol	7.7 mg/mL (20 mM)

■ **Precision**

- Between lots

One person tested three different lots of **ichroma™ COVID-19 Ab**, ten times at each concentration of the control standard.

- Between persons

Three different persons tested one lot of **ichroma™ COVID-19 Ab**, ten times at each concentration of the control standard.

- Between days

One person tested one lot of **ichroma™ COVID-19 Ab** during three days, ten times at each concentration of the control standard.

- Between sites

One person tested **ichroma™ COVID-19 Ab** at three different site, ten times at each concentration of the control standard.

[IgG result]

Cal	Between lot		Between person	
No.	Positive/No.	Positive rate	Positive/No.	Positive rate
1	0/30	0%	0/30	0%
2	30/30	100%	30/30	100%
3	30/30	100%	30/30	100%
Cal	Between day		Between site	
No.	Positive/No.	Positive rate	Positive/No.	Positive rate
1	0/30	0%	0/30	0%
2	30/30	100%	30/30	100%
3	30/30	100%	30/30	100%

[IgM result]

Cal	Between lot		Between person	
No.	Positive / No.	Positive rate	Positive / No.	Positive rate
1	0/30	0%	0/30	0%
2	30/30	100%	30/30	100%
3	30/30	100%	30/30	100%
Cal	Between day		Between site	
No.	Positive / No.	Positive rate	Positive / No.	Positive rate
1	0/30	0%	0/30	0%
2	30/30	100%	30/30	100%
3	30/30	100%	30/30	100%

■ **Clinical performance evaluation**

ichroma™ COVID-19 Ab has demonstrated the following clinical performance results.

		RT-PCR		
		Positive	Negative	Total
ichroma™ COVID-19 Ab	Positive	46	0	46
	Negative	0	131	131
	Indeterminate	2	4	6
	Total	48	135	183

- Clinical sensitivity: 95.8%
- Clinical specificity: 97.0%

REFERENCES

1. Huang LR *et al.* Evaluation of Antibody Responses Against SARS Coronaviral Nucleocapsid or Spike Proteins by Immunoblotting or ELISA (2004) J Med Virol. 73: 33.
2. Woo PC et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the (SARS) coronavirus nucleocapsid protein in patients with pneumonia (2004) Clin Diagn Lab Immunol. 11: 665.
3. Wu HS et al. SARS-Associated Coronavirus Diagnostic kit_ development of an ELISA-based antibody detection test with a cocktail of nucleocapsid and spike SARS-CoV proteins (2008) J Clin Microbiol. 43: 3054.
4. Trivedi SU et al. Development and Evaluation of a Multiplexed Immunoassay for Simultaneous Detection of Serum IgG Antibodies to Six Human Coronaviruses (2019) Sci Rep. 9: 1390

Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices