DENGUE IgG *i*Clia

Chemiluminesence Immunoassay for the Detection of ***Dengue IgG Antibodies in Human Serum/Plasma***

1. INTRODUCTION

The mosquito-borne dengue viruses (serotype 1-4) cause dengue fever, a severe flu-like illness. The disease is prevalent in third world tropical regions and spreading to sub-tropical developed countries - including the United States. WHO estimates that 50-80 million cases of dengue fever occur worldwide each year, including a potentially deadly form of the disease called dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting. Secondry infection is the more common form of the disease in many parts of Southeast Asia and South America. This form of the disease is more serious and can result in DHF and DSS. The major clinical symptoms can include high fever, haemorrhagic evets, and circulatory failure, and the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 hours if appropriate treatment is not administered.

Primary dengue virus infection is characterized by elevations in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days. IgM levels also become elevated after 10 to 14 days and remain detectable for life. During secondary infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly from 1 to 2 days after the onset of symptoms.

2. INTENDED USE

Dengue IgG iClia is a chemiluminiscent microparticle immunoassay designed for *in vitro* qualitative detection of Dengue IgG antibodies in human serum or plasma and is used as a screening test for testing of collected blood samples suspected for Dengue. The kit detects all four subtypes; DEN1, DEN2, DEN3 & DEN4 of Dengue Virus. This kit is only operational in connection with CLIA 181 Analyzer.

3. PRINCIPLE

Dengue IgG iClia is chemiluminescent immunoassay based on the "GAC CAPTURE" principle. The magnetic microparticles are coated with Anti-Human IgG antibodies.

The samples are added in the assay cup containing coated microparticles. Antibodies to Dengue IgG if present in the specimen, will bind to the Anti human IgG antibodies absorbed onto the surface of the microparticles. Unbound antibodies are then washed off with wash buffer followed by addition of AE conjugate (Dengue antigen (DEN1-4) linked to acridinium ester) to assay cup . This dengue antigen conjugate will bind to dengue specific IgG antibodies which is complexed with anti- human IgG antibodies. The amount of bound AE conjugate is proportional to the concentration of dengue IgG Antibodies present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs). There is a direct relationship between the amount of dengue IgG Antibodies present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the Calibrator.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	8°C 2°C	Temperature Limitation
	Manufacturing Date		Caution - See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 50 Test Pack
- 100 Test Pack
- 150 Test Pack

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer	Magnetic microparticles coated with anti-Human IgG antibodies with preservatives.
Assay Buffer	Buffer containing protein stabilizers & antimicrobial agents as preservative.
AE Conjugate	Dengue Antigen linked to acridinium ester with protein stabilizers.
Diluent	Buffer containing protein stabilizers & antimicrobial agents as preservative.
Control-1	Normal human plasma negative for Dengue IgG Antibodies with preservative.
Control-2	Positive for Dengue IgG Antibodies with preservative.
Calibrator	Cut-off Calibrator, Positive for Dengue IgG Antibodies with preservative.
Reagent Sealers	Adhesive sheets to cover the opened reagents.

7. STORAGE AND STABILITY

The shelf-life of the kit is 12 months from the date of manufacturing, when stored at 2-8°C. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8°C.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **CLIA 181 Analyzer**

All materials and analyzer to be used for running the Dengue IgG iClia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer.
4. Specimens should be free of microbial contamination and may be stored at 2-8^{0}C for one week, or frozen at -20^{0}C or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscous/ thick or turbid specimen at 10,000 RPM for 15 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Dengue IgG iClia test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. Centrifuge the sample at 10,000 rpm for 15 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121^{0}C at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8^{o}C.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microparticles that may have settled during transport or storage.
5. Once reagents are opened, reagent Sealer must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent sealers are not used according to the instructions given.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. To avoid contamination, wear clean gloves when placing a reagent sealer on an uncapped reagent bottle.
8. Once a reagent sealer has been placed on an open reagent bottle,do not invert the bottle as this will result in reagent leakage and may compromise assay results.
9. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent Sealers) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent Sealer placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run control-1 & control-2 in each assay to evaluate validity of the kit.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Avoid strong light exposure during the assay.
13. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia-181 user manual for detailed information on preparing the analyzer.
2. Before loading the Dengue IgG iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticle buffer that may have settled during transporation/ storage. Once the microparticles have been loaded, no further mixing is required.

   Note: Swirl the microparticle bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. Once the microspheres have been resuspended, place a reagent sealer on the bottle.
3. Load the Dengue IgG iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 μL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 5 μL.
7. Ensure sample positons are properly define at the time of loading in the analyzer.

8. The Dengue IgG test-specific parameters are stored in barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Mix Dengue IgG iClia calibrator and controls by gentle inversion before use. Open the cap and place the calibrator and control-1 & control-2 vials into each respective assigned positions. Read the barcode for calibrator and controls provided with the kit.
10. Run calibration as mentioned in heading **calibration** below.
11. Press Run. The test result for first sample will be obtained at 55 minutes.
12. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Calibration

1. Test Calibrator in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that calibrator and controls values are within the validity range specified in this instruction manual.
2. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless, recalibration is required.
3. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and Pre-Trigger/ Trigger solution/wash buffer).
   b) Every week and/or at the time of any component to be changed.
   c) Controls are out of validation range.
   d) Required by pertinent regulations.
   e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the Dengue IgG iClia.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) Mean Calibrator Sample to cut-off ratio (S/CO) must be between 1.1 - 2.5. If it is not so, the run is invalid and must be repeated.

ii) Sample to cut-off ratio (S/CO) of control-1 must be between 0.05 to 0.50. If it is not so, the run is invalid and must be repeated.

iii) Sample to cut-off ratio (S/CO) of control-2 must be between 5 to 20. If it is not so, the run is invalid and must be repeated.

RESULT CALCULATION:

The analyzer automatically calculates the sample to cut-off ratio (S/CO) of each sample based on cut-off value using formulas.

a. Cut off value = mean RLU of calibrator x Calibration Factor (F)

b. Calculation of Sample to cut-off Ratio:

Sample cut-off Ratio (S/CO) = RLU of Sample / Cut-off value

Note: Calibration Factor (F) is batch specific and is provided in the calibrator barcode.

14. INTERPRETATION OF RESULTS

a. If the Dengue IgG S/CO is $\leq$ 0.9 then interpret the sample as Negative for Dengue IgG antibodies.

b. If the Dengue IgG S/CO is between 0.9 - 1.1 then interpret the sample as Equivocal for Dengue IgG antibodies and sample should be re-tested.

c. If the Dengue IgG S/CO is $\geq$ 1.1 then interpret the sample as Positive for Dengue IgG antibodies.

15. PERFORMANCE CHARACTERISTICS

A) In-house Evaluation:

Diagnostic Sensitivity and Specificity: The Performance of the Dengue IgG iClia with reference to sensitivity and specificity was evaluated in-house with the panel of 90 negative and 10 Dengue IgG Antibodies positive samples. The performance is also checked with fresh clinical negative (100) and Dengue IgG Antibodies Positive (15) samples. The results of all the positive and negative samples were compared with commercially available licensed test kit. The results of the in-house study done are as follows:

No. of Samples	Status	Dengue IgG iClia		Commercially available Dengue IgG ELISA	
		Positive	Negative	Positive	Negative
25	Dengue IgG Positive	25	0	25	0
190	Dengue Negative	0	190	0	190

Sensitivity : 100% **Specificity** : 100%

B) Analytical Specificity :

The analytical specificity of the Dengue IgG iClia Test kit is checked to check the potential for false results with 10 cross-reacting specimen; HIV, HCV ,HBsAg, Chikungunya and Leptospira. The specificity on all above samples tested is 100%. The analytical specificity of the test kit is also checked with potentially interfering substances /samples card to check the potential for false results arising from interference from potentially interfering substance .There was no interference with the test results when biomolecules; Bilirubin (20mg/dl), Hemoglobin(500mg/ dl),Triglyceride(1000mg/dl), Total protein(10mg/dl), RF(1000mg/ml), ANA(400mg/ml) & HAMA positive human plasma(600mg/mL) were added to the test specimen with much higher level in normal human blood.

C) External Evaluation:

The performance of Dengue IgG iClia with reference to Sensitivity & Specificity is also evaluated from NABL lab using clinical patient samples; Dengue positive (12) & negative (148). Following results are observed:

Sensitivity : 100% **Specificity** : 100%

Precision: Precision is checked by running Dengue IgG iClia test in 10 replicates (Intra assay variation, Inter assay variation) and Inter Machine variation with Kit controls(Control-1 & Control-2), 2 Dengue positive samples; one strong positive and one weak positive .The CV% in Sample RLU to Cutoff ratio (S/CO) of both the controls and positive samples is within 10% .

16. LIMITATION OF THE TEST

1. The test should be used for detection of Dengue IgG Antibodies in serum or plasma only and not in other body fluids.
2. **This is only a screening test** and will only indicate the presence or absence of Dengue IgG antibodies in the specimen. All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patients clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with the above procedure.
3. False positive results can be obtained due to cross reaction with Murray Valley and encephalitis, Japanese encephalitis, yellow fever and West Nile viruses. This occurs in less then 1% of the sample tested.

17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or

refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

18. REFERENCES

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2. Gubler DJ, Trent DW: Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. Infect Agents Dis 2:383-393, 1993.
3. Wu SJ Hanson B,Paxton H,Nisalak A, Vaugha DW, Rossi C, Henchal EA, Porter KR,Watts DM,Hayes CG.Evaluation of a dipstickelisa for detection of antibodies to dengue virus.Clin Diagn Lab Immunol 1997; 4(4):452-7.

19. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls deterioration due to improper storage or used after expiry.	Use controls within 30 days once opened and Check storage temp. It should be 2-8^{0}C.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8^{0}C.
2) False Positive results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
3) False negative results	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identitification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

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