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 screening of 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 dose 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 detectible for months. There are currently no validated commercial assays for Zika serological diagnosis [(3)].

PRINCIPLE

The test uses a sandwich immunodetection method; Dried antibodies in the detector tube, once diluted with the diluent, bind with antigens in the sample to form antigen-antibody complexes. These complexes then migrate through the nitrocellulose matrix and are captured by another sets of immobilized antibodies on the test line.

The more antigens in the sample, the more antigen-antibody complexes, which leads to a stronger fluorescence signal. This signal then is interpreted by the reader to display the Zika IgG/IgM concentration in the sample.

COMPONENTS

ichroma™ Zika IgG/IgM consists of 'Cartridge', 'Detection buffer tube', 'Detection diluent vial' 'ID chip' and 'Instruction for use'.

- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- Cartridge part contains a test strip, the membrane which has anti-human IgM and anti-human IgG at the each test lines (1 and 2), respectively, while Chicken IgY at the control line.
- Detector part contains recombinant Zika NS1 antigen fluorescence conjugate, anti-chicken IgY fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer, diluent) should agree.
- Do not interchange test components between different lots or use test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with applicable local requirement. A Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, the detection buffer and the sample reach the room temperature by leaving them in the room for approximately 30 minutes.
- **ichroma™ Zika IgG/IgM** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ Zika IgG/IgM** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Zika IgG/IgM** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **K-2 EDTA, heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in the original aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer is stable for 20 months if stored at 2-8 °C.
- The diluent dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- 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 antigens.
- The test may yield false negative result. The non-responsiveness of the antibodies to the antigens is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antibody unrecognizable by the antigens.
- 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 including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-59

Components of **ichroma™ Zika IgG/IgM**

- Cartridge Box:
  - Cartridges 25

- ID Chip 1
- Instruction For Use 1

■ Aluminum Pouch containing Detection Buffer Tubes

- Detection Buffer tubes 25

■ Dilution Buffer Vial Pouch

- Diluent Vial 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Zika IgG/IgM**. Please contact our sales division for more information.

■ Instrument for ichroma™ tests

- **ichroma™ II** REF FPRR021

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.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- Samples 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, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 2 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be thawed only once and only for test, because repeated freezing and thawing can result in the changed test values.
- Samples containing precipitates must be clarified by centrifugation.

TEST SETUP

- Check the contents of **ichroma™ Zika IgG/IgM**: Sealed Cartridge, Detection Buffer Tubes, Diluent Vial and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip, the detection buffer as well as the diluent.
- Keep the sealed cartridge (if stored in refrigerator), the detection buffer tube and the diluent at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the Instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the Instrument for ichroma™ tests**.**
- Press the 'Select' button on the Instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Transfer 150 µL of the diluent using a pipette to the detection buffer tube.
2) Transfer 30 µL of sample (whole blood/serum/plasma/ control) using a pipette to the detection buffer tube.
3) Dissolve the granulized detection buffer thoroughly by pipetting 20 times.
   (The sample mixture must be used immediately.)
4) Load 75 µL of a sample mixture into a sample well in the cartridge.
5) Leave the sample-loaded cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact 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 has been marked on the cartridge especially for this purpose.
7) Press 'Select' button on the instrument for ichroma™ test to start the scanning process.
8) Instrument for ichroma™ test should start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays "IG value: Positive / Negative / Indeterminate, IM value: Positive / Negative / Indeterminate".
- Ancillary value is served in the form of a cut-off index (COI).

Cut-off index (COI)	Result
≤ 0.90	Negative for Zika IgG/IgM
> 0.90, < 1.1	Indeterminate
≥ 1.1	Positive for Zika IgG/IgM

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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided 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 instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

■ **Analytical Sensitivity**

- Limit of Detection (LOD)

To determine the limit of detection (LOD), the panel was tested by serial dilution with **ichroma™ Zika IgG/IgM**

Zika IgG positive panel				
	Commercial ELISA		Ichroma™ Zika IgG/IgM	
Dilution Factor	ISR of ELISA	Results	COI	Results
2^{0}	3.23	Positive	56.94	Positive
2^{-1}	2.72	Positive	53.69	Positive
2^{-2}	2.15	Positive	27.59	Positive
2^{-3}	1.49	Positive	22	Positive
2^{-4}	1.04	Positive	21.14	Positive
2^{-5}	0.62	Negative	19.37	Positive
2^{-6}	0.37	Negative	19.21	Positive
2^{-7}	0.22	Negative	7.46	Positive
2^{-8}	0.14	Negative	2.95	Positive
2^{-9}	0.11	Negative	2.53	Positive
2^{-10}	0.09	Negative	0.68	Negative
Zika IgM positive panel				
	Commercial ELISA		Ichroma™ Zika IgG/IgM	
Dilution Factor	ISR of ELISA	Results	COI	Results
2^{0}	0.91	Negative	10.54	Positive
2^{-1}	0.36	Negative	6.01	Positive
2^{-2}	0.2	Negative	0.76	Negative
2^{-3}	0.13	Negative	0.37	Negative
2^{-4}	0.08	Negative	0.43	Negative
2^{-5}	0.07	Negative	0.22	Negative
2^{-6}	0.06	Negative	0.23	Negative
2^{-7}	0.06	Negative	0.21	Negative
2^{-8}	0.06	Negative	0.07	Negative

- **Analytical Specificity**

- Cross-reactivity
There was no significant cross reactivity from these materials with the **ichroma™ Zika IgG/IgM** test.

Clinical category	ichroma™ Zika IgG/IgM		
	Number of samples	Negative	Positive
DENV	10	10	0
CMV	19	19	0
EBV	20	20	0
HAV	28	28	0
HCV	10	10	0
HSV	19	19	0
Rubella	20	20	0
VZV	20	20	0
Syphilis	17	17	0
ANA	23	23	0
RF	23	23	0
Early stage of pregnancy	17	17	0
Middle stage of pregnancy	22	22	0
Total	238	238	0

- Interference
There was no significant interference from these materials with the **ichroma™ Zika IgG/IgM** test.

Materials	Concentration
Heparin	100,000 U/L
EDTA	5 μM
Sodium citrate	0.085 M
Bilirubin	500 μM
Hemoglobin	2 g/L
Triglycerides	1.5 mg/mL
Cholesterol	20 mM
Albumin	60 mg/mL

- **Precision**

- Between Lot
One person tested three different lots of **ichroma™ Zika IgG/IgM**, ten times at each concentration of the control standard.
- Between person
Three different persons tested same lot of **ichroma™ Zika IgG/IgM**, five times at each concentration of the control standard.
- Between day
One person tested same lot of **ichroma™ Zika IgG/IgM** during three days, five times at each concentration of the control standard.
- Between site
One person tested same lot of **ichroma™ Zika IgG/IgM** at three different sites, five times at each concentration of the control standard.

Zika IgG/IgM Cal	Between lot		Between person		Between day		Between site	
	Positive/ Number of test	Positive	Positive/ Number of test	Positive	Positive/ Number of test	Positive	Positive/ Number of test	Positive
Negative	0/10	0 %	0/5	0 %	0/5	0 %	0/5	0 %
High	10/10	100 %	5/5	100 %	5/5	100 %	5/5	100 %
Mid	10/10	100 %	5/5	100 %	5/5	100 %	5/5	100 %
Low	10/10	100 %	5/5	100 %	5/5	100 %	5/5	100 %

- **Comparability with reference product**

		Commercial IgG ELISA result			
		Positive	Equivocal	Negative	Total
ichroma™ Zika IgG/IgM	Positive	24	0	0	24
	Equivocal	0	0	0	0
	Negative	0	0	62	62
	Total	24	0	62	86

		Commercial IgM ELISA result			
		Positive	Equivocal	Negative	Total
ichroma™ Zika IgG/IgM	Positive	24	0	0	24
	Equivocal	0	0	0	0
	Negative	0	0	62	62
	Total	24	0	62	86

- IgG sensitivity = 24/24 x 100 = 100 %
- IgG specificity = 62/62 x 100 = 100 %
- IgM sensitivity = 24/24 x 100 = 100 %
- IgM specificity = 62/62 x 100 = 100 %

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.

Σ	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

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