Snibe Diagnostic

CE REF 130256014M:100 tests/kit
130656014M: 50 tests/kit
130756014M: 30 tests/kit

MAGLUMI® hs-cTnI (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the high sensitivity quantitative determination of cardiac troponin I (cTnI) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis and treatment of individuals with suspected or confirmed myocardial infarction and cardiac muscle damage.

SUMMARY

Troponins are regulatory proteins that control the calcium-mediated interaction of actin and myosin, which results in contraction and relaxation of striated muscle[1,2]. The troponin complex is made up of three subunits: troponin C, troponin I and troponin T[2,3,4]. Each troponin is encoded by separate genes, the amino acid sequences of troponins I is unique to cardiac muscle. This difference has allowed for the development of rapid, quantitative assays to detect elevations of cardiac troponins in the serum[3].

Cardiac troponin I (cTnI) is the biomarker of choice for the diagnosis of myocardial necrosis because it is the most sensitive and specific biochemical marker of myocardial injury/necrosis available[1]. When compared to CK-MB and other cardiac biomarkers, cTnI has demonstrated nearly absolute myocardial tissue specificity as well as high clinical sensitivity for myocardial ischemia[3]. In cases of AMI, cTnI levels in serum rise within hours after the onset of cardiac symptoms, peak at 12-16 hours, and can remain elevated for 4-9 days[5]. Elevated level of cTnI is an adverse prognostic indicator, even after adjustment for clinical predictors and electrocardiogram findings[6]. It is also a class I indication for risk stratification in patients with ACS and the diagnosis of MI[3]. In patients with ACS, increased concentrations of troponin closely correlate with the presence, complexity, and severity of epicardial coronary artery disease, as well as decreased microvascular myocardial perfusion[3]. In patients with unstable angina, recurrent ischemia patients with unstable angina, recurrent ischemia is predictive of major cardiac events. Likewise, elevated plasma levels of cTnI of myocardial injury can be used for risk stratification, to select patients for adjunctive therapy with new antithrombotic agents and/or for early angiography and revascularization[7].

Cardiac troponins have not only diagnostic value, but yield prognostic information as well. Patients presenting with clinical evidence of ischemia and increased troponins have worse outcomes than those without detectable troponin in the circulation[3]. With MI, any troponin level above the reference range is associated with an increased risk of adverse events in both the short- and long-term[8] Even in patients with stable coronary artery disease, high-sensitivity assays have demonstrated that detectable concentrations of cardiac troponin portend a higher incidence of heart failure and cardiovascular death[3]. Non-ischaemic myocardial injury may arise secondary to many cardiac conditions such as myocarditis, or may be associated with non-cardiac conditions such as renal failure[9]. Therefore, for patients with increased cTn values, clinicians must distinguish whether patients have suffered a non-ischaemic myocardial injury or one of the MI subtypes[9]. If there is no evidence to support the presence of myocardial ischaemia, a diagnosis of myocardial injury should be made[9].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, magnetic microbeads coated with cTnI monoclonal antibody, ABEI labeled with another cTnI monoclonal antibody and buffer are mixed thoroughly and incubated, reacting to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of cTnI present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with cTnI monoclonal antibody (~10.0 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of cTnI antigen in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Calibrator High	A high concentration of cTnI antigen in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Buffer	HCl (0.08%).	6.5 mL	4.0 mL	3.0 mL
ABEI Label	ABEI labeled with cTnI monoclonal antibody (~0.417 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	7.5 mL	4.5 mL	3.3 mL
Control 1	A low concentration of cTnI antigen (10.0 pg/mL) in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Control 2	A middle concentration of cTnI antigen (21.0 pg/mL) in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Control 3	A high concentration of cTnI antigen (175 pg/mL) in PBS buffer, NaN_3 (<0.1%).	1.5 mL	1.5 mL	1.5 mL
All reagents are provided ready-to-use.				

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA, Li-heparin

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. 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.
- The sample volume required for a single determination of this assay is 100 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 10-30°C or 48 hours at 2-8°C, or 6 months frozen at -20°C. Frozen specimens subjected to up to 2 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, with cTnI concentrations above the analytical measuring interval, can be diluted with manual dilution procedure. The recommended dilution ratio is 1:10. The concentration of the diluted sample must be >5000 pg/mL.
- For manual dilution, multiply the result by the dilution factor.
- Please choose applicable diluents or ask Snibe for advice before manual dilution.

PROCEDURE

Materials Provided

hs-cTnI (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer MAGLUMI X8, MAGLUMI X3, MAGLUMI X6 or Integrated System Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the NIST SRM 2921.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[10].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the hs-cTnI assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order hs-cTnI (CLIA) Controls (REF: 160201493MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the cTnI concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/mL. For further information please refer to the Analyzer Operating Instructions.

Conversion factor: pg/mL×1=ng/L

Interpretation of Results

The expected range for the hs-cTnI assay was obtained by testing 617 apparently healthy individuals in China, gave the following expected value:

Apparently healthy population	N	99th percentile (pg/mL)
Female	304	11.8
Male	313	20.1
Total	617	17.5

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Cardiomyocyte damage caused by any factor will increase cardiac troponin-level[11,12].
- When diagnosing myocardial infarction, high-sensitivity troponin-test results need to be judged comprehensively with other information such as ECG, clinical observations and symptoms. Only relying on troponin results is not sufficient to evaluate myocardial infarction. It is recommended to evaluate the condition of patients with acute coronary syndrome (ACS) through continuous sampling[11,13,14].
- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the cTnI results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- 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 which employ mouse monoclonal antibodies[15,16]. 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 animal serum products can be prone to this interference and anomalous values may be observed[17].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (pg/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (pg/mL)	%CV	SD (pg/mL)	%CV	SD (pg/mL)	%CV
Serum Pool 1	11.978	0.670	5.59	0.287	2.40	1.052	8.78
Serum Pool 2	20.033	0.720	3.59	0.359	1.79	1.099	5.49
Serum Pool 3	198.630	11.043	5.56	4.372	2.20	16.090	8.10
Plasma Pool 1	11.890	0.680	5.72	0.585	4.92	1.035	8.70
Plasma Pool 2	19.614	0.811	4.13	0.637	3.25	1.313	6.69
Plasma Pool 3	196.700	11.060	5.62	5.673	2.88	15.378	7.82
Control 1	9.831	0.438	4.46	0.246	2.50	0.557	5.67
Control 2	21.028	0.509	2.42	0.418	1.99	0.977	4.65
Control 3	174.294	4.692	2.69	2.039	1.17	6.576	3.77

Linear Range

2.00-50000 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

1.00-500000 pg/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.500 pg/mL.

Limit of Detection (LoD) =1.00 pg/mL.

Limit of Quantitation (LoQ) =2.00 pg/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1000 mg/dL	Biotin	0.5 mg/dL
Bilirubin	60 mg/dL	Digoxin	7.5 μg/mL
Intralipid	3000 mg/dL	Acetaminophen	500 μg/mL
HAMA	40 ng/mL	Nifedipine	60 μg/mL
ANA	398 AU/mL	Acetylsalicylic acid	600 μg/mL
Rheumatoid Factor	1500 IU/mL	Propranolol	5 μg/mL
Human albumin	12 g/dL	Erythromycin	200 μg/mL
IgG	12 g/dL	Nitrofurantoin	64 μg/mL
K2-EDTA	22.75 μmol/mL	Methyldopa	25 μg/mL
Heparin lithium salt	80 IU/mL	Nystatin	20 μg/mL

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactant in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
Cardiac Troponin T	1000 ng/mL	Myoglobin	1000 ng/mL
Cardiac Troponin C	1000 ng/mL	Myosin	1000 ng/mL
Skeletal troponin I	1000 ng/mL	Tropomyosin	1000 ng/mL
Myosin Light Chain	1000 ng/mL	CK-MB	1000 ng/mL
Actin	1000 ng/mL	TPA	2.5 μg/mL

High-Dose Hook

No high-dose hook effect was seen for cTnI concentrations up to 1000000 pg/mL.

Method Comparison

A comparison of the hs-cTnI assay with a commercially available immunoassay, gave the following correlations (pg/mL):

Number of samples measured: 299

Passing-Bablok: $\hat{y}$=0.9942x+0.1601, τ=0.984.

The clinical specimen concentrations were between 2.2 and 48198.2 pg/mL.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE	CE marking		

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