

PROBLEM	POSSIBLE CAUSE	SOLUTION
2) False Positive results	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test 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 test results	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 identification	Make sample I.D. at the time of sample Collection.
	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 identification.	Mark the sample I.D. at the time of sample collection.

8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Dengue IgM 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.

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Tests are for in vitro diagnostic use only and should be run by competent person only.
- Do not pipette by mouth.
- 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°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.
- 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.
- Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

1 2 . PRECAUTIONS FOR USE & REAGENT HANDLING

- Do not use kit components beyond the expiration date which is printed on the kit.
- Store the reagents & samples at 2-8°C.
- Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
- 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.
- Once reagents are opened, reagent Plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
- Mark the test specimen with patient’s name or identification number. Improper identification may lead to wrong result reporting.
- To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
- Once a reagent plug 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.
- 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

Plugs) 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 Plug placed) while in refrigerated storage off the system, the reagent kit must be discarded.

- Run control-1 & control-2 in each assay to evaluate validity of the kit.
- Distilled or deionised water must be used for wash buffer preparation.
- Avoid strong light exposure during the assay.
- In case of any doubt the run should be repeated.

1 3 . TEST PROCEDURE

Preparation of dengue antigen:

- Use 3 ml of antigen diluent to dissolve lyophilized antigen well and desolve completely. Put the cap and let it stand for 10 minutes. Mix solution thoroughly before use. **The working antigen is stable for 10 days at 2-8°C and 2 months at -20°C (only 10 freeze thaw of liquid antigen are allowed at -20°C).**
- Transfer reconstituted antigen into the RC bottle by opening soft purple cap. After transferring reconstituted antigen and place the purple plug before use.

Assay Procedure

- Refer to the Clia Analyzer user manual for detailed information on preparing the analyzer.
- Before loading the Dengue IgM iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticles buffer that may have settled during transporation/ storage. Once the microparticles have been loaded, no further mixing is required.
Important Note: Swirl the microparticle (RA) 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, remove the cap and place the the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB), (RC) and (RD) bottles and place the reagent plugs before use as follow:

(RA) & (RB)

:

Natural color plug

(RC)

:

Purple color plug

(RD)

:

Brown color plug
- Load the Dengue IgM iClia reagent kit on the Chemiluminescence immunoassay analyzer.
- Verify that all necessary reagents are available in the reagent tray.
- Ensure that adequate sample volume (not less than 250 µL) is present in sample tube prior to running the test.
- Sample volume required for each additional test from same sample tube is 5 µL.
- Ensure sample positions are properly define at the time of loading in the Analyzer.

8. The Dengue IgM 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.

- Mix Dengue IgM 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.
- Run calibration as mentioned in heading calibration below.
- Press Run. The test result for first sample will be obtained at 45 minutes.
- The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Calibration

- Test both calibrators 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 for use.

- Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless, recalibration is required.
- Recalibrate the analyzer in following conditions:
 - After each exchange/use of new lot (Test reagent and pre-trigger/ Trigger solution/wash buffer).
 - Every week and/or at the time of any component to be changed.
 - Controls are out of validation range.
 - Required by pertinent regulations.
 - After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the Dengue IgM iClia.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

- Sample to cut-off ratio (S/CO) of calibrator-1 (C0) must be between 0.05 to 0.5. If it is not so, the run is invalid and must be repeated.
- Sample to cut-off ratio (S/CO) of calibrator-2 (C1) must be > 0.8. If it is not so, the run is invalid and must be repeated.
- Sample to cut-off ratio (S/CO) of control-1 (Q1) must be between 0.05 to 0.50. If it is not so, the run is invalid and must be repeated.
- Sample to cut-off ratio (S/CO) of control-2 (Q2) must be between 2.5 to 7.5. 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.

- Cut off value = mean RLU of calibrator-1 + mean RLU of calibrator-2 x calibration factor (F)
- 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

- If the Dengue IgM S/CO is < 0.9 then interpret the sample as Negative for Dengue IgM antibodies.
- If the Dengue IgM S/CO is between 0.9 - 1.1 then interpret the sample as Equivocal for Dengue IgM antibodies and sample should be re-tested.
- If the Dengue IgM S/CO is > 1.1 then interpret the sample as Positive for Dengue IgM antibodies.

15 . PERFORMANCE CHARACTERISTICS

A) In-house Evaluation:

Diagnostic Sensitivity and Specificity: The Performance of the Dengue IgM iCLIA with reference to sensitivity and specificity was evaluated in-house with the panel of 90 negative and 10 Dengue IgM positive samples. The performance is also checked with fresh clinical negative (100) and Dengue IgM 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 IgM iClia		Commercially available Dengue IgM ELISA	
		Positive	Negative	Positive	Negative
25	Dengue IgM 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 IgM 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 IgM iClia with reference to Sensitivity & Specificity is also evaluated with 85 negative and 20 dengue IgM antibodies positive clinical patient samples suspected for Dengue. The evaluation also included cross-reacting samples; malaria, leptospira & chikungunya positive. The results observed are as follows:

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

Precision: Precision is checked by running Dengue IgM CLIA test in 10 replicates (Intra assay variation, Inter assay variation) and Inter Machine variation with Kit controls(Control 1& Control 2) , 2 Dengue IgM 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% .

1 6 . LIMITATION OF THE TEST

- The test should be used for detection of Dengue IgM Antibodies in serum or plasma only and not in other body fluids.
- This is only a screening test** and will only indicate the presence or absence of Dengue IgM 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.
- 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 for use, 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

- Pinheiro FP, Corber SJ: Global situation of dengue and dengue haemorrhagic fever and its emergence in the Americas. World Health Stat ! 50(3/4):161-169, 1997.
- 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.
- 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/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8°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°C.