Infection

ichroma™ Zika IgG/IgM

INTENDED USE

ichroma™ Zika IgG/IgM is a fluorescence immunoassay (FIA) for the qualitative determination of IgG/IgM antibodies against Zika virus in human whole blood/serum/plasma. It is useful as an aid in the determination of immune status to Zika virus infection.

For *in vitro* diagnostic use only.

INTRODUCTION

Zika virus (ZIKV) is a mosquito-borne virus that was first identified in a monkey in Uganda in 1947. Zika is an RNA virus (non-segmented positive-sense RNA genome) that belongs to the Flaviviridae family and *Flavivirus* genus [(1)]. Phylogenetically, the virus is closely related with other members of the genus *Flavivirus*, including dengue virus, West Nile virus, yellow fever virus, and Japanese encephalitis virus [(2)]. Zika virus infection can be diagnosed primarily by RT-PCR. The viremic period is believed to be small, as the virus can be detected in the blood from day 0 to 4 after the onset of symptoms. The time required for the recognition of viral RNA in blood may also depend on the viral load during the acute phase of the disease, because viremia decrease over time. A negative PCR result in blood collected 5-7 days after the onset of symptoms does not exclude the *Flavivirus* infection. Specific Zika IgM antibodies can be detected by enzyme-linked immunosorbent assay (ELISA) or immunofluorescent assays (IFA) from day 4 to 5 after onset of symptoms. Specific IgM for flaviviruses can be detected usually for 2 to 3 months but sometimes for a much longer period of time. Specific IgG antibodies appear later, usually from day 8 to 10 and remain detectable for months. There are currently no validated commercial assays for Zika serological diagnosis [(3)].

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antigens in buffer bind to antibodies in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-streptavidin 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 'Zika IgG/IgM positive' in the sample.

COMPONENTS

ichroma™ Zika IgG/IgM consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin, anti-human IgG at each test line respectively 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 2 granules containing Zika antigen-fluorescent conjugate, anti-human IgM Biotin conjugate, anti-chicken IgY fluorescent conjugate and sodium azide as a preservation in MES buffer. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide (NaN_3) as a preservative in KPI buffer, 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'.
- 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).
- 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.
- The cartridge should remain sealed in its original pouch until just before use. Do not use 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.
- 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 tube and the detector diluent contain sodium azide(NaN_3), and it may cause certain health issues like convulsions, low blood pressure and low 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™ Zika IgG/IgM** when biotin concentration in the sample was below 5 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ Zika IgG/IgM** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Zika IgG/IgM** should be used only in conjunction with the instrument for ichroma™ tests
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Na EDTA, K_2EDTA, 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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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
	2 - 30°C	12 months	Opened

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

MATERIALS SUPPLIED

REF CFPC-59
Components of **ichroma™ Zika IgG/IgM**

- 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 with **ichroma™ Zika IgG/IgM**.
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 PLUS** REF FPRR036

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Zika IgG/IgM** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection, when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The sample (serum, plasma) may be stored for up to a week at 2 - 8 °C prior to being tested. If testing will be delayed more than a week, it should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 8 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™ Zika IgG/IgM**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and an 'instructions for use'.
- Ensure that the lot number of the cartridges matches that of the detector tube, the diluent tube 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.
- Turn on the Instrument for ichroma™ tests.
- Insert 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) Select the sample type (whole blood or serum/plasma) on the screen.
2) 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.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Take 30 µL of sample (whole blood/serum/plasma/ control) using a pipette and dispense it to the detector tube and mix the sample thoroughly by shaking it about 10 times.
4) 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.)
5) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
6) Leave the cartridge at room temperature for 12 minutes.

⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*

7) 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.
8) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
   (ichroma™ M2 will start the test automatically after inserting.)
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) 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 the 'Multi test mode 1) – 5)'.
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™ tests.
   (ichroma™ M2 will start the test automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 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 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) Select the sample type (whole blood or serum/plasma) on the screen.
10) 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 'G (COI value): P / N / I' ,'M (COI value): P / N / I'.
- Ancillary value is served in the form of a cut-off index (COI).

Cut-off index (COI)	Result	Note
< 0.9	Negative for Zika IgG/IgM	No need to additional test.
0.9 ≤ COI < 1.1	Indeterminate	Need to retest.
≥ 1.1	Positive for Zika IgG/IgM	Need to confirmation test.

※ If test results are shown 'Negative' or 'Indeterminate' repeatedly, these samples are considered Zika IgG/IgM antibody negative.

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™ Zika IgG/IgM**. 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 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. ichroma™ **ichroma™ Zika IgG/IgM** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	
#1	Human immunodeficiency virus(HIV)
#2	Cytomegalovirus (CMV)
#3	Epstein-Barr virus (EBV)
#4	Hepatitis A virus (HAV)
#5	Anti-Hepatitis B virus (Anti-HBS)
#6	Hepatitis B virus S Antigen (HBsAg)
#7	Hepatitis C virus (HCV)
#8	Herpes simplex virus(HSV)
#9	Rubella
#10	varicella-zoster virus(VZV)
#11	Anti-Nuclear antibody (ANA)
#12	Syphilis
#13	Toxoplasma
#14	Rheumatoid factor (RF)
#15	Samples of pregnant women

*** Antibodies to the Zika virus commonly cross-react with antibodies to the Chikungunya virus and antibodies to other viruses of the Flavivirus genus.**

- **Interference**

Interferents listed in the following table were added to the test sample mentioned below. **ichroma™ Zika IgG/IgM** test results did not show any significant interference with these materials.

No.	Interference material	Concentration
1	Lithium Heparin	100,000 U/L
2	Sodium Heparin	100,000 U/L
3	Na EDTA	2 mg/mL (5 μM)
4	K_2 EDTA	2 mg/mL (5 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**

- Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of ichroma™ Zika IgG/IgM were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

- Multi-site study

Between day

One person tested 1 lot of **ichroma™ Zika IgG/IgM** for 5 days. Each standard material was tested 2 times per day. For each test, each material was repeated three times.

Between person

Three persons tested 1 lot of **ichroma™ Zika IgG/IgM** for 5 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Between site

One person tested 1 lot of **ichroma™ Zika IgG/IgM** for 5 days at three sites. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Ig G	**Repeatability**		Total precision		**Lot to lot precision**	
	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Neg	0/42	0%	0/84	0%	0/252	0%
Low	42/42	100%	84/84	100%	252/252	100%
Mid	42/42	100%	84/48	100%	252/252	100%

Multi -site study						
Ig G	Between-person		Between-site		Between-reader	
	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Neg	0/30	0%	0/30	0%	0/30	0%
Low	30/30	100%	30/30	100%	30/30	100%
Mid	30/30	100%	30/30	100%	30/30	100%

Single-site study						
Ig M	**Repeatability**		Total precision		**Lot to lot precision**	
	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Neg	0/42	0%	0/84	0%	0/252	0%
Low	42/42	100%	84/84	100%	252/252	100%
Mid	42/42	100%	84/48	100%	252/252	100%

Multi -site study						
Ig M	Between-person		Between-site		Between-reader	
	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Neg	0/30	0%	0/30	0%	0/30	0%
Low	30/30	100%	30/30	100%	30/30	100%
Mid	30/30	100%	30/30	100%	30/30	100%

■ **Comparability**

Zika IgG		Comparator A (ELISA)		
		Positive	Negative	Total
ichroma™ Zika IgG/IgM	Positive	38	3	41
	Negative	2	117	119
	Total	40	120	160

Zika IgM		Comparator A (ELISA)		
		Positive	Negative	Total
ichroma™ Zika IgG/IgM	Positive	37	5	64
	Negative	3	115	118
	Total	40	120	160

- Percent positive agreement (PPA) of IgG = 95.00%
- Percent positive agreement (PPA) of IgM = 92.50%
- Percent negative agreement (PNA) of IgG = 97.50%
- Percent negative agreement (PNA) of IgM = 95.83%

REFERENCES

1. Zika virus disease: a current review of the literature. Muhammad Atif et al., Infection, 2016
2. Zika virus: a new arboviral public health problem. Tulin Demir et al., Folia Microbiol, 2016
3. European Centre for Disease Prevention and Control. Interim guidance for healthcare provider and Zika virus laboratory diagnosis. ECDC, Stockholm. 2016

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

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

For technical assistance, please contact:
Boditech Med Inc.'s Technical Services
Tel: +(82) -33-243-1400
E-mail: TS@boditech.co.kr

Boditech Med Inc.
43, Geodudanji 1-gil, Dongnae-myeon, Chuncheon-si, Gang-won-do, 24398, Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, Belgium
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE IVD