Infection

ichroma™ Dengue IgG/IgM

INTENDED USE

ichroma™ Dengue IgG/IgM is a fluorescence Immunoassay (FIA) for the qualitative determination of IgG/IgM antibodies against dengue virus in human whole blood/serum/plasma. It is useful as an aid in screening of Dengue virus infection.

For *in vitro* diagnostic use only.

INTRODUCTION

Dengue virus (DENV), a mosquito-borne virus, phylogenetically belongs to the genus Flavivirus, including Zika virus, West Nile virus, yellow fever virus, and Japanese encephalitis virus. Dengue virus comprises of 4 serotypes distinct in the infection tendency and immune responses. Dengue virus infection causes clinically a wide range of human diseases from mild Dengue Fever (DF) to severe Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS).[1] Several lines of evidences show that secondary Dengue virus infection, with different serotypes from the primary infection, is relevant to severe Dengue diseases.[2] The immune response to primary or secondary virus infection varies. In the case of primary infection, specific IgM is higher titre during 4-10 days after onset of illness than IgG. IgG response become permanent for whole life of patient with primary infection. During secondary infection, in contrast, the titre of virus-specific IgG is higher than IgM titre in whole of serological period.[2,3] Although there are many types of serological diagnostic reagents including enzyme-linked immunosorbent assay (ELISA) or immunofluorescent assays (IFA), development of simultaneous and accurate detection kit of IgG and IgM is required.[4]

PRINCIPLE

The test uses a sandwich immunodetection method; granulized detector antigen in cartridge binds to antibody in the sample, forming antibody-antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antigen on the test strip.

The more antibody in sample forms the more antigen-antibody complex, which leads to stronger intensity of fluorescence signal on detector antigen, which is processed by the instrument for ichroma™ tests to show 'dengue IgG/IgM positive' in sample.

COMPONENTS

ichroma™ Dengue IgG/IgM consists of 'Cartridges', 'Detection Buffer Tubes', 'Diluent vial' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti-human IgM and anti-human IgG at each test lines respectively, while Nus antigen at the control line.
- 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.
- The detection buffer contains granules. There are contain anti-Dengue antigen fluorescence conjugate, anti-Nus fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- 25 detection buffer tubes are packed in an aluminum foil pouch.
- The diluent contains a detergent and bovine serum albumin (BSA) as a stabilizer and sodium azide in sodium borate buffer as a preservative. The diluent is dispensed in a vial.

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) 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 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 the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Dengue 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™ Dengue IgG/IgM** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Dengue IgG/IgM** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an 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-60

Components of **ichroma™ Dengue 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™ Dengue IgG/IgM**.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
- **ichroma™ Printer** REF FPRR007

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Dengue 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 one time 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™ Dengue 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 10 µL of sample (whole blood/serum/plasma/ control) using a pipette to the sample well on the cartridge.
2) Transfer 150 µL of the diluent using a pipette to the detection buffer tube.
3) Dissolve the granulized detection buffer thoroughly by tapping 10 times and pipetting 10-20 times. (The sample mixture must be used immediately.)
4) Pipette out 75 µL of a sample mixture and dispense it into the DB well on 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) Tab the 'Start' icon on the screen.
8) 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.

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	Note
≤ 0.9	Negative for Dengue IgG Ab / IgM Ab	No need to additional test.
> 0.9, < 1.1	Indeterminate	Need to retest. If test results are shown 'Negative' or 'Indeterminate' repeatedly, these samples are considered dengue IgG/IgM antibody negative.
≥ 1.1	Positive for Dengue IgG Ab / IgM Ab	Need to confirmation test.

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™ Dengue 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™ Dengue IgG/IgM**

Dengue IgG positive panel				
	Commercial ELISA		Ichroma™ Dengue IgG/IgM	
Dilution Factor	ISR of ELISA	Results	COI	CV (%)
2^{0}	8.24	Positive	18.45	3.4
2^{-1}	6.56	Positive	10.21	5.1
2^{-2}	4.84	Positive	6.98	8.3
2^{-3}	3.84	Positive	5.43	4.3
2^{-4}	2.84	Equivocal	3.85	13.6
2^{-5}	2.18	Equivocal	2.16	10
2^{-6}	2.14	Equivocal	1.01	5.8
2^{-7}	2.01	Equivocal	0.98	44.2
2^{-8}	1.07	Negative	0.99	19.7

Dengue IgM positive panel				
	FDA-cleared MAC ELISA		ichroma™ Dengue IgG/IgM	
Dilution Factor	ISR of ELISA	Results	COI	CV (%)
2^{0}	10.66	Positive	7.84	1.1
2^{-1}	4.6	Positive	1.97	7.1
2^{-2}	3.02	Positive	0.98	7.4
2^{-3}	1.75	Equivocal	1.01	25.1
2^{-4}	0.38	Negative	0.87	35.2
2^{-5}	0.13	Negative	0.89	28.2

* In the case of Dengue IgG panel, **ichroma™ Dengue IgG/IgM** shows more sensitive analytical reactivity by 16 times than result of reference ELISA.

* In the case of Dengue IgM panel, **ichroma™ Dengue IgG/IgM** shows similar sensitivity to result of reference ELISA.

- **Analytical Specificity**

- Cross-reactivity
There was no false positive result from 169 samples containing potentially interfering substances with the **ichroma™ Dengue IgG/IgM** test. The overall specificity was 100 %.

Clinical category	ichroma™ Dengue IgG/IgM		
	Number of samples	Negative	Positive
ZIKV	17	17	0
CMV	20	20	0
EBV	20	20	0
HAV	28	28	0
HCV	15	15	0
HBV	21	21	0
ANA	23	23	0
RF	25	25	0
Total	238	238	0

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

Materials	Concentration
Heparin	100,000 U/L
EDTA	4 μM
Sodium citrate	0.085 M
Bilirubin	200 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™ Dengue IgG/IgM**, ten times at each concentration of the control standard.
- Between person
Three different persons tested same lot of **ichroma™ Dengue IgG/IgM**, five times at each concentration of the control standard.
- Between day
One person tested same lot of **ichroma™ Dengue IgG/IgM** during three days, five times at each concentration of the control standard.
- Between site
One person tested same lot of **ichroma™ Dengue IgG/IgM** at three different sites, five times at each concentration of the control standard.

Dengue 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™ Dengue IgG/IgM	Positive	40	0	0	40
	Equivocal	0	0	0	0
	Negative	0	0	50	50
	Total	40	0	50	90
		FDA-cleared MAC-ELISA result			
		Positive	Equivocal	Negative	Total
ichroma™ Dengue IgG/IgM	Positive	26	0	1	27
	Equivocal	0	0	0	0
	Negative	1	0	49	50
	Total	27	0	50	77

- IgG sensitivity = 40/40 x 100 = 100 %
- IgG specificity = 50/50 x 100 = 100 %
- IgM sensitivity = 25/26 x 100 = 96.15 %
- IgM specificity = 48/49 x 100 = 97.95 %

REFERENCES

1. Evaluation of diagnostic test: Dengue. Rosanna W. P. et al., Nature, 2010
2. Immunoglobumin G (IgG) to IgM ratio in secondary adult dengue infection using samples from early days of symptoms onset. Cucunawangsih et al., BMC Infectious Diseases, 2015
3. Dengue Viraemia Titer, Antibody Response Pattern and Virus Serotype Correlate with Disease Severity: Vaughn. D. W. et. al., Journal of Infectious Diseases. 2000
4. Current Global Status of Dengue Diagnostics: Miranda D. S. et. al., Journal of Advance in Biology and Biotechnology, 2015

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: sales@boditech.co.kr

Boditech Med Incorporated
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