PROBLEM	POSSIBLE CAUSE	SOLUTION
	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.
3) Low hs-Troponin-I test 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 identification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

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hs-TROPONIN-I *i*Clia

Chemiluminescence microparticle immunoassay for Quantitative Measurement of hs-cTnI in Human Serum/Plasma

1. INTRODUCTION

Cardiac Troponin-I (cTnI) is a cardiac contractile regulatory protein. It is rich in myocardium with a molecular weight of 22.5KD. There are two skeletal muscle isomers. Compared with the isomers, cTnI shows 40% different origin in amino acid sequence, and there are 31 additional amino acid residues at the N-terminal, making cTnI a myocardial specific antigen. Studies have shown that the release of CTnI into the blood circulation as a unique structural protein component of myocardial cells is a highly sensitive and specific sign of myocardial cell injury, and is of great significance for the diagnosis, prognosis and curative effect of acute myocardial infarction (AMI).

2. INTENDED USE

hs-Troponin-I iClia is a chemiluminiscent microparticle immunoassay (CMIA)designed the quantitative measurement of High Sensitive Cardiac Troponin I (hs-cTnI) in human serum/plasma as an aid in the diagnosis of Myocardial Infarction in conjunction with other laboratory and clinical findings. The assay kit is intended for in-vitro diagnostic use. This kit is only operational in conjuction with J.Mitra CLIA Analyzer.

3. PRINCIPLE

hs-Troponin-I iClia Diagnostic Kit is a sandwich immunoassay for determination of hs-cTnI in human serum and plasma using chemiluminescent technology.

In the first step, anti-cTnI labeled magnetic microparticle , human serum, assay buffer and an anti- cTnI labeled acridinium ester (AE Conjugate) are mixed and incubated in an assay tube, which allows patient specific hs-cTnI to bind to microparticle. After sample matrix is removed by washing, the Microparticle-anti-cTnI antibody/antigen/antibody immune complex is kept with the help of a magnetic separator. Excess acridinium ester conjugate is removed by washing and finally the bound enzyme is detected by addition of chemiluminescent substrate.

The relative light unit (RLU) intensity is proportional to the amount of hs-cTnI. According to the hs-Troponin-I RLU-concentration standard curve, the RLU tested can be interpreted to hs-Troponin-I concentration in the sample expressed as pg/mL.

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 European Standard EN ISO 15223-1:2021.

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

5. KIT PRESENTATION

- 50 Tests
- 100 Tests

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic Microparticles coated with anti-cTnI antibodies with preservatives.
Assay Buffer (RB)	Tris buffer and BSA with preservative.
AE Conjugate (RD)	anti-cTnI antibodies linked to acridinium ester with Protein stabilizers.
Calibrator-1 (C0)	Low concentration of cTnI antigen in human serum containing preservatives.
Calibrator-2 (C1)	High concentration of cTnI antigen in human serum containing preservatives.
Control-1 (Q1)	Purified cTnI antigen in Tris buffer (pH7.4) with stabilizer.
Control-2 (Q2)	Purified cTnI antigen in Tris buffer (pH7.4) with stabilizer.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The kit should be stored at 2-8ºC in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8ºC.**

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**
- **J. Mitra CLIA Analyzer**

All materials and analyzer to be used for running the hs-Troponin-I 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 2-8ºC for one week, or frozen at -20ºC 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 viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes or 5,000 RPM for 30 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

hs-Troponin-I 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 or 5,000 rpm for 30 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 121ºC 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 2-8ºC.
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 plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugss 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 plug on an uncapped reagent bottle.

8. 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.
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 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 plugs placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run TROPONIN Control-1 & TROPONIN 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 Analyzer user manual for detailed information on preparing the analyzer.
2. Before loading the hs-Troponin-I iClia reagent tray on the analyzer for the first time, mix contents of the microparticle buffer bottle to resuspend microparticles 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) and (RD) bottles and place the reagent plugs before use as follow:*

(RA) & (RB) : Natural color plug

(RD) : Brown color plug

3. Load the hs-Troponin-I iClia reagent tray 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 50 μL.
7. The TROPONIN test-specific parameters are stored in reagent 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.
8. Run calibration, if required.
9. Mix Troponin iClia calibrators and controls by gentle inversion before use. Open the the cap and place the TROPONIN Calibrator-1 & TROPONIN Calibrator-2 and TROPONIN Control-1 & TROPONIN Control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
10. Press START. The test result for first sample will be obtained at 20 minutes.
11. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculate the results.

Calibration

1. Traceability: This assay has been standardized against the Roche TROPONIN reagent kit.
2. Every hs-Troponin-I iClia kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
3. Test all 2 Calibrators in triplicate. Both TROPONIN Control-1 and TROPONIN Control-2 must be tested in each run to evaluate the assay calibration. Ensure that controls values are within the validity range specified in the hs-Troponin-I iClia QC data sheet given in the Clia Analyzer.
4. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
5. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and pritrigger/ Trigger solution/wash buffer).
   b) Every 15 days 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 hs-Troponin-I iClia.

RESULT CALCULATION:

The analyzer automatically calculates the concentration of each sample. The results are given in pg/ml.

Result Interpretation

If sample concentration is lower than the lower limit of the linear range, report the result < 25 pg/ml, while > 50000 pg/ml when it is higher than the upper limit of linear range. Sample with concentration higher than linear range can be further diluted 1:5 or 1:10 with Sample diluent.

For the calculation of the concentration, this dilution factor has to be taken into account.

Determination of Reference Interval

Reference Interval of Troponin iCLIA is considered as < 80 pg/ml for healthy people, which is established referring to literatures, based on the rest results of more than 60 clinical samples.

Due to the differences in geography, race, gender or age, it is suggested that each laboratory should establish its own reference range or conduct verification of the existing reference interval.

14. PERFORMANCE CHARACTERISTICS

- Assay results obtained in individual laboratories may vary from data presented in this product insert.

Limit of Blank (LoB)

- The Limit of Blank was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
- The Limit of Blank is the 95th percentile value from n > 20 measurements of analyte free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.
- The observed LoB value was < 20 pg/ml.

Accuracy: The accuracy of Troponin iClia was detected with 60 clinical specimens and compared with Roche CLIA. The co-relation co-efficient is > 0.95.

Precision

Intra Assay Variation

Within run variation was determined by 10 replicate measurements of two different TROPONIN control sera(Low) and (High) in one assay in 3 different lots. The within assay variability is <10%.

Inter Assay Variation

Between run variation was determined by 10 replicate measurements in 10 sequential days of two different control sera(Low) and (High)in 3 different lots.The between assay variability is <10%.

Intra-Assay, n=10			Inter-Assay, n=10×3		
Control	Mean (pg/ml)	CV	Sample	Mean (pg/ml)	CV
1	490	4.37%	1	520	6.19%
2	22700	3.80%	2	22840	7.56%

Inter machine(J. Mitra CLIA Analyzer) Variation 2

Between machine variation was determined by 3 replicate measurements of two different TROPONIN control sera(Low) and (High)in 3 different lots in 3 different CLIA Analyzer. The between machine variability is <10.0 %.

Linearity

The linearity was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP6-A requirements.

The linearity range was verified by more than 6 concentration levels which encompass or be equal to the minimum and the maximum values of linearity range and duplicate assays in triplicate in single run for each lot at all 6 levels.

The Troponin iClia kit has been demonstrated to be linear from is 25 pg/ml to 50000 pg/ml, regression (R2) of more than >0.990.

Interference

A study was performed based on guidance from CLSI EP7-A2.

Potentially interfering substances were evaluated to determine whether hs-Troponin-I concentrations were affected when using the hs-Troponin-I iClia kit. Samples containing the potential interferents were prepared at two hs-Troponin-I concentrations. The samples were assayed, and the hs-Troponin-I concentrations of the spiked samples were compared to the reference samples.

Potential Interferent	Interferent Concentration	% Interferent Bias
Bilirubin	20 mg/dL	<10%
Hb	500 mg/dL	<10%
Triglyceride	1000 mg/dL	<10%
Total protein	10 g/dL	<10%
RF	1000IU/mL	< 10%
ANA	400AU/mL	< 10%
HAMA	600pg/ml	< 10%

15. LIMITATION OF THE TEST

- The hs-Troponin-I iClia should be used for detection of troponin in serum or plasma only and not in other body fluids.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions, If the Troponin results are inconsistent with clinical evidence, additional testing is recommended.
- Clinical diagnosis should not be made on the findings of a single test result, but should be integrated with all clinical and laboratory findings.
- Samples of lipid, hemolysis or jaundice may result in incorrect results. Hemoglobin (150 mg/dL), triglyceride (1000 mg/dL), or bilirubin (40 mg/dL) will have no significant interference for the results.

16. 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.

17. REFERENCES

1. Bluestein, B.I., Famulare, A.J. and Worthy, T.E., (1988). Heterogeneous fluorescence assays using controlled pore glass particles. U.S. Patent, 4,780,423.
2. Garcia-Casal, M.N., Pena-Rosas, J.P., Urrechaga, E., Escanero, J.F., Huo, J., Martinez, R.X. and Lopez-Perez, L., (2018). Performance and comparability of laboratory methods for measuring TROPONIN concentrations in human serum or plasma: A systematic review and meta-analysis. PloS one, 13(5), p.e0196576.
3. Casiday, R., and Frey, R., (2007). Iron use and storage in the body: TROPONIN. Department of Chemistry, Washington University, 1-12.
4. Knovich, M. A., Storey, J. A., Coffman, L. G., Torti, S. V., and Torti, F. M., (2009). TROPONIN for the clinician. Blood reviews, 23(3), 95-104.

18. 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 2-8ºC.
	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 2-8ºC.
	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.
2) High hs-Troponin-I test results	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.