BIO TECHNOLOGY boditech

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.

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-antibodies on a test strip.

More antibodies in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal which is processed by the instrument for ichroma™ tests to show the 'dengue IgG/IgM positive' in the sample.

COMPONENTS

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

- The cartridge contains the membrane called a test strip which has anti-human IgM, anti-human IgG at each test lines 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 a granule containing Dengue antigen-fluorescent conjugate, anti-chicken IgY fluorescent conjugate, bovine serum albumin (BSA) as a stabilizer in Tris. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative in Tris, 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 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, 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, diluent tubes and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector diluent contains sodium azide(NaN_3), and it 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™ Dengue IgG/IgM** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Dengue IgG/IgM** should be used only in conjunction with the instrument for ichroma™ tests
  - Have to use recommended anticoagulant.

Recommended anticoagulant
EDTA, K_2EDTA, K_3EDTA, 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 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.

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	20 months	Opened

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

MATERIALS SUPPLIED

REF CFPC-60

Components of **ichroma™ Dengue 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 from **ichroma™ Dengue 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
- **Boditech Dengue IgG/IgM Control** REF CFPO-280

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 sample (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 month at 2 - 8 °C prior to being tested. If testing will be delayed more than a month, it should be frozen at -20 °C.
- The sample (serum, plasma) stored frozen at -20 °C for 1 year showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ Dengue IgG/IgM**: Sealed cartridges, detector tubes, detector diluent, ID chip and an 'instructions for use'.
- Ensure that the lot number of the cartridges matches that of the diluent tube as well as an ID chip.
- If the sealed cartridge and the detection buffer 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) 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.)
2) Take 5 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 - 20 times
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) 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.*

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™ tests 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 the '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™ tests.
(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 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) 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 Dengue IgG/IgM	No need to additional test.
≥ 0.9, < 1.1	Indeterminate	Need to retest.
≥ 1.1	Positive for Dengue IgG/IgM	Need to confirmation test.

※ If test results are shown 'Negative' or 'Indeterminate' repeatedly, these samples are considered dengue 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™ Dengue 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 Sensitivity**
  - **Cut-off**
    The **ichroma™ Dengue IgG/IgM** 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
COI ≥ 1.1	Pos. for Dengue IgG / IgM
0.9 ≤ COI < 1.1	Indeterminate
COI < 0.9	Neg. for Dengue IgG / IgM

- **Analytical specificity**
  - **Cross-reactivity**
    There was no false positive result from 8 species virus samples containing potentially interfering substances with the **ichroma™ Dengue IgG/IgM** test.

Cross-reactivity Materials	
#1	Cytomegalovirus (CMV)
#2	Epstein-Barr virus (EBV)
#3	Hepatitis A virus (HAV)
#4	Hepatitis C virus (HCV)
#5	Hepatitis B virus (HBV)
#6	Anti-Nuclear antibody (ANA)
#7	Rheumatoid factor (RF)
#8	Zika

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

Interferent	Conc.
Sodium Heparin	100,000 U/L
K_2 EDTA	5 μM
Sodium citrate	0.17 M
Bilirubin	500 μM
Hemoglobin	2 g/L
Triglycerides	1.5 g/L
Cholesterol	20 mM
BSA	60 g/L

- **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**, ten times at each concentration of the control standard.
- Between day
  One person tested same lot of **ichroma™ Dengue IgG/IgM** for three days, ten times at each concentration of the control standard.
- Between site
  One person tested same lot of **ichroma™ Dengue IgG/IgM** at three different sites, ten times at each concentration of the control standard.

Ig G		
Standard material	Between lot	Between person
	Positive ratio	Positive ratio
IgG(Neg.), IgM(Neg.)	0%	0%
IgG(Pos.), IgM(inde.)	100%	100%
IgG(Pos.), IgM(Pos.)	100%	100%
Standard material	Between day	Between site
	Positive ratio	Positive ratio
IgG(Neg.), IgM(Neg.)	0%	0%
IgG(Pos.), IgM(inde.)	100%	100%
IgG(Pos.), IgM(Pos.)	100%	100%
Ig M		
Standard material	Between lot	Between person
	Positive ratio	Positive ratio
IgG(Neg.), IgM(Neg.)	0%	0%
IgG(Pos.), IgM(inde.)	0%	0%
IgG(Pos.), IgM(Pos.)	100%	100%
Standard material	Between day	Between site
	Positive ratio	Positive ratio
IgG(Neg.), IgM(Neg.)	0%	0%
IgG(Pos.), IgM(inde.)	0%	0%
IgG(Pos.), IgM(Pos.)	100%	100%

■ **Comparability**

Dengue IgG		Comparator A (ELISA)		
		Positive	Negative	Total
ichroma™ Dengue IgG/IgM	Positive	60	0	60
	Negative	1	21	22
	Total	61	21	82
Dengue IgM		Comparator A (ELISA)		
		Positive	Negative	Total
ichroma™ Dengue IgG/IgM	Positive	18	1	19
	Negative	2	32	34
	Total	20	33	53

- Percent positive agreement (PPA) of IgG = 98.4 %
- Percent positive agreement (PPA) of IgM = 90 %
- Percent negative agreement (PNA) of IgG = 100 %
- Percent negative agreement (PNA) of IgM = 96.97 %

REFERENCES

1. Evaluation of diagnostic test: Dengue. Rosanna W. P. et al., Nature, 2010
2. Immunoglobulin 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:
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