REF	Σ
AU242028	50

Intended Use

The Aurora High Sensitive Cardiac Troponin I Assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of Cardiac Troponin I (cTnI) in human serum and plasma. This assay is intended for in vitro diagnostic use.

Summary

Cardiac troponin I, often denoted as cTnI, is presented in cardiac muscle tissue by a single isoform with a molecular weight of 23.9 kDa. It consists of 209 amino acid residues. The theoretical pI of cTnI is 9.05. cTnI differs from other troponins due to its N-terminal extension of 26 amino acids. cTnI has been known as a reliable marker of cardiac muscle tissue injury. It is considered to be more sensitive and significantly more specific in diagnosis of the myocardial infarction than the "golden marker" of last decades- CK-MB, as well as total creatine kinase, myoglobin and lactate dehydrogenase isoenzymes. The high-sensitivity cardiac troponin test (hs-cTnI) is the latest generation of the cardiac enzyme testing that allows for detection of very low levels of troponin I, helping to diagnose heart attacks more quickly.

Test Principle

The Aurora High Sensitive Cardiac Troponin I assay is a quantitative sandwich immunoassay to determine the presence of cTnI in human serum and plasma using CMIA technology with flexible assay protocols.

1. Sample, paramagnetic anti-cTnI coated microparticles and a conjugate containing acridinium-labeled anti-cTnI are mixed, cTnI present in the sample binds to anti-cTnI coated microparticles and acridinium-labeled anti-cTnI, forming an antigen antibody complex.
2. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
3. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of cTnI in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the previously established calibration curve.

Reagents

R1	3.0 mL
R2	3.0 mL
CAL	Contains 3 levels, Reconstitute each vial with 1 mL of double deionized water
CON	Contains 2 levels, 1.0 mL each level

R1: Microparticles. Anti- cTnI coated microparticles. Preservative: 0.05% ProClin 300

R2: Conjugate. Acridinium-labeled anti- cTnI. Preservative: 0.05% ProClin 300

CAL: Calibrator. Lyophilized of different concentrations of cTnI. Preservative: 0.05% ProClin 300

CON: Control. Tris buffer solution for quality control of High Sensitive Cardiac Troponin I (hs-cTnI)

Required Materials

- Pre-Trigger Solution: Hydrogen peroxide solution.
- Trigger Solution: Sodium hydroxide solution.
- Wash Buffer: Phosphate buffered saline solution with 0.05% ProClin 300.

Safety Precautions

- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Wear gloves when handling specimens or reagents.
- Clean and disinfect all spills of specimens or reagents using a suitable disinfectant.
- Trigger solution contains sodium hydroxide (NaOH) and should be avoided contact with eyes.

Warning（Contains Proclin 300）

Hazardous Component: 0.05% Proclin 300

- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin [EC no. 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC no. 200-239-6] (3:1)

Hazard Statement

- H317: May cause an allergic skin reaction.
- H319: Causes serious eye irritation.
- H410: Very toxic to aquatic life with long-lasting effects.

Precautionary Statement

- P261: Avoid breathing dust/fume/gas/mist/vapors/spray.
- P264: Wash hands thoroughly after handling.
- P272: Contaminated work clothing should not be allowed out of the workplace.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.
- P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P337+P313: If eye irritation persists: Get medical advice/attention.
- P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
- P302+P352: IF ON SKIN: Wash with plenty of soap and water.
- P321: Seek immediate care from a doctor.
- P363: Wash contaminated clothing before reuse.
- P273: Avoid release to the environment.
- P391: Collect spillage.
- P501: Dispose of contents/container in a safe way.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- Do not pool reagents within a kit or between kits.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment.
- Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum 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.

Reagent Storage

REAGENT	Storage Temperature	Maximum Storage Time
Unopened	2℃～8℃ Do not freeze.	12 months
On board/ Opened	2℃～8℃ Do not freeze.	28 days

- 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 septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.

Calibrator & Control Storage

CAL	CON	Storage Temperature	Maximum Storage Time
Unopened		2℃～8℃	12 months
Opened		2℃～8℃	30 days

Applicable Analyzer

Automatic Chemiluminescence Immunoassay Analyzer (model: Aurora S-01 System).

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum separator tubes (SST)
Plasma	Dipotassium EDTA Tripotassium EDTA Sodium heparin Lithium heparin powder Plasma separator tubes (PST) -lithium heparin gel

- Other specimen collection tube types have not been tested with this assay.
- Liquid anticoagulants may have a dilution effect

resulting in lower concentrations for individual patient specimens.

- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

Do not use specimens with the following conditions:

- Heat-inactivated
- Pooled
- Grossly hemolyzed (> 500 mg/dl hemoglobin)
- Obvious microbial contamination
- Fungal growth
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- Avoid more than 3 freeze/thaw cycles.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged for a minimum of 30,000 g-minutes before testing if they contain fibrin, red blood cells, or other particulate matter, they were previously frozen.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below. Centrifugation time using alternate Relative Centrifugal Force values (RCF) can be calculated using the following formula:

Centrifugation Time (Minutes)	RCF (×g)	g × minutes
10	3,000	30,000
15	2,000	30,000
20	1,500	30,000

- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	Room temperature	≤ 4 hours
	2℃～8℃	≤24 hours
	-20℃	≤ 90 days

- Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum room temperature storage time.
- Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum 2-8°C storage time and store frozen.
- Frozen specimens must be mixed thoroughly after thawing.
- Use caution in handling patient specimens to prevent cross-contamination.
- Do not exceed the storage limitations listed above.

Assay Procedure

- Refer to the system operating instruction or the online help system for detailed information on preparing the system.
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
- Invert the microparticle bottle 30 times. Visually inspect the bottle to ensure microparticles are

resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended. If the microparticles do not resuspend, DO NOT USE. Once the microparticles have been resuspended, place a septum on the bottle.

- Load the reagent kit on the chemiluminescence immunoassay analyzer.
- Verify that all necessary reagents are present.
- Verify adequate sample volume is present prior to running the test.
- Sample volume for first test: 250 µL
- Sample volume for each additional test from same sample cup: 80 µL
- The test-specific parameters stored in barcode on the reagent pack are read in. In cases the barcode cannot be read, enter the sequence numbers
- Order calibration, if necessary.
- Prepare High Sensitive Cardiac Troponin I Calibrators and Controls.
- Mix calibrator(s) and controls by gentle inversion before use.
- Hold bottles vertically and dispense recommended volumes into each respective sample cup.
- Place the calibrators in the calibrator rack in the sample zone.
- Calibration.
- Load samples. For information on loading samples, refer to the Analyzer's Operations Manual.
- Press RUN.
- The chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

For optimal performance, it is important to perform routine maintenance as described in the Analyzer's Operations Manual. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

- Samples cannot be diluted for cTnI determinations. Samples which read > 50000 pg/mL should be reported as such.

Calibration

- Traceability: This assay has been standardized against the International Standard NIST SRM 2921.
- Every hs-cTnI assay kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
- Test Calibrators in duplicate. The calibrators should be priority loaded. A replicate of each control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Once calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
- After 28 days when using the same lot reagent.
- A reagent kit with a new lot number is used.
- Controls are out of range.
- Required by pertinent regulations.
- Assay may also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the assay. For detailed information on how to perform an assay calibration, refer to the Analyzer's Operations Manual.
- Calibration Range: 6 pg/mL～50000 pg/mL.

Quality Control Procedures

- Order Control, if necessary.
- The recommended control requirement for the hs-cTnI assay is that a single replicate of each control level be tested:
- Once every 24 hours or each day of use
- After performing calibration
- After instrument service procedures or maintenance that may affect assay performance have been performed.
- If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.
- Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be

indicated.

• These results should be applied to your laboratory's quality control practices. In addition, the laboratory must ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

• Unless specified, target values and ranges provided with the commercial control product insert are guidelines only and should not be used for quality control purposes.

• Refer to Clinical and Laboratory Standards Institute (CLSI) Document C24-A3, or other published guidelines for general quality control recommendations.

Results

Calculation

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

Limits

• Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.

• If the cTnI results are inconsistent with clinical evidence, additional testing is recommended.

• Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.

• Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.

• Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Additional information may be required for diagnosis.

• The Aurora High Sensitive Cardiac Troponin I assay is susceptible to interference effects from triglycerides at > 1000 mg/dL.

• There is no high-dose HOOK effect at cTnI concentrations up to 1000000 pg/mL.

Expected Values

• It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical, season, patient, dietary, or environmental factors. A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) C28-A3c.

• Human serum specimens from apparently healthy individuals were collected the 128 specimens, 64 were female and 64 were male，age between 21 and 90 years.

The population	n	Reference range*
Apparently healthy individuals	128	≤ 60 pg/mL

*According to the 95th percentile.

*Representative data; results in individual laboratories and in different geographical areas may vary from these data.

Specific Performance Characteristics

• Assay results obtained in individual laboratories may vary from data presented.

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 ≤6 pg/mL.

Accuracy

Intra Assay Variation

• Within run variation was determined by replicate

determination (n=10) of two different control sera in one assay. The within assay variability is ≤8.0%.

Inter Assay Variation

- Inter assay variation was determined by replicate measurements (n=10) of two different control sera in 3 different lots. The inter assay variation is ≤10.0%.

Intra-Assay, n=10			Inter-Assay, n=10×3		
Sample	Mean (pg/mL)	CV	Sample	Mean (pg/mL)	CV
1	489	4.75%	1	501	5.84%
2	25776	4.50%	2	25114	6.02%

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 (n=3) at all levels.
- The Aurora High Sensitive Cardiac Troponin I assay has been demonstrated to be linear from 20 pg/mL to 50000 pg/mL, regression ≥0.99 and max diff ≤ 15% in this interval.

Specificity

Cross-Reactivity

- A study was performed based on guidance from CLSI EP7-A2.
- The cross-reactants listed below were evaluated to determine whether cTnI concentrations were affected when using the Aurora High Sensitive Cardiac Troponin I assay.

Cross-Reactant	Cross-Reactant Concentration	% Cross-Reactant Reactivity
cTnC	1000 ng/mL	≤ 0.05%
cTnT	1000 ng/mL	≤ 0.05%
sTnI	1000 ng/mL	≤ 0.05%

Interference

- A study was performed based on guidance from CLSI EP7-A2.
- Potentially interfering substances were evaluated to determine whether cTnI concentrations were affected when using the Aurora High Sensitive Cardiac Troponin I assay. Samples containing the potential interferents were prepared at two cTnI concentrations. The samples were assayed, and the cTnI 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%
Intralipid	1000 mg/dL	≤ 10%
Total protein	10 g/dL	≤ 10%
RF	1000IU/mL	≤ 10%
ANA	400AU/mL	≤ 10%
HAMA	600ng/mL	≤ 10%

Reference

1. Lee GR, Browne TC, Guest B, et al. Transitioning high sensitivity cardiac troponin I (hs-cTnI) into routine diagnostic use: More than just a sensitivity issue. Pract Lab Med. 2016; 4:62-75.

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3. Beatty AL, Ku IA, et al. High-sensitivity cardiac troponin T levels and secondary events in outpatients with coronary heart disease from the Heart and Soul Study. JAMA Intern Med. 2013 May 13;173(9):763-9.

4. Kozinski M, Krintus M, Kubica J, Sypniewska G. High-sensitivity cardiac troponin assays: From improved analytical performance to enhanced risk stratification. Crit Rev Clin Lab Sci. 2017 May;54(3):143-172.

5. Zhang P, Kirk JA, et al. Multiple reaction monitoring to identify site-specific troponin I

phosphorylated residues in the failing human heart. Circulation. 2012 Oct 9;126(15):1828-37.

6. Schulz, O., Reinicke, M., et al. High-sensitive cardiac troponin I (hs-cTnI) values in patients with stable cardiovascular disease: An initial foray. Clinica Chimica Acta, 2010,411(11-12), 812-817.

Symbols

	Manufacturer
	Date of manufacture
	Use-by date
	Contains sufficient for <n> tests
	Consult instructions for use
	Biological risks
	Temperature limit
	CE Marking
EC REP	EU Representative
IVD	In Vitro diagnostic medical device
REF	Catalogue Number
LOT	Batch code
REAGENT	Reagent
R1	Microparticles
R2	Conjugate
CAL	Calibrator
CON	Control

Contact Information

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CE
IVD

Release Date: … … … Date of Manufacture: … … …