BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

ACCESS hsTnI
High Sensitivity Troponin I

REF B52699

FOR PROFESSIONAL USE ONLY

Rx Only

For use on Access 2 Immunoassay Systems with test name: TnIhs

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

Access hsTnI is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the Access 2 Immunoassay Systems to aid in the diagnosis of myocardial infarction (MI).

SUMMARY AND EXPLANATION

The troponins (I, C, and T) are members of a complex of proteins that modulate the calcium-mediated interaction between actin and myosin within muscle cells.[1] The nomenclature of these distinct proteins of the troponin complex is derived from their respective function in muscle contraction. Troponin T anchors the troponin complex to tropomyosin of the thin filament, whereas troponin I inhibits actomyosin ATPase, and troponin C is a calcium-binding subunit. Three isoforms of troponin I (TnI) have been identified: one associated with fast–twitch skeletal muscle, one with slow–twitch skeletal muscle, and one with cardiac muscle. The slow and fast–twitch isoforms have a similar molecular weight of approximately 20,000 dalton (Da) each. The cardiac–specific TnI isoform has a molecular weight of approximately 24,000 Da and contains a post–translational tail of 31 amino acids on the N–terminus of the molecule.[2,3] This sequence and the 42% and 45% dissimilarity with the sequences of the other two isoforms have made possible the generation of highly specific monoclonal antibodies without cross–reactivity with other non–cardiac TnI forms.[4,5] As a result of its high tissue specificity cTnI is a cardio–specific, highly sensitive marker for myocardial injury. The Access hsTnI assay uses monoclonal antibodies specifically directed against human cTnI.

In myocardial infarction, cTnI levels rise in the hours after the onset of cardiac symptoms, reaching a peak at 12–16 hours and can remain elevated for 4–9 days post MI.[6,7] Numerous pathologies can potentially cause troponin elevations without overt ischemic heart disease.[8,9] These pathologies include, but are not limited to, congestive heart failure, acute and chronic trauma, electrical cardioversion, hypertension, hypotension, arrhythmias, pulmonary embolism,

severe asthma, sepsis, critical illness, myocarditis, stroke, non–cardiac surgery, extreme exercise, drug toxicity (adriamycin, 5–fluorouracil, herceptin, snake venoms), end stage renal disease, and rhabdomyolysis with cardiac injury.[9,10] Importantly, these other etiologies rarely demonstrate the classic rising and falling pattern experienced with a MI, which highlights the importance of serial monitoring when the clinical scenario is unclear.[8,11]

Definition of Myocardial Infarction

In 2012, a Task Force of the Joint European Society of Cardiology (ESC), American College of Cardiology Foundation (ACCF), American Heart Association (AHA), and World Heart Federation (WHF) published an updated redefinition of MI in which cardiac troponin (cTn) plays a central role.[11]

The 2012 Third Universal Definition of Myocardial Infarction document states that in patients presenting to the Emergency Department with chest pain, or other ischemic symptoms, the criteria for diagnosis of MI are:

Detection of a rise and/or fall of cardiac biomarkers values [preferably cardiac troponin] with at least one value above the 99th percentile of the upper reference limit (URL) and with at least one of the following:

- Symptoms of ischemia;
- New or presumed new ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB);
- Development of pathological Q waves in the ECG;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality;
- Identification of an intracoronary thrombus by angiography or autopsy.[11]

Additionally, the Third Universal Definition of Myocardial Infarction document recommends an optimal imprecision level (coefficient of variation, or CV) for troponin assays ≤ 10% at the 99th percentile URL of a healthy population.

Cardiac troponin should be measured upon admission, and then serially at regular intervals to demonstrate a rise and/or fall in cTn values. When an increased cTn value does not support the diagnosis of acute myocardial ischemia, a careful search for other possible etiologies of myocardial injury should be undertaken.[12]

High Sensitivity Troponin Assays

The International Federation of Clinical Chemistry (IFCC) has issued guidance on high sensitivity troponin assays. In order to be classified as a high sensitivity assay, two performance requirements must be met:

- The assay must have analytical imprecision ≤ 10% CV at the 99th percentile URL of a healthy population.
- The assay must be able to measure cTn above the Limit of Detection (LOD) in ≥ 50% of a healthy population.[13]

Compared to contemporary troponin assays, high sensitivity assays demonstrate significantly improved precision at and below the 99th percentile URL, allowing better discrimination of small differences in cTn values between serial measurements.[14] More precise determination of the 99th percentile URL has also led to an ability to report distinct reference ranges for male and female subjects.[15]

METHODOLOGY

The Access hsTnI assay is a two–site immunoenzymatic ("sandwich") assay. Monoclonal anti–cTnI antibody conjugated to alkaline phosphatase is added to a reaction vessel along with a surfactant–containing buffer and sample. After a short incubation, paramagnetic particles coated with monoclonal anti–cTnI antibody are added. The human cTnI binds to the anti–cTnI antibody on the solid phase, while the anti–cTnI antibody–alkaline phosphatase conjugate reacts with different antigenic sites on the cTnI molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional

to the concentration of cTnI in the sample. The amount of analyte in the sample is determined from a stored, multi–point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and lithium heparin plasma are the recommended sample types. **Lithium heparin plasma and serum samples should not be used interchangeably.**[16]
2. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[17,18] To minimize the effect of preanalytical factors observe the following recommendations for handling, processing, and storing blood samples:[17]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation in a vertical, closure-up position.
     - Nonanticoagulated tubes containing gel separator should be stored in an upright position as soon as the mixing is complete.
     - Precentrifugation serum/cells contact time is according to tube manufacturer's recommendations. Clotting may be slowed at cooler temperatures or if the patient is on anticoagulant therapy.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 25°C) for up to 4 hours.
   - If the assay will not be completed within 4 hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, freeze at -20°C or colder.
   - Frozen specimens can be stored up to 180 days before testing.
   - Thaw samples only once. Frozen samples should be thawed at room temperature, mixed thoroughly by gentle inversion, and centrifuged per tube manufacturer's recommendations prior to analysis.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis. Failure to do so can contribute to falsely elevated results.[19]
   - For plasma, avoid transferring material from the white blood cell/platelet layer located just above the red blood cells. If a fixed angle rotor is used for centrifugation, be careful not to resuspend platelets.
   - Transfer turbid serum or plasma samples from their original tube and centrifuge again prior to assay. Never centrifuge a specimen in an original tube that contains a separating device (gel barrier) more than once.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

REAGENTS

PRODUCT INFORMATION

Access hsTnI Reagent Pack

Cat. No. B52699: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.

- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 64 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

Well	Ingredients
R1a:	Dynabeads* paramagnetic particles coated with mouse monoclonal anti-human cTnI antibody suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide and 0.1% ProClin** 300.
R1b:	0.1N NaOH
R1c:	TRIS buffered saline, surfactant, protein (mouse), < 0.1% sodium azide and 0.1% ProClin 300.
R1d:	Sheep monoclonal anti-human cTnI alkaline phosphatase conjugate diluted in ACES buffered saline, with surfactant, BSA matrix, protein (bovine, sheep, mouse), < 0.1% sodium azide and 0.25% ProClin 300.

*Dynabeads is a registered trademark of Dynal A.S., Oslo, Norway.

**ProClin™ is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

hsTnI PMP (Compartment R1a)	WARNING

	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Ethoxylated lauryl alcohol 1 - <3%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
0.1N NaOH (Compartment R1b)	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Sodium Hydroxide 0.1 - 1%
hsTnI Reagent Buffer (Compartment R1c)	WARNING	
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.

		3-((3-Cholamidopropyl)dimethylammonio)-propanesulfonate 1 - 5% reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
hsTnI Conjugate (Compartment R1d)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access hsTnI Calibrators
   Provided at zero and approximately 30.7, 144, 567, 2,293, 9,280 and 27,027 pg/mL (ng/L).
   Cat. No. C26909
2. Quality Control (QC) materials: commercial control material.
3. Access Sample Diluent A
   Cat. No. 81908
4. Access Substrate
   Cat. No. 81906
5. Access 2 Immunoassay Systems:
   Access Wash Buffer II, Cat. No. A16792

EQUIPMENT AND MATERIALS

R1 Access hsTnI Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

An active calibration curve is required for all tests. For the Access hsTnI assay, calibration is required every 63 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[20] Include commercially available quality control materials that cover at least two levels of analyte. It is recommended that at least one level is targeted near the MI cutoff. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Native human cTnI was used in development of the assay. Quality control materials containing TnI from other sources (e.g. recombinant antigens) may behave differently. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes when requesting the Access hsTnI assay. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), ng/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 1.

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

SEE LABNET FOR REFERENCE RANGE

REPORTING RESULTS

EXPECTED RESULTS

A multicenter prospective study was conducted to establish the 99th percentile URL in a population of apparently healthy adults. Lithium heparin plasma and serum samples were evaluated. Subjects ranging from 21 to 99 years of age were enrolled at five geographically diverse locations throughout the United States. Forty five percent of subjects were ≥ 60 years of age.

Subjects were surveyed and were excluded if they met any of the following criteria:

- Disease(s) of/or affecting the cardiovascular system
- Currently taking a medication for cardiovascular disease
- Diabetes
- Chronic kidney disease.
- Other serious chronic disease(s) (e.g. cancer, COPD, HIV, lupus erythematosus, etc.)
- Acute bacterial or viral infection
- Pregnancy

The overall observed 99th percentile URL in 1,089 lithium heparin plasma samples is 17.5 pg/mL (ng/L) (95% CI: 12.6 – 20.7). The overall observed 99th percentile URL in 1,088 serum samples is 18.2 pg/mL (ng/L) (95% CI: 13.1 - 23.1).

The 99th percentile URL values determined for lithium heparin plasma (females, males, and overall), and serum (females, males, and overall) are shown in the following table. All values were determined using the non-parametric statistical method.

Table 1.0 99th Percentile URL of a Healthy Population

Sample Type	Population	N	99th percentile URL pg/mL (ng/L)	95% CI pg/mL (ng/L)
Lithium heparin plasma	Females	595	11.6	8.4 - 18.3
	Males	494	19.8	14.0 - 42.9
	Overall	1,089	17.5	12.6 - 20.7
Serum	Females	595	11.8	8.7 - 18.7
	Males	493	19.7	14.3 - 44.3
	Overall	1,088	18.2	13.1 - 23.1

IFCC guidance states high sensitivity assays must have analytical imprecision ≤ 10% CV at the 99th percentile URL of a healthy population.[13] For Access hsTnI on the Access 2 Immunoassay System, the 10% CV limit of quantitation (LoQ) was measured to be 4.1 pg/mL (ng/L).

The study presented above also demostrated > 50% of healthy subjects had cTnI levels above the observed limit of detection.

Imprecision at the Established 99th Percentile URLs

The expected imprecision in the clinically relevant concentration range was plotted, using data from the LOQ studies, to create a best fit regression describing the relationship of %CV and cTnI concentration. The regression analysis was evaluated to estimate imprecision at the established 99th percentile values (Table 2.0).

Table 2.0 Imprecision at the Established 99th Percentile URLs

Sample Type	Population	99th percentile URL pg/mL (ng/L)	% CV based on LoQ imprecision profile
Lithium heparin plasma	Females	11.6	4.2
	Males	19.8	3.6
	Overall	17.5	3.7
Serum	Females	11.8	6.9
	Males	19.7	5.8
	Overall	18.2	6.0

PROCEDURAL NOTES

LIMITATIONS

1. The validated operational temperature range of the Access 2 Immunoassay Systems is 18°C to 28°C (66.4°F and 82.4°F). Ambient laboratory temperature should be maintained within this range while conducting patient sample testing.
2. The performance of Access hsTnI represented in these Instructions for Use is reflective of use on Access 2 Immunoassay Systems only. Performance on UniCel DxI Access Immunoassay Systems is not interchangeable. When using results from different systems, comparability of patient results should be verified within the laboratory following guidelines such as those described in CLSI EP31-A-IR.[21]
3. Samples can be accurately measured within the analytical range of the Limit of Quantitation (LoQ) and the highest (S6) calibrator value (approximately 2.0 to 27,027 pg/mL [ng/L]).
   - If a sample contains less than the LoQ for the assay, the result will be reported as less than that value (i.e., < 2.0 pg/mL [ng/L]).
   - If a sample contains more than the stated value of the highest Access hsTnI Calibrator (S6), the result will be reported as greater than that value. Alternatively, dilute one volume of sample with 9 volumes of Access Sample Diluent A.
   - Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
4. Samples with very high cTnI concentrations may cause carryover into the Access hsTnI reagent pack. The extent of carryover observed is directly proportional to the cTnI concentration that is present in the high sample. If a sample with cTnI >270,000 pg/mL (ng/L) is tested, clinically significant carryover may be observed with all subsequent samples that are tested from the same reagent pack. In one study, the estimated carryover (based upon the upper and lower limits of the 95% CI) was 3-5 pg/mL (ng/L) from a high sample at 270,000 pg/mL (ng/L) and 5-8 pg/mL (ng/L) from a high sample at 500,000 pg/mL (ng/L). If there is suspected carryover into the reagent pack, use a fresh reagent pack and repeat all samples that were tested after the high cTnI sample.
5. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[22,23] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
6. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor and fibrin.[24] Carefully evaluate results if the sample is suspected of having these types of interferences.

7. Endogenous alkaline phosphatase (ALP), exogenous ALP and proteins capable of binding to ALP may cause interference.[25] Elevated ALP levels are commonly observed in patients with hepatobiliary disease and bone disease associated with increased osteoblastic activity. Alkaline phosphatase levels above 400 U/L may cause false positive results. In one study, a sample with cTnI concentration of approximately 8 pg/mL demonstrated an increase of 4 pg/mL when spiked with 800 U/L of alkaline phosphatase.
8. Access hsTnI should not be used for patients taking asfotase alfa (i.e. Strensiq).[26]
9. Native human cardiac troponin I was used in development of this assay. Troponin I not from this source (e.g. recombinant antigens) may behave differently.
10. The Access hsTnI results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
11. Positive predictive values (PPV) demonstrated for female subjects using the established female 99th percentile URL values were lower than the PPV values obtained using the overall 99th percentile URL values. Using the lower female 99th percentile URLs may result in a higher proportion of positive test results for females that are non-MI. Taking into consideration the lower bound of the 95% CI, in the worst-case scenario (serum drawn at 6-9 hours after presentation) up to 78% of positive test results for females may be non-MI.
12. Troponin results differ between methods due to selection of standardization or traceability.[27,28] Do not use results between troponin methods interchangeably.
13. The Access hsTnI assay does not demonstrate any "hook" effect up to 2,000,000 pg/mL (ng/L).

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

CLINICAL PERFORMANCE EVALUATION

A multicenter prospective study was conducted to evaluate the diagnostic accuracy of the Access hsTnI assay using the established 99th percentile URLs. The study was designed to establish the clinical performance of Access hsTnI as an aid in the diagnosis of MI.

The study included 1,851 evaluable subjects from ED patients presenting with chest pain or equivalent ischemic symptoms suggestive of Acute Coronary Syndromes (ACS). A total of 14 geographically diverse, primary care hospital-associated emergency departments participated, reflecting regional, urban, suburban, and rural patient populations.

True MI statuses of all subjects were adjudicated by an independent panel of expert physicians using criteria consistent with the Universal Definition of Myocardial Infarction.[29] Adjudicators were blinded to the Beckman Coulter assay results and the attending physicians' diagnosis. All results presented below were based on the adjudicated diagnoses. The MI incidence was 13% (238/1851).

Samples were tested at three independent clinical laboratories on Access 2 Immunoassay Systems. Testing was performed using serum and lithium heparin plasma samples. Study results are shown in Table 3.0 (lithium heparin plasma) and Table 4.0 (serum). Results are presented for the following time intervals between ED admission and specimen collection:

- Time of admission (baseline), ≥ 1 - 3 hours, ≥ 3 - 6 hours and ≥ 6 - 9 hours after admission.

Clinical Sensitivity and Specificity

Diagnostic sensitivity (% MI correctly diagnosed) and specificity (% Non-MI correctly diagnosed) were calculated per CLSI Guideline I/LA21-A2.[30] Estimates of sensitivity and specificity were determined by dividing the number of patients correctly diagnosed by the Access hsTnI assay (n) by the total number of patients with an adjudicated diagnosis (N).

Positive Predictive Value (PPV) and Negative Predictive Value (NPV)

PPV (probability of MI diagnosis in patients with cTnI > 99th percentile URL) and NPV (probability of non-MI diagnosis in patients with cTnI ≤ 99th percentile URL) were calculated per CLSI Guideline I/LA21-A2.[30] Estimates of PPV were determined by dividing the number of patients with elevated cTnI values and adjudicated MI diagnoses (n) by the total number of patients with elevated cTnI values (N). Estimates of NPV were determined by dividing the number of patients with non-elevated cTnI values and adjudicated non-MI diagnoses (n) by the total number of patients with non-elevated cTnI values (N).

Predictive value analysis is directly related to the prevalence of disease in the intended use population. The overall MI prevalence of 13% in this study is consistent with literature and public health findings, and indicates that the study population is representative of the intended use population. Since predictive value analysis is prevalence dependent; results will vary by region and facility.

Table 3.0 Clinical Performance of Access hsTnI Using the Calculated 99th Percentile URL Cutoffs for Lithium Heparin Plasma. Presented at Multiple Time Intervals After Admission to the Emergency Department

99th percentile URL cutoff, pg/mL (ng/L)	Hours After Admission to ED	Sensitivity		Specificity		PPV		NPV	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
Lithium heparin plasma									
Overall: 17.5	Baseline	86 (86/100)	78 - 92	90 (510/564)	88 - 93	61 (86/140)	53 - 70	97 (510/524)	96 - 99
	≥ 1-3 hour	95 (128/135)	90 - 98	90 (985/1090)	89 - 92	55 (128/233)	48 - 61	99 (985/992)	99 - 100
	≥ 3-6 hour	93 (140/151)	87 - 96	90 (1045/1161)	88 - 92	55 (140/256)	48 - 61	99 (1045/1056)	98 - 100
	≥ 6-9 hour	99 (70/71)	92 - 100	86 (387/451)	82 - 89	52 (70/134)	43 - 61	100 (387/388)	99 - 100
Females: 11.6	Baseline	90 (27/30)	74 - 98	90 (228/254)	85 - 93	51 (27/53)	37 - 65	99 (228/231)	96 - 100
	≥ 1-3 hour	98 (42/43)	88 - 100	90 (482/533)	88 - 93	45 (42/93)	35 - 56	100 (482/483)	99 - 100
	≥ 3-6 hour	98 (48/49)	89 - 100	89 (497/557)	86 - 92	44 (48/108)	35 - 54	100 (497/498)	99 - 100
	≥ 6-9 hour	100 (22/22)	85 - 100	84 (188/225)	78 - 88	37 (22/59)	25 - 51	100 (188/188)	98 - 100
Males: 19.8	Baseline	87 (61/70)	77 - 94	88 (272/310)	84 - 91	62 (61/99)	51 - 71	97 (272/281)	94 - 99
	≥ 1-3 hour	95 (87/92)	88 - 98	89 (494/557)	86 - 91	58 (87/150)	50 - 66	99 (494/499)	98 - 100
	≥ 3-6 hour	92 (94/102)	85 - 97	89 (535/604)	86 - 91	58 (94/163)	50 - 65	99 (535/543)	97 - 99
	≥ 6-9 hour	98 (48/49)	89 - 100	83 (188/226)	78 - 88	56 (48/86)	45 - 67	100 (188/189)	97 - 100

Table 4.0 Clinical Performance of Access hsTnI Using the Calculated 99th Percentile URL Cutoffs for Serum. Presented at Multiple Time Intervals After Admission to the Emergency Department

99th percentile URL cutoff, pg/mL (ng/L)	Hours After Admission to ED	Sensitivity		Specificity		PPV		NPV	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
Serum									
Overall: 18.2	Baseline	84 (91/108)	76 - 91	91 (541/595)	88 - 93	63 (91/145)	54 - 71	97 (541/558)	95 - 98
	≥ 1-3 hour	94 (132/141)	88 - 97	91 (1009/1108)	89 - 93	57 (132/231)	51 - 64	99 (1009/1018)	98 - 100
	≥ 3-6 hour	93 (142/152)	88 - 97	90 (1082/1202)	88 - 92	54 (142/262)	48 - 60	99 (1082/1092)	98 - 100
	≥ 6-9 hour	96 (64/67)	88 - 99	86 (403/469)	82 - 89	49 (64/130)	40 - 58	99 (403/406)	98 - 100
Females: 11.8	Baseline	86 (25/29)	68 - 96	90 (236/263)	85 - 93	48 (25/52)	34 - 62	98 (236/240)	96 - 100
	≥ 1-3 hour	98 (42/43)	88 - 100	91 (491/542)	88 - 93	45 (42/93)	35 - 56	100 (491/492)	99 - 100
	≥ 3-6 hour	98 (49/50)	89 - 100	88 (512/579)	86 - 91	42 (49/116)	33 - 52	100 (512/513)	99 - 100
	≥ 6-9 hour	100 (20/20)	83 - 100	83 (196/235)	78 - 88	34 (20/59)	22 - 47	100 (196/196)	98 - 100
Males: 19.7	Baseline	86 (68/79)	77 - 93	88 (292/332)	84 - 91	63 (68/108)	53 - 72	96 (292/303)	94 - 98
	≥ 1-3 hour	94 (92/98)	87 - 98	89 (501/566)	86 - 91	59 (92/157)	51 - 66	99 (501/507)	97 - 100
	≥ 3-6 hour	93 (95/102)	86 - 97	89 (553/623)	86 - 91	58 (95/165)	50 - 65	99 (553/560)	97 - 100
	≥ 6-9 hour	96 (45/47)	86 - 100	83 (193/234)	77 - 87	52 (45/86)	41 - 63	99 (193/195)	96 - 100

Note: The Access hsTnI assay is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and clinical information.

Non-MI Patients with Elevated cTnI Values (Myocardial Injury)

Of the 1613 non-MI patients in the Beckman Coulter prospective multicenter pivotal trial with lithium heparin plasma samples available, 210 (13%) had at least one positive cTnI value (≥99th percentile URL) on one or more of the serial draws. Of the 1634 non-MI patients with serum samples available, 226 (14%) had at least one positive cTnI value on one or more of the serial draws. Among these patients, 97-98% (204/210: plasma, 221/226: serum) were found to have cardiac conditions such as angina, atrial fibrillation, cardiomyopathy, carditis, heart failure, severe coronary artery disease, tachycardia; or non-cardiac conditions such as renal failure or pulmonary embolism that may result in myocardial damage. Results are consistent with literature findings that cTnI may be elevated in non-MI patients with coronary and non-coronary disease in the presence of myocardial injury.[31, 32] Elevated cTnI values in a non-MI patient should not be disregarded. Troponin is specific for myocardial injury; serial samples and clinical context allow identification of patients with acute and chronic conditions causing myocardial injury.

LINEARITY

The Access hsTnI assay demonstrated acceptable linearity throughout the analytical measuring range. Linearity was tested using a protocol based on CLSI EP6-A.[33] Serum and lithium heparin plasma samples were evaluated. In each study one high sample approximately at the highest calibrator and one low sample approximately at the limit of detection were mixed to make 7 sample concentrations evenly distributed across the analytical measuring range. Four replicates of the 7 mixed samples, 8 replicates of the low sample and 4 replicates of the high sample were tested on a single Access 2 Immunoassay System.

The Access hsTnI assay was designed to be linear, with a maximum percent bias of 10% for samples across the analytical measuring range. One study, analyzed using a linear regression method, demonstrated a maximum deviation from linearity of 10% for samples across the analytical measuring range.

DILUTION RECOVERY

The Access hsTnI assay exhibits mean dilution recovery within ±10% of expected concentration, and individual sample recovery within ±15% of expected concentration.

Six samples containing elevated cTnI levels were diluted 1:10 with Access Sample Diluent A. Five replicates of each sample run on an Access 2 Immunoassay System resulted in the following data:

Table 5.0 Dilution Recovery Study Results

Sample	Expected Concentration pg/mL (ng/L)	Determined Mean Concentration pg/mL (ng/L)	Individual Mean Recovery (%)
Serum 1	140,680	144,004	102
Serum 2	144,042	152,412	106
Serum 3	137,333	148,814	108
		Mean % Recovery	106
Lithium Heparin 1	140,545	142,983	102
Lithium Heparin 2	145,324	141,317	97
Lithium Heparin 3	145,405	144,797	100
		Mean % Recovery	100

IMPRECISION

Imprecision was tested using a protocol based on CLSI EP05-A3.[34] Studies were performed using a total of 3 reagent lots, 1 calibrator lot and multiple Access 2 Immunoassay Systems. Serum and Lithium heparin plasma samples were evaluated.

Representative data is shown in Table 6.0. Five patient pools were assayed in duplicate, on 3 reagent lots, in 4 runs per day, over 10 days generating a total of 40 runs and 240 replicates for each sample.

Table 6.0 Imprecision Study Results

Sample	Mean pg/mL (ng/L) (n=240)	Within-Run		Between-Run		Between-Day		Within-Lab		Total Imprecision*	
		%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)
Lithium heparin plasma											
Pool 1 native	8.9	5	0.45	3	0.29	1	0.12	6	0.55	10	0.89
Pool 2 native	17.8	4	0.75	1	0.12	1	0.24	5	0.80	6	1.1
Pool 3 spiked	79	3	2.5	1	1.0	2	1.5	4	3.1	5	4.1
Pool 4 spiked	4,540	4	165	3	130	3	144	6	254	7	337
Pool 5 spiked	16,090	4	569	2	303	2	352	5	735	7	1,038
Serum											
Pool 1 native	9.7	5	0.47	3	0.27	3	0.26	6	0.60	10	0.95
Pool 2 native	12.0	4	0.48	3	0.35	0.1	0.02	5	0.59	7	0.89
Pool 3 spiked	99	3	3.2	1	1.3	1	1.1	4	3.6	5	4.5
Pool 4 spiked	3,984	4	143	2	69	2	79	5	177	6	253
Pool 5 spiked	18,922	4	714	1	248	1	220	4	787	7	1,342

*Total imprecision estimate includes within-run, between-run, between-day, between-lot, between-instrument and between-calibration variance components.

A reproducibility study was also conducted at all three independent testing facilities used in the clinical trial, in order to determine reproducibility across sites. The study was based on CLSI EP05-A3[34] guidelines and used four patient pools covering the measuring range of the assay, including one pool with concentration targeted near the 99th percentile URL, and four commercial controls. Samples were assayed in duplicate with 2 runs per day for 5 days at the 3 sites, generating a total of 30 runs and 60 replicates.

Table 7.0 Reproducibility Study Results

Sample	Mean pg/mL (ng/L)	N	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
			%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)
Lithium heparin plasma												
Pool 1 native	11.3	60	3	0.32	2	0.22	1	0.08	1	0.11	4	0.41

Table 7.0 Reproducibility Study Results, Continued

Sample	Mean pg/mL (ng/L)	N	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
			%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)	%CV	SD pg/mL (ng/L)
Pool 2 spiked	29.7	60	3	0.93	1	0.32	1	0.38	0	0.00	4	1.1
Pool 3 spiked	98	60	2	1.9	2	1.5	0	0.00	2	1.6	3	2.9
Pool 4 spiked	18,565	60	4	687	0	0.00	0	0.00	1	157	4	705
Serum												
Pool 1 native	11.8	60	3	0.30	2	0.22	1	0.06	0.4	0.05	3	0.38
Pool 2 spiked	27.5	60	2	0.67	1	0.36	0	0.00	0.1	0.04	3	0.76
Pool 3 spiked	100	60	3	2.9	2	1.6	0	0.00	0	0.00	3	3.3
Pool 4 spiked	16,374	60	3	477	1	229	1	170	1	85	3	562
QC material												
QC 1	19.2	60	3	0.63	2	0.31	1	0.16	3	0.56	5	0.91
QC 2	54	60	2	1.1	2	0.93	4	2.0	0	0.00	5	2.4
QC 3	1,088	60	2	23	1	10	3	31	0.3	3.0	4	40
QC 4	14,543	60	2	344	2	310	2	290	1	154	4	567

Analytical Specificity / Interferences

Lithium heparin plasma and serum samples containing cTnI concentrations of approximately, 10 pg/mL (ng/L) and 100 pg/mL (ng/L) were spiked with the substances below and run on a single Access 2 Immunoassay System. Values were calculated as described in CLSI EP7-A2.[35] Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). There was no significant interference observed at the levels tested in Table 8.0. The change in concentration between the controls and test samples was within ±10% for samples > 11.5 pg/mL (ng/L). For samples ≤ 11.5 pg/mL (ng/L) the change in concentration between controls and test samples was within 2SD, where 2SD is defined as 2.30 pg/mL (ng/L).

Table 8.0 Interfering substances tested

Substance	Concentration Added	Substance	Concentration Added
Acetaminophen	50 mg/dL	Fibrinogen	1,000 mg/dL
Acetylsalicylic Acid	65 mg/dL	Furosemide	40 mg/dL
Atenolol	1 mg/dL	Hemoglobin	4 mg/mL
Atorvastatin	20 µg/mL	Human Serum Albumin	6,000 mg/dL
Bilirubin (conjugated)	40 mg/dL	Ibuprofen	50 mg/dL
Bilirubin (unconjugated)	20 mg/dL	Intralipid	3,000 mg/dL
Bivalirudin	42 µg/mL	Sodium Heparin	28.8 U/mL
Caffeine	10 mg/dL	Methyldopa	2.5 mg/dL
Captopril	5 mg/dL	Nitrofurantoin	6.4 mg/dL
Cinnarizine	40 mg/dL	Nystatin	2 mg/dL
Clopidogrel	75 µg/mL	Phenobarbital	20 µg/mL
Cocaine	2 mg/dL	Rifampicin	60 µg/mL
Cyclosporine	5 µg/mL	Rosuvastatin	20 µg/mL
Digoxin	200 ng/mL	Tissue Plasminogen Activator (TPA)	2.5 µg/mL
Dopamine	65 mg/dL	Verapamil	16 mg/dL

A study was performed to evaluate the potential cross-reactivity of the assay with other substances that are similar in structure to cTnI. Lithium heparin plasma and serum samples containing cTnI concentrations of approximately 10 pg/mL (ng/L) and 100 pg/mL (ng/L) were spiked with the substances below and run on a single Access 2 Immunoassay System. Values were calculated as described in CLSI EP7-A2.[35] There was no significant cross-reactivity observed at the levels tested in Table 9.0. The change in concentration between the controls and test samples was within ±10% for samples > 11.5 pg/mL (ng/L). For samples ≤ 11.5 pg/mL (ng/L) the change in concentration between controls and test samples was within 2SD, where 2SD is defined as 2.30 pg/mL (ng/L).

Table 9.0 Cross-reactants tested

Substance	Concentration Added (ng/mL)
Actin	1,000
CK-MB	1,000
Myoglobin	1,000
Myosin	1,000
Cardiac troponin C	250
Skeletal troponin I	250
Tropomyosin	1,000
Cardiac Troponin T	125

LIMIT OF BLANK

Limit of Blank (LoB) was tested using a protocol based on CLSI EP17-A2.[36] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple Access 2 Immunoassay Systems. In each study, 5 replicates of four zero analyte samples (S0 Calibrator & Sample Diluent A) were measured in 3 runs. The LoB for the Access hsTnI assay

ranged from 0.0 to 0.8 pg/mL (ng/L) across the studies performed. The maximum observed LoB for Access hsTnI is 0.8 pg/mL (ng/L).

LIMIT OF DETECTION

Limit of Detection (LoD) was tested using a protocol based on CLSI EP17-A2.[36] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple Access 2 Immunoassay Systems. Serum and lithium heparin plasma samples were evaluated. In each study, 5 replicates from five low-level samples were measured in 10 runs. The LoD for the Access hsTnI assay ranged from 1.0 to 2.0 pg/mL (ng/L) across the studies performed. The maximum observed LoD for Access hsTnI is 2.0 pg/mL (ng/L).

LIMIT OF QUANTITATION

Limit of Quantitation (LoQ) was tested using a protocol based on CLSI EP17-A2.[36] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple Access 2 Immunoassay Systems. Serum and Lithium heperin plasma samples were evaluated. In each study, 5 replicates of 13 samples were measured in 10 runs. LoQ was determined as the lowest concentration which met the design requirements of total imprecision ≤ 20% CV. The 20% CV LoQ for the Access hsTnI assay ranged from 0.9 to 2.0 pg/mL (ng/L) across the studies performed. The maximum observed 20% CV LoQ for Access hsTnI is 2.0 pg/mL (ng/L).

ADDITIONAL INFORMATION

REVISION HISTORY

Revision C

Added a Limitation, added revision history and patent statement.

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

ACCESS hsTnI Calibrators
High Sensitivity Troponin I

REF C26909

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access hsTnI Calibrators are intended to calibrate the Access hsTnI assay for quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert Relative Light Unit (RLU) measurements of patient samples to specific quantitative analyte concentrations.

TRACEABILITY

The analyte in the Access hsTnI 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 Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

REAGENTS

PRODUCT INFORMATION

Access hsTnI Calibrators

Cat. No. C26909: S0-S2, 1.5 mL/vial; S3-S6, 1 mL/vial

- Provided ready to use.
- Store upright.
- Freeze upon receipt at -15 to -30°C.
- Thaw at room temperature. Mix contents thoroughly by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at -15 to -30°C.
- Vial is stable at 2 to 10°C for 64 days after initial use.
- Signs of possible deterioration are quality control values out of range.
- Thaw only once.
- Refer to calibration card for exact concentrations.

S0:	Buffered bovine serum albumin (BSA) matrix with surfactant < 0.1% sodium azide, and 0.1% ProClin* 300.
S1, S2, S3, S4, S5, S6:	Recombinant troponin complex at cTnI levels of approximately 30.7, 144, 567, 2,293, 9,280 and 27,027 pg/mL in buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide and 0.1% ProClin 300.
Calibration Card:	1

*ProClin™ is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

hsTnI Calibrator S0	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
hsTnI Calibrators S1, S2, S3, S4, S5, S6	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

CALIBRATION

CALIBRATION INFORMATION

Run the Access hsTnI Calibrators S0 – S2 in quadruplicate, and the Calibrators S3-S6 in duplicate.

The Access hsTnI Calibrators are provided at 7 levels – zero and 30.7, 144, 567, 2,293, 9,280 and 27,027 pg/mL (ng/L). Assay calibration data are valid up to 63 days.

TESTING PROCEDURE(S)

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

PROCEDURAL NOTES

LIMITATIONS

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision B

IFU updated to change copyright, add revision history and add patent statement.

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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Document History Page

Change type: New, Major, Minor etc.	Changes Made to Document – describe	Signature responsible person/Date	Lab Operations Director Review/ Date	Laboratory Medical Director Review/ Date	Date change implemented
New	• New Test: High Sensitivity Troponin I	3/23/21	3/26/21		3/26/21