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ACCESS
Immunoassay Systems

Instructions For Use

ACCESS hsTnI
High Sensitivity Troponin I

REF B52699

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access hsTnI assay is a paramagnetic particle, chemiluminescent immunoassay for high sensitivity quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the Access 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 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] Multiple studies have confirmed high sensitivity assays detect cTn release earlier, and increase sensitivity for MI diagnosis at presentation. This may facilitate earlier 'rule-in' and 'rule-out' of MI.[14,16,17]

METHODOLOGY

The Access hsTnI assay is a sequential two–step 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 preferred samples. EDTA plasma is also an acceptable sample type. **Lithium heparin plasma, EDTA plasma and serum samples should not be used interchangeably.**[18] The 99th percentile values presented in the Expected Results section apply to lithium heparin plasma and serum samples. A conversion factor of 0.90 should be applied to the 99th percentile for EDTA plasma samples.

EXAMPLE: 0.90 x [lithium heparin plasma 99th percentile URL] = [EDTA plasma 99th percentile URL].

2. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[19,20] To minimize the effect of preanalytical factors observe the following recommendations for handling and processing blood samples:[21]
   - 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 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 30°C) for up to 8 hours.
   - If the assay will not be completed within 8 hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, freeze serum and heparin plasma at -20°C or colder. EDTA plasma should not be frozen.
   - Frozen specimens can be stored up to 180 days before testing.
   - Thaw samples only once.
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.[22]
   - 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. B52700
2. Quality Control (QC) materials: commercial control material.
3. Access Sample Diluent A
   Vial Cat. No. 81908
   Diluent Pack Cat. No. A79783 (For use with the UniCel DxI system onboard dilution feature.)
4. Access Substrate
   Cat. No. 81906
5. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

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.[23] 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 hsTnI assay. Use fifty (50) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature (test name: dhsTn). 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.

- Select hsTnI as the test name for assaying samples containing cTnI concentrations up to the concentration of the Access hsTnI S6 calibrator.
- UniCel DxI users may use the UniCel DxI onboard dilution feature (Test name: dhsTn) for assaying samples containing cTnI concentrations greater than the Access hsTnI S6 calibrator.

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.

REPORTING RESULTS

EXPECTED RESULTS

A multicenter prospective study was conducted to establish the 99th percentile URL in a population of apparently healthy adults. Serum and lithium heparin plasma samples were evaluated. Subjects ranging from 21 to 99 years of age were enrolled at five geographically diverse locations throughout the United States. A total of 494 males and 595 females were included with 45% ≥ 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 observed 99th percentile URL in 1,089 lithium heparin plasma samples measured using the non-parametric method is 17.5 pg/mL (ng/L) (95% CI: 12.6 - 20.7). No quantitative differences in results were observed between serum and lithium heparin plasma samples.

Table 1.0 99th percentile URL of a healthy population

Population	N	99th percentile URL pg/mL (ng/L)	95% CI pg/mL (ng/L)
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

Note: Each laboratory should validate or establish its own reference intervals to assure proper representation of specific populations.

Current guidance from the IFCC states high sensitivity assays must have analytical imprecision ≤ 10% CV at the 99th percentile URL of a healthy population.[13] For Access hsTnI, the 10% CV limit of quantitation (LoQ) was measured to be 5.6 pg/mL (ng/L).

In addition, IFCC states that a high sensitivity assay must be able to measure cTn above the limit of detection (LoD) in ≥ 50% of a healthy population. In the study presented above, > 50% of 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 using

lithium heparin plasma samples was evaluated to estimate imprecision at the established 99th percentile values (Table 2.0).

Table 2.0 Imprecision at the established 99th percentile URLs

Population	99th percentile URL pg/mL (ng/L)	% CV based on LoQ imprecision profile
Females	11.6	4.2
Males	19.8	3.6
Overall	17.5	3.7

PROCEDURAL NOTES

LIMITATIONS

1. Samples can be accurately measured within the analytical range of the lower Limit of Detection (LoD) and the highest (S6) calibrator value (approximately 27,027 pg/mL [ng/L]).
   - If a sample contains less than the lower Limit of Detection (LoD) for the assay, report the result as less than that value (i.e., < 2.3 pg/mL [ng/L]).
   - If a sample contains more than the stated value of the highest Access hsTnI Calibrator (S6), report the result as greater than that value. Alternatively, dilute one volume of sample with 9 volumes of Access Sample Diluent A.
2. 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.
3. **Onboard Dilution Feature for use on UniCel DxI systems:**

   The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 9 volumes of Access Sample Diluent A, allowing samples to be quantitated up to 10X the stated value of the highest calibrator (S6). 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.[24,25] 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, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[26,27] Carefully evaluate the results of patients suspected of having these types of interferences.
7. 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.

8. 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.
9. Troponin results differ between methods due to selection of standardization or traceability.[28,29] Do not use results between troponin methods interchangeably.
10. 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.[30] 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% (238/1,851).

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

- 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.[31] 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.[31] 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. 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
Overall: 17.5	Baseline	90 (198/219)	86 - 94	90 (1,390/1,539)	89 - 92	57 (198/347)	52 - 62	99 (1,390/1,411)	98 - 99
	≥ 1-3 hour	97 (116/120)	92 - 99	90 (873/970)	88 - 92	55 (116/213)	48 - 61	100 (873/877)	99 - 100
	≥ 3-6 hour	94 (130/138)	89 - 98	88 (767/871)	86 - 90	56 (130/234)	49 - 62	99 (767/775)	98 - 100
	≥ 6-9 hour	94 (34/36)	81 - 99	88 (183/208)	83 - 92	58 (34/59)	44 - 70	99 (183/185)	96 - 100
Females: 11.6	Baseline	94 (66/70)	86 - 98	90 (666/738)	88 - 92	48 (66/138)	39 - 57	99 (666/670)	99 - 100
	≥ 1-3 hour	98 (39/40)	87 - 100	90 (446/494)	87 - 93	45 (39/87)	34 - 56	100 (446/447)	99 - 100
	≥ 3-6 hour	100 (43/43)	92 - 100	88 (351/401)	84 - 91	46 (43/93)	36 - 57	100 (351/351)	99 - 100
	≥ 6-9 hour	93 (13/14)	66 - 100	84 (81/97)	75 - 90	45 (13/29)	26 - 64	99 (81/82)	93 - 100
Males: 19.8	Baseline	91 (136/149)	86 - 95	88 (707/801)	86 - 90	59 (136/230)	53 - 66	98 (707/720)	97 - 99
	≥ 1-3 hour	96 (77/80)	89 - 99	88 (419/476)	85 - 91	58 (77/134)	49 - 66	99 (419/422)	98 - 100
	≥ 3-6 hour	93 (88/95)	85 - 97	86 (404/470)	83 - 89	57 (88/154)	49 - 65	98 (404/411)	97 - 99
	≥ 6-9 hour	96 (21/22)	77 - 100	87 (96/111)	79 - 92	58 (21/36)	41 - 75	99 (96/97)	94 - 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.

Analysis of Delta Values

Delta values indicate a significant rise or fall between serial cTnI measurements. The use of delta values may improve clinical specificity and PPV for acute MI compared to an evaluation based upon the 99th percentile URL cutoff alone.[32,33] When serial samples are obtained and cTnI deltas are considered in the clinical context of each patient, acute MI may be more rapidly distinguished from other conditions causing myocardial injury. Delta values must be defined specifically for each manufacturer's assay, and there must be clear criteria around the calculation method.[13]

An analysis was performed to evaluate diagnostic accuracy of potential delta values for Access hsTnI, when used in conjunction with the 99th percentile URL; 1,721 subjects from the multicenter prospective study were included. Lithium heparin plasma samples were used in the analysis. Two groups were assessed:

- Subjects with a cTnI value > the 99th percentile URL and maximum observed cTnI change ≥ delta value between time points (positive result)
- Subjects who did not have a cTnI value > the 99th percentile URL, or did not have a cTnI change ≥ delta value between time points, or both (negative result)

Results were compared to true MI statuses of all subjects, as determined by the independent adjudication panel. Sensitivity, specificity, PPV, and NPV are reported in Table 4.0. Delta values were also assessed in conjunction with the sex-specific 99th percentile URLs, evaluating males and females separately; there was not found to be a significant impact to diagnostic accuracy.

Specificity using the 99th percentile URL cutoff alone ranged from 84-90%. When a delta value was added to the analysis, Specificity ranged from 92-99% depending on the magnitude of the delta value considered. PPV using the 99th percentile URL cutoff alone ranged from 45-59%. When a delta value was added to the analysis, PPV ranged from 62-90% depending on the magnitude of the delta value considered.

Table 4.0 Diagnostic accuracy of delta change values used in conjunction with elevations > the overall 99th percentile URL cutoff: 17.5 pg/mL (ng/L)

Delta change value (≥) pg/mL (ng/L)	Timing of serial draws	Sensitivity % (n/N)	Specificity % (n/N)	PPV % (n/N)	NPV % (n/N)
3	Baseline vs. 1-3 hours	76 (94/123)	95 (938/986)	66 (94/142)	97 (938/967)
	Baseline vs. 3-6 hours	87 (109/125)	92 (759/826)	62 (109/176)	98 (759/775)
5	Baseline vs. 1-3 hours	71 (87/123)	97 (959/986)	76 (87/114)	96 (959/995)
	Baseline vs. 3-6 hours	78 (97/125)	95 (788/826)	72 (97/135)	97 (788/816)
11	Baseline vs. 1-3 hours	61 (75/123)	99 (971/986)	83 (75/90)	95 (971/1,019)
	Baseline vs. 3-6 hours	60 (75/125)	98 (805/826)	78 (75/96)	94 (805/855)
22	Baseline vs. 1-3 hours	50 (62/123)	99 (979/986)	90 (62/69)	94 (979/1,040)
	Baseline vs. 3-6 hours	54 (67/125)	99 (817/826)	88 (67/76)	93 (817/875)

Note: PPV and NPV values are prevalence dependent and results will vary by facility and region. Each laboratory should validate these data or establish its own delta values to assure proper representation of specific populations. Delta values are not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and clinical information.

LINEARITY

Representative data for linearity are provided for illustration only. Performance obtained in individual laboratories may vary.

Based on CLSI EP6-A[34], 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 and a single DxI 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 percent bias of 6% for samples across the analytical measuring range.

DILUTION RECOVERY

Representative data for dilution recovery are provided for illustration only. Performance obtained in individual laboratories may vary.

Five samples containing elevated cTnI levels were diluted 1:10 with Access Sample Diluent A. Five replicates of each sample run on a DxI 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	21,304	21,902	103
Serum 2	14,573	13,271	91
Lithium Heparin 1	17,513	20,030	114
Lithium Heparin 2	23,324	21,246	91
Lithium Heparin 3	13,404	11,940	89

IMPRECISION

Representative data for imprecision are provided for illustration only. Performance obtained in individual laboratories may vary.

The Access hsTnI assay exhibits within-laboratory (total) imprecision of ≤ 10% CV at concentrations ≥ 11.5 pg/mL (ng/L), and within-laboratory standard deviation (SD) ≤ 1.15 pg/mL (ng/L) for concentrations < 11.5 pg/mL (ng/L).

One study, based on CLSI EP5-A3[35] guidelines, provided the following data. This study used three spiked patient pools assayed in duplicate, in 2 runs per day, over 20 days generating a total of 40 runs and 80 replicates.

Table 6.0 Imprecision study results

Sample	Mean pg/mL (ng/L) (n=80)	Within-Run %CV	Between-Run %CV	Within-Laboratory (Total Imprecision) %CV
Low Pool	30.3	4	6	7
Medium Pool	110	4	4	6
High Pool	17,667	4	3	5

A separate study was performed at an independent testing facility. The study was based on CLSI EP05-A3 guidelines and used five spiked 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 20 days generating a total of 40 runs and 80 replicates.

Table 7.0 Imprecision study results

Sample	Mean pg/mL (ng/L) (n=80)	Total Imprecision SD pg/mL (ng/L)	Total Imprecision (%CV)
Pool 1	7.8	0.62	8
Pool 2	13.0	0.73	6

Table 7.0 Imprecision study results, Continued

Sample	Mean pg/mL (ng/L) (n=80)	Total Imprecision SD pg/mL (ng/L)	Total Imprecision (%CV)
Pool 3	32	1.64	5
Pool 4	107	4.22	4
Pool 5	20,268	949	5
QC 1	25.6	1.54	6
QC 2	62	3.22	5
QC 3	1,242	43	3
QC 4	15,415	740	5

Analytical Specificity / Interferences

Representative data for analytical specificity/interferences are provided for illustration only. Performance obtained in individual laboratories may vary.

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 and a single DxI Immunoassay System. Values were calculated as described in CLSI EP7-A2.[36] 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 and a single DxI 800 Immunoassay System. Values were calculated as described in CLSI EP7-A2.[36] 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

Representative data for Limit of Blank, Limit of Detection and Limit of Quantitation are provided for illustration only. Performance obtained in individual laboratories may vary.

LIMIT OF BLANK

Limit of Blank (LoB) was tested using a protocol based on CLSI EP17-A2.[37] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple DxI and 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 1.7 pg/mL (ng/L) across the studies performed. The observed LoB for Access hsTnI is 1.7 pg/mL (ng/L).

LIMIT OF DETECTION

Limit of Detection (LoD) was tested using a protocol based on CLSI EP17-A2.[37] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple Access 2 and DxI 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 2.3 pg/mL (ng/L) across the studies performed. The observed LoD for Access hsTnI is 2.3 pg/mL (ng/L).

LIMIT OF QUANTITATION

Limit of Quantitation (LoQ) was tested using a protocol based on CLSI EP17-A2.[37] Studies were performed using a total of 3 reagent lots, 3 calibrator lots and multiple Access 2 and DxI Immunoassay Systems. In each study, 5 replicates of 13 samples were measured in 10 runs. Serum and lithium heparin plasma samples were evaluated. LoQ was determined as the lowest concentration which could be measured with total imprecision ≤ 20% CV. The 20% CV LoQ for the Access hsTnI assay ranged from 1.0 to 2.3 pg/mL (ng/L) across the studies performed. The observed 20% CV LoQ for Access hsTnI is 2.3 pg/mL (ng/L).

ADDITIONAL INFORMATION

May be covered by one or more pat. -see www.beckmancoulter.com/patents.

REVISION HISTORY

Revision K

IFU updated to add a Limitation. Add Dutch, Finnish, Macedonian, and Estonian. Add revision history and patent statement and update copyright.

Revision L

Update Spanish to close CASE-2020-00656557

SYMBOLS KEY

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

REFERENCES

1. Perry SV. The regulation of contractile activity in muscle. Biochem Soc Trans 1979; 7: 593-617.

2. Vallins WJ, Brand NJ, Dabhade N, Butler-Browne G, Yacoub MH, Barton PJ. Molecular cloning of human cardiac troponin I using polymerase chain reaction. FEBS Lett 1990; 270: 57-61.

3. Perry SV. Troponin I: inhibitor or facilitator. Mol Cell Biochem 1999; 190: 9-32.

4. Katrukha A. Antibody selection strategies in cardiac troponin assay. In: Wu AHB, editor. Cardiac markers, 2nd ed. Totowa (NJ): Humana Press Inc., 2003, 173-185.

5. Larue C, Defacque-Lacquement H, Calzolari C, Le Nguyen D, Pau B. New monoclonal antibodies as probes for human cardiac troponin I: epitopic analysis with synthetic peptides. Mol Immunol 1992; 29: 271-278.

6. Mair J, Morandell D, Genser N, Lechleitner P, Dienstl F, Puschendorf B. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. Clin Chem 1995; 41: 1266-1272.

7. Mair J, Genser N, Morandell D, Maier J, Mair P, Lechleitner P, Calzolari C, Larue C, Ambach E, Dienstl F, Pau B, Puschendorf B. Cardiac troponin I in the diagnosis of myocardial injury and infarction. Clin Chim Acta. 1996; 245: 19-38.

8. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol 2006; 48: 1-11.

9. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 2005; 173: 1191-1202.

10. Jeremias A, Gibson CM. Narrative Review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. Ann Intern Med 2005; 142: 786-91.

11. Thygesen K, Alpert JS, Jaffe AS. Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Third universal definition of myocardial infarction. Eur Heart J 2012; 33: 2551-67. J Am Coll Cardiol 2012; 60: 1581-98. Circulation 2012; 126: 2020-35. Available online at: www.escardio.org/guidelines.

12. Hamm CW, Giannitsis E, Katus HA. Cardiac troponin elevations in patients without acute coronary syndrome. Circulation 2002; 106: 2871-2872.

13. Clinical Applications of Cardiac Bio-markers. IFCC: International Federation of Clinical Chemistry and Laboratory Medicine, 26 July 2014. Web. 14 Feb. 2017.

14. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012; 33: 2252-7.

15. Apple F, Ler R, Murakami M. Determination of 19 Cardiac Troponin I and T Assay 99th Percentile Values from a Common Presumably Healthy Population. Clinical Chemistry 58:11, 1574–1581 (2012).

16. Korley FK, Jaffe AS. Preparing the United States for high-sensitivity cardiac troponin assays. J Am Coll Cardiol 2013; 61:1753-8.

17. Roffi M, Patrono C, Collet JP, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2016 Jan 14;37(3):267-315.

18. Wu AH, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes R Jr. National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery disease. Clin Chem 1999; 45: 1104-1121.

19. Approved Guideline - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, GP44-A4. 2010. Clinical and Laboratory Standards Institute.

20. Approved Standard - Sixth Edition, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, H3-A6. 2007. Clinical and Laboratory Standards Institute.

21. Approved Guideline - Seventh Edition, Collection of Diagnostic Venous Blood Specimens, GP41. 2017. Clinical and Laboratory Standards Institute.

22. Marks V. False-Positive Immunoassays Results; a Multicenter Survey of Erroneous Immunoassay Results from Assays of 74 Analytes in 10 Donors from 66 Laboratories in Seven Countries. Clin Chem 2002;48:2008-16

23. Cembrowski GS, Carey RN. Laboratory quality management: QC ⇌ QA. ASCP Press, Chicago, IL, 1989.

24. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.

25. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613-621.

26. Lum G, Solarz D, Farney L. False Positive Cardiac Troponin Results in Patients Without Acute Myocardial Infarction. Labmedicine 2006; 37(9): 546-550.

27. Lingwood D, Ballantyne JS. Alkaline phosphatase-immunoglobulin conjugate binds to lipids in vitro, independent of antibody selectivity. Journal of Immunological Methods 2006; 311: 174-177.

28. Christenson RH, Bunk DM, Schimmel H, Tate JR. Put Simply, Standardization of Cardiac Troponin I Is Complicated. Clin Chem 2012; 58:1 165-168.

29. Tate JR, Bunk DM, Christenson RH, Katrukha A, Noble JE, Porter RA, Schimmel H, Wang L, Panteghini M. Standardization of cardiac troponin I measurement: past and present. Pathology 2010, 42(5): 402-8.

30. Thygesen K, Alpert JS, White HD; Joint ESC/ACC/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J 2007; 28: 2525-38. J Am Coll Cardiol 2007; 50: 2173-95. Circulation 2007; 116: 2634-53.

31. CLSI. Clinical Evaluation of Immunoassays; Approved Guideline-Second Edition. CLSI document I/LA21-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2008.

32. Cullen L, Parsonage WA, Greenslade J, et al. Delta troponin for the early diagnosis of AMI in emergency patients with chest pain. Int J Cardiol. 2013 Oct 3; 168(3):2602-8

33. Morrow DA, Bonaca MP, Real World Application of “Delta” Troponin. JACC Vol 62, No. 14, 2013

34. Approved Guideline - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, EP6-A. April 2003. Clinical and Laboratory Standards Institute.

35. Approved Guideline – Evaluation of Precision of Quantitative Measurement Procedures, EP5-A3. 2014. Clinical and Laboratory Standards Institute.

36. Approved Guideline - Interference Testing in Clinical Chemistry, EP7-A2. November 2005. Clinical and Laboratory Standards Institute.

37. Approved Guideline . Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, EP17-A2. June 2012. Clinical and Laboratory Standards Institute.

IMMUNOTECH S.A.S. a Beckman Coulter Company, 130, avenue de Lattre de Tassigny,
BP 177, 13276 Marseille cedex 9, France, 33-491 172 727