Infection

ichroma™ Dengue NS1 Ag

INTENDED USE

ichroma™ Dengue NS1 Ag is a fluorescence immunoassay (FIA) for the qualitative determination of NS1 (Dengue Virus Non-structural protein 1) Antigen in human whole blood/serum/plasma during dengue virus infection. It is useful as an aid in screening of early Dengue virus infection.

For *in vitro* diagnostic use only.

INTRODUCTION

Nonstructural protein 1 (NS1), one of the dengue viral nonstructural proteins, plays a role in supporting the replication complex and attenuating the host immune response against viral infection.[1] Several lines of evidence show that plasma level of secreted NS1 (sNS1) correlated with viremia levels in dengue virus infected patients. The more generated dengue virus, the higher concentration of sNS1 occurred after onset of illness.[2,3] The amount of sNS1 is decreased when plasma viral level is reduced. Thus, it is reasonable to detect the sNS1 in patient blood, which makes it possible to early diagnosis of dengue virus infection.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens 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 antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show 'dengue NS1 Ag positive' in the sample.

COMPONENTS

ichroma™ Dengue NS1 Ag of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has anti-Dengue NS1 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 has 2 granules containing anti-Dengue NS1-fluorescence conjugate and anti-chicken IgY fluorescence conjugate and sodium azide as a preservative in Tris. All detector tubes are packed in a pouch.
- The detector diluent contains tween 20 as a detergent, sodium azide as a preservative in potassium phosphate 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 test components between different lots or use 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, 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.
- **ichroma™ Dengue NS1 Ag** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Dengue NS1 Ag** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Na_2 EDTA, K_2 EDTA, 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-62

Components of **ichroma™ Dengue NS1 Ag**

- Cartridge Box:
  - Cartridges 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™ Dengue NS1 Ag**.

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 NS1 Ag Control** REF CFPO-282

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Dengue NS1 Ag** 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 sample (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 up to a week at 2 - 8°C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) 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™ Dengue NS1 Ag**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent 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 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 100 µL of detector diluent using a pipette and dispense it to the detector tube containing 2 granules. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately.)
2) Take 50 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 will start the test 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 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) 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 "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 NS1 Ag	No need to additional test.
≥ 0.9, < 1.1	Indeterminate	*Need to retest.
≥ 1.1	Positive for Dengue NS1 Ag	Need to confirmation test.

* If retest results are shown 'Negative' or 'Indeterminate' repeatedly, these samples are considered dengue NS1 antigen 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 NS1 Ag**. 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**
  - **Limit of Detection (LOD)**
  The LOD of **ichroma™ Dengue NS1 Ag** was determined using of Dengue NS1 recombinant antigen. The LoD is determined as 50 ng/mL through 20 repetitive tests.
  - **Cut-off**
  The **ichroma™ Dengue NS1 Ag** 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 NS1 Ag
0.9 ≤ COI < 1.1	Indeterminate
COI < 0.9	Neg. for Dengue NS1 Ag

- **Analytical Specificity**
  - Cross-reactivity
  There was no significant cross-reactivity on 36 viruses and bacteria with the **ichroma™ Dengue NS1 Ag**.

No.	Cross-reactants	Concentration
1	*Influenza A virus (A/Kansas/14/2017)*	1.4 x 10^6 PFU/mL
2	*Influenza B virus (B/Phuket/3073/201)*	7.6 x 10^6 PFU/mL
3	*Respiratory Syncytial virus A2*	8.5 x 10^5 PFU/mL
4	*Respiratory Syncytial virus B*	2 x 10^6 pfu/mL
5	*Human rhinovirus B-Type 14*	6 x10^8 pfu/mL
6	*Human Metapneumovirus*	1.17 X 10^5 $TCID_{50}$/mL
7	*Human Enterovirus A Type 71*	4.5 x 10^6 $TCID_{50}$/mL
8	*Parainfluenza virus Type 2*	1.51 X 10^6 $TCID_{50}$/mL
9	*Parainfluenza virus Type 3*	4.57 X 10^6 $TCID_{50}$/mL
10	*Parainfluenza virus Type 4B*	1.70 X 10^5 $TCID_{50}$/mL
11	*Japanese encephalitis virus*	2.4 x 10^5 pfu/mL
12	*Human Cytomegalovirus*	1 x 10^6 pfu/mL
13	*Measles virus*	3 x 10^7 pfu/mL
14	*Mumps Orthorubulavirus*	4.0 x 10^{10} pfu/mL
15	*Adenovirus type1*	1 X $10^{6.8}$ $TCID_{50}$/mL

16	*Adenovirus type2*	1 X $10^{4.3}$ $TCID_{50}$/mL
17	*Adenovirus type3*	1 x 10^{6} pfu/mL
18	*Coxsackievirus A2*	1 X 10^{8} $TCID_{50}$/mL
19	*Coxsackievirus A4*	1 X $10^{5.7}$ $TCID_{50}$/mL
20	*Coxsackievirus B1 - conn5*	8.7 X 10^{-7} $CCID_{50}$/mL
21	*Coxsackievirus B3 – nancy (5A1)*	2.3 X 10^{-8} $CCID_{50}$/mL
22	*Rhinovirus - RV21*	5.6 X 10^{4} $CCID_{50}$/mL
23	*Pseudomonas aeruginosa*	1 x 10^{6} CFU/mL
24	*Staphylococcus aureus*	1 x 10^{6} CFU/mL
25	*Streptococcus pyogenes*	6 x 10^{8} CFU/mL
26	*Haemophilus influenzae*	1 x 10^{6} CFU/mL
27	*Corynebacterium diphteria*	1 x 10^{6} CFU/mL
28	*Legionella pneumophila*	1.6 x 10^{7} CFU/mL
29	*Streptococcus pneumoniae*	6 x 10^{8} CFU/mL
30	*West Nile virus*	Positive Plasma
31	*Hepatitis A virus*	Positive Plasma
32	*Hepatitis B virus*	Positive Plasma
33	*Hepatitis C virus*	Positive Plasma
34	*Epstein Barr virus*	Positive Plasma
35	*Chikungunya virus*	Positive Plasma
36	*Zikavirus*	Positive Plasma

- Interference
  Interferents listed in the following table were added to the test sample mentioned below. **ichroma™ Dengue NS1 Ag** 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_2 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)
11	Rheumatoid Factor	200 mg/mL

■ **Precision**

- Between day
  One person tested 1 lot of **ichroma™ Dengue NS1 Ag** for 5 days. Each standard material was tested 2 times per day. For each test, each material was repeated three times.
- Between lot
  One person tested 3 lot of **ichroma™ Dengue NS1 Ag** for 5 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.
- Between person
  Three persons tested 1 lot of **ichroma™ Dengue NS1 Ag** 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™ Dengue NS1 Ag** for 5 days at three sites. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Dengue NS1 Ag	Between day		Between lot	
	Pos./Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Negative	0/30	0 %	0/60	0 %
Low Positive	30/30	100 %	60/60	100 %
Mid Positive	30/30	100 %	60/60	100 %
High Positive	30/30	100 %	60/60	100 %

Dengue NS1 Ag	Between person		Between site	
	Pos./ Number of tests	Pos. rate	Pos./ Number of tests	Pos. rate
Negative	0/30	0 %	0/60	0 %
Low Positive	60/60	100 %	60/60	100 %
Mid Positive	60/60	100 %	60/60	100 %
High Positive	60/60	100 %	60/60	100 %

■ **Comparability**

		Reference reagent (RT-PCR)		
		Positive	Negative	Total
ichroma™ Dengue NS1 Ag	Positive	60	0	60
	Negative	0	200	200
	Total	60	200	260

- Percent positive agreement (PPA) = 100 %
- Percent negative agreement (PNA) = 100 %
- Total agreement = 100 %

REFERENCES

1. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. Daniel H. Library et al., The Journal of Infectious Diseases, 2002.
2. Potential application of nonstructural protein NS1 serotype-specific immunoglobulin G enzyme-linked immunosorbent assay in the seroepidemiologic study of dengue virus infection: Correlation of results with those of the plaque reduction neutralization test. Pei-Yun S. et al., Journal of Clinical Microbiology, 2002.
3. Evaluation of diagnostic tests: Dengue, Rosanna W. P. et. al., Nature, 2010.

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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