Infection

ichroma™ COVID-19 nAb

INTENDED USE

ichroma™ COVID-19 nAb is a fluorescence immunoassay (FIA) for the qualitative determination of neutralizing antibodies against SARS-CoV-2 (or 2019-nCoV) that block the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein with the ACE-2 cell surface receptor in human whole blood/serum/plasma.

For *in vitro* diagnostic use only.

INTRODUCTION

COVID-19 is an infectious disease caused by SARS-CoV-2, a new member of the same coronavirus family that caused SARS and MERS. Entry of SARS-CoV-2 into human host cells occurs through binding of surface unit S1 of its spike protein to the cell receptor angiotensin-converting enzyme 2 (ACE-2), which leads to endocytosis, viral replication, and then spreads SARS-CoV-2 infection. Such infection typically induces antibody response. The antibody can bind to the SARS-CoV-2 spike, and only a small part of the antibody is with neutralizing function. Only neutralizing antibody can block the action of SARS-CoV-2 spike and inhibits the SARS-CoV-2 virus to infect new cells.

ichroma™ COVID-19 nAb test can help to identify whether the infected individuals with or without symptoms have acquired protective immunity from COVID-19 and how long the neutralizing antibodies persist after infection.

* The benefits of using this product are;

1) It can also help to accurately evaluate therapeutic antibodies against SARS-CoV-2,
2) It will greatly help the development of the effective vaccine.

PRINCIPLE

The test uses a competitive immunodetection method.

The SARS-CoV-2 neutralizing antibody in the sample binds to the fluorescence-labeled (FL) detector SARS-CoV-2 Spike RBD antigen in detection buffer, forming the complexes as a sample mixture. They will migrate onto the nitrocellulose matrix where the covalent couple of ACE-2 is immobilized and interfere with the binding of analyte and Fluorescence-labeled (FL) antigen.

More SARS-CoV-2 neutralizing antibodies in the sample will result in less detection of antigen to accumulate, which leads to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for ichroma™ tests to show SARS-CoV-2 neutralizing antibody concentration in the sample.

COMPONENTS

ichroma™ COVID-19 nAb consists of 'cartridges', 'detector tubes A', 'detector tubes B' and 'detector diluents'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line, 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 A contains anti-chicken IgY-fluorescence conjugate, the viral antigen-fluorescence conjugate, mouse IgG, bovine serum albumin (BSA) as a stabilizer, sucrose, sodium chloride in Tris-HCl. All detector tubes are packed in a pouch.
- The detector tube B contains ACE-2-biotin conjugate, bovine serum albumin (BSA) as a stabilizer, sucrose, sodium chloride in Tris-HCl. All detector tubes are packed in a pouch.
- The detector diluent contains tween 20, sodium chloride and sodium azide as a preservative in Tris-HCl, and it is pre-dispensed in 2 vials. The detector diluents are 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).
- 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.
- 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.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests 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 relevant local regulations.
- The detector diluents 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.

- No Biotin interference was observed in **ichroma™ COVID-19 nAb** when biotin concentration in the sample was below 500 ng/mL. If a patient has been taking biotin, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ COVID-19 nAb** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ COVID-19 nAb** 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

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube A & B	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.

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 the false negative result as it makes the 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.

MATERIALS SUPPLIED

REF CFPC-120

Components of **ichroma™ COVID-19 nAb**

- Cartridge Box:
  - Cartridge 25
  - Detector tube A 25
  - Detector tube B 25
  - Detector diluent 2
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

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

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
- **Boditech COVID-19 nAb Control** REF CFPO-303

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ COVID-19 nAb** 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.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 24 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 nAb**: Sealed cartridges, detector tubes A & B, detector diluents, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridges matches that of the detector tubes, the detector diluents as well as an ID chip.
- If the sealed cartridge, the detector tubes 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.
- Turn on the instrument for ichroma™ test.
- Insert the ID chip into the 'ID chip port'.

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

TEST PROCEDURE

▸ **ichroma™ II, ichroma™ M2**

Multi test mode / Read now mode

1) Take 200 µL of detector diluent using a pipette and dispense it to the **detector tube A** containing a SARS-CoV-2 spike RBD conjugate granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
2) Take 50 µL of sample (whole blood/ serum/ plasma/ control) using a pipette and dispense it to the detector tube A.
3) Close the lid of the detector tube A and mix the sample

thoroughly by shaking it about 10 times.
4) Leave the detector tube A + sample mixture (①) at room temperature for 5 minutes.
5) Take 150 µL of sample mixture (①) using a pipette and dispense it to the **detector tube B** containing an ACE-2-biotin conjugate granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
6) Close the lid of the detector tube B and mix the sample thoroughly by shaking it about 10 times.
7) Take 75µL of the sample mixture and dispense it into the sample well of the cartridge.
8) Leave the cartridge at room temperature for 15 minutes before inserting the device into the holder.
⚠ *Scan the sample loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
9) 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.
10) Press 'Select' or tap the 'Start' button on the instrument for ichroma™ test to start the scanning process.
11) The instrument for ichroma™ tests will start scanning the sample loaded cartridge immediately.
12) 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". (Multi Test mode 1) - 7))
2) Insert the sample-loaded cartridge into the holder immediately 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) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ test.
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 15 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'.

INTERPRETATION OF TEST RESULT

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

※ The cut-off index means 30% interference with the SARS-CoV-2 spike RBD protein and ACE-2 receptor by neutralizing antibodies.

Cut-off index (COI, %)	Result	Note
< 30	Negative	No need to retest
≥ 30	Positive	Need to confirmation test

- A "Negative" test result means no detectable SARS-CoV-2 neutralizing antibody.
- A "Positive" test result means SARS-CoV-2 neutralizing antibody detected.
- The accurate determination of test result as "Positive" should be confirmed by additional clinical evaluation.

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 nAb**. 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**
- **LoD (limit of detection)**

Type of materials	Human anti-SARS-CoV-2 RBD IgG (COI %)
LoD	7.683%

Standard material (20/130) $PRNT_{50}$ titer	WHO 1st International Standard for anti-SARS-CoV-2 immunoglobulin (20/136) (IU/mL)
1:20	16 IU/mL

- **Cut-off**

Cut-off index (COI, %)	Result
< 30	Negative
≥ 30	Positive

- **Analytical specificity**
- Cross-reactivity
Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. i**chroma™ COVID-19 nAb** test results did not show any significant cross-reactivity with these biomolecules.

No.	Name	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(s) at the concentration mentioned below. **ichroma™ COVID-19 nAb** test results did not show any significant interference with these materials.

No.	Interferents	Concentration
1	Distilled water	Undiluted
2	DMSO	Undiluted
3	DMF	Undiluted
4	Heparin	100,000 U/L
5	EDTA	1.6 mg/mL (4 μM)
6	Sodium citrate	25 mg/mL (0.085 M)
7	Hemoglobin	2 mg/mL
8	BSA	60 mg/mL
9	Bilirubin	0.24 mg/mL (400 μM)
10	Triglycerides	1.5 mg/mL
11	Cholesterol	7.7 mg/mL (20 mM)

■ **Precision**

- Between lot

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

- Between person

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

- Between days

One person tested 10 times per concentration of control standard using one lot of **ichroma™ COVID-19 nAb** for three days.

- Between sites

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

Std.	Between lot		Between person	
	# Pos./ # Tot.	Pos. rate (%)	# Pos./ # Tot.	Pos. rate (%)
Neg.	0/30	0	0/30	0
Low	30/30	100	30/30	100
Mid	30/30	100	30/30	100
Std.	Between day		Between site	
	# Pos./ # Tot.	Pos. rate (%)	# Pos./ # Tot.	Pos. rate (%)
Neg.	0/30	0	0/30	0
Low	30/30	100	30/30	100
Mid	30/30	100	30/30	100

■ **Clinical performance evaluation**

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

		ELISA		
		Positive	Negative	Total
ichroma™ COVID-19 nAb	Positive	34	0	34
	Negative	3	48	51
	Total	37	48	85

- Clinical sensitivity: 91.9%
- Clinical specificity: 100%

		$PRNT_{50}$		
		Positive (>1:20)	Negative (≤1:20)	Total
ichroma™ COVID-19 nAb	Positive	97	8	105
	Negative	1	171	172
	Total	98	179	277

- Clinical sensitivity: 99.0% (95% CI: 94.4-99.8%)
- Clinical specificity: 95.5% (95% CI: 91.4-97.7%)
- Consistent with PRNT: 96.8% (Kappa=0.93007)

		$PRNT_{90}$		
		Positive (>1:10)	Negative (≤1:10)	Total
ichroma™ COVID-19 nAb	Positive	105	0	105
	Negative	3	169	172
	Total	108	169	277

- Clinical sensitivity: 97.2% (95% CI: 92.2-99.1%
- Clinical specificity: 100% (95% CI: 97.8-100.0%)
- Consistent with PRNT: 98.9% (Kappa=0.97712)

REFERENCES

1. Peng Zhou *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020 Mar;579(7798):270-273.
2. Shibo Jiang *et al.* Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. *Trends Immunol*. 2020 May;41(5):355-359.
3. Guangyu Zhou *et al*. Perspectives on therapeutic neutralizing antibodies against the novel coronavirus SARS-CoV-2. *Int J Biol Sci*. 2020 Mar 15;16(10):1718-1723.
4. Rui Shi *et al.* A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature*. 2020 Aug;584(7819):120-124.

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

For technical assistance, please contact:
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