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Immunoassay

REF **CMH0201/CMH0202/CMH0203/CMH0204/CMH0205**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

cTnI CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of cardiac troponin I (cTnI) concentration in human serum and plasma (heparin or sodium citrate).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

Cardiac troponin I (cTnI) is a contractile protein exclusively present in the cardiac muscle[1, 2]. It is one of three subunits of the troponin complex (I, T, C), which with tropomyosin are bound to actin in the thin filament of the myofibril. Its physiological role is to inhibit the ATPase activity of the actin-myosin complex in the absence of calcium, and therefore, to prevent muscular contraction[3]. The content of cardiac troponin in normal serum is much lower than other cardiac enzymes, and the concentration in cardiac muscle cells is very high.[4-5]

cTnI levels in acute myocardial infarction (AMI) exhibit similar rise and fall patterns to those found in CK-MB. The collection of at least three blood samples during the early triage period has been recommended[6]. cTnI is 13 times more abundant in the myocardium than CK-MB and does not normally circulate in the blood, so the signal to noise ratio is more favorable for the detection of myocardial necrosis[7].

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, cTnI antibody coated microparticles and enzyme-labeled anti-cTnI are added to the reaction vessel. During incubation, cTnI present in the sample is allowed to react simultaneously with the two antibodies, resulting in the cTnI being sandwiched between the microparticles-coated antibodies and enzyme-labeled antibodies. After washing, a complex is generated among the solid phase, the cTnI within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of cTnI in the samples.

Materials Provided

1. Calibrators

6 vials lyophilized Calibrator A through F with corresponding approximate cTnI concentrations. The matrix is Tris-NaCl buffer. Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 5 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

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- Microparticles Solution

Mouse monoclonal anti-cTnI coated microparticles in PBS (phosphate buffered saline) buffer. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal anti-cTnI in Tris buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

The analyte in these cTnI calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain human sourced components, which have been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and Syphilis. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not mix or use components from kits with different batch codes.
10. When storing the calibrators, be certain the vials are securely sealed.
11. Ensure the microparticles are resuspended before loading it on the analyzer.
12. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
13. Do not substitute any reagent in this kit from other manufacturers or other lots.
14. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
15. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light.
2. The expiry date is shown on the container label.
3. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
4. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at

2-10 ℃ in an upright position.

5. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 10 days.

Sample

1. Collect serum or plasma samples in accordance with correct medical practices (the Plain Vacuum Tube and Coagulating Vacuum Tube can be used to collect the serum samples, the Heparin and Citrate Anti-coagulant Vacuum Tube can be used to collect the plasma samples).
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃ Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy etc. in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tube(s) or cup(s) on the sample rack, 50 μL of samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticle Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of cTnI in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a cTnI value exceeding 100 ng/mL may be diluted manually.
Low-value sample is used to dilute the samples.
After dilution, multiply the result by the dilution factor.

Measurement Results

The sample test results are determined automatically by the system software. The amount of cTnI in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored results.
The default unit for this assay is ng/mL.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. The negative results cannot be completely ruled out the possibility of myocardial infarction. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. This assay was designed and validated for use with human serum and plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 0.1-100 ng/mL. If cTnI concentrations above the measuring range to be expected, it is recommended to dilute samples with low-value sample, the maximum dilution is 1:9 of this test, allowing samples to be quantitated up to approximately 1000 ng /mL.

Biological Reference Interval

The suggested normal range (97.5% confidence interval)) was obtained by testing 354 physical examination serums samples from 186 normal adult males and 168 normal adult females (no cardiovascular disease and at the age 19-80). It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.
Thus the normal range with 97.5% confidence interval is 0.11 ng/mL.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	0.32	3.45	6.71
2	80	9.02	2.14	5.12
3	80	30.51	3.42	7.29

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank=0.1ng/mL
Limit of Detection=0.15ng/mL
Limit of Quantitation= 0.25ng/mL with a coefficient of variation of ≤ 20%.

3. Analytical Specificity

Cross reaction: the following substances and concentrations were tested and found cross reaction rate is ≤ 0.05% with the test;

Substances	Concentration
cTnC	1000 ng/mL
cTnT	1000 ng/mL
sTnI	1000 ng/mL

Interference: this assay is designed to have an acceptable interference with the substances listed below, at the concentration levels listed:

Interferent	Concentration
Bilirubin	40 mg/dL
Triglyceride	3000 mg/dL
Hemoglobin	125 mg/dL

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and a cTnI reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	275	0.0648	1.0201	0.9171

Literature References

1. Wilkinson JM, Grand RJA. 1978. Comparison of amino acid sequence of troponin I from different striated muscles. Nature. 271: 31-35.
2. Wade R, Eddy R, Shows TB, Kedes L. 1990. cDNA sequence, tissue-specific expression and chromosomal mapping of the human slow-twitch skeletal muscle isoform of troponin I. Genomics. 7: 346-357.
3. Perry SV. 1979. The regulation of contractile activity in muscle. Biochem Soc Trans 7: 593-617.
4. Solaro RJ, Moir AJG, Perry SV. 1976. Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. Nature 262: 615–616.
5. Zhang P, Kirk, JA, Ji W, dos Remedios CG, Kass DA, Van Eyk JE, Murphy AM. 2012. Multiple Reaction Monitoring to Identify Site-Specific Troponin I Phosphorylated Residues in the Failing Human Heart. Circulation 126: 1828–1837.
6. Wu HBA, Apple FS, Gibler B, Jesse RL et al.1999. National Academy of Clinical Biochemistry Standards of Laboratory Practice: Recommendations for the use of cardiac markers in coronary artery disease. Clin. Chem; 45(7):1104-1121.
7. Adams JE, Schechtman KB, Landt Y, Ladenson JH, Jaffe AS. 1994. Comparable detection of acute myocardial infarction by creatine kinase MB isoenzyme and cardiac troponin I. Clin. Chem. 40:1291-1295.