BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access AccuTnI+3 Troponin I

REF A98264

FOR PROFESSIONAL USE ONLY

Rx Only

For Use on UniCel DxI Access Immunoassay Systems† with test name: TnIDx

PRINCIPLE

INTENDED USE

The Access AccuTnI+3 Reagent is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the UniCel DxI Access Immunoassay Systems to aid in the diagnosis of myocardial infarction.

† UniCel DxI 600, UniCel DxI 800, UniCel DxC 880i, UniCel DxC 860i, UniCel DxC 680i, UniCel DxC 660i

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 isotypes 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 (cTnI) 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 damage. The Access AccuTnI+3 assay uses monoclonal antibodies specifically directed against human cardiac troponin I.

In myocardial infarction (MI), 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 confusing.[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 biomarkers play a central role.[12] Professional groups recognize cardiac troponin (cTn) as the preferred biomarker for MI diagnosis.

The 2012 Third Universal Definition of Myocardial Infarction document states that the following is one criterion for the diagnosis of MI:

- "Detection of a rise and/or fall of cardiac biomarkers values [preferably cardiac troponin (cTn)] 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."[12]

Because cTn may not appear in blood within the first hours after myocardial injury,[13] cTn 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 is encountered in the absence of myocardial ischemia, a careful search for other possible etiologies of cardiac damage should be undertaken.[14]

METHODOLOGY

The Access AccuTnI+3 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. For use on serum and lithium heparin plasma samples. Do not dilute patient samples, as this could lead to lower than expected troponin results. Matched serum and lithium heparin plasma samples were tested using the Access AccuTnI+3 assay. Regression and correlation analysis are shown below:

n	Range of Observations (ng/mL)	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient (r)
118	0.03 - 61	0.00 (-0.01 - 0.00)	0.99 (0.98 - 1.00)	1.00
55	0.03 - 0.50	0.00 (0.00 - 0.00)	0.96 (0.93 - 0.98)	1.00

   Heparin plasma and serum samples **should not be used interchangeably**.[15]

2. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[16,17,18] To minimize the effect of preanalytical factors, observe the following recommendations for handling, processing, and storing blood samples:
   - Collect all blood samples observing routine precautions for venipuncture.[16]
   - Allow serum samples to clot completely before centrifugation. Time to clot may be prolonged in serum samples due to the patient's clinical condition or in patients receiving anticoagulant therapy.[16]

- Keep tubes stoppered at all times.[16]
- Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than two hours.
- Samples should be centrifuged, physically separated from contact with cells, and refrigerated as soon as possible.
- Remove any residual fibrin or cellular matter. Failure to do so can contribute to falsely elevated results.
- 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, care should be taken to avoid resuspending platelets.
- Turbid serum or plasma samples containing particulate matter should be transferred from the original tube and recentrifuged prior to assay. A specimen (original tube) that contains a separating device (gel barrier) is never to be recentrifuged.
- If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C or colder.[16]
- Follow blood collection tube manufacturer's recommendations for centrifugation.
- Samples may be stored for six months at -20°C.

3. 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.
4. Thaw samples only once and centrifuge all thawed samples prior to analysis. Do not thaw in a water bath.

REAGENTS

PRODUCT INFORMATION

Access AccuTnI+3 Reagent Pack (for use on UniCel DxI Access Immunoassay Systems)

Cat. No. A98264: 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 56 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

R1a:	Paramagnetic particles coated with mouse monoclonal anti-human cardiac troponin I (cTnI) suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA) matrix, < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	0.1 N NaOH.
R1c:	TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.
R1d:	Mouse monoclonal anti-human cTnI alkaline phosphatase conjugate diluted in ACES buffered saline, with surfactant, BSA matrix, protein (bovine, goat, mouse), < 0.1% sodium azide, and 0.25% ProClin 300. Mouse monoclonal anti-human cTnI alkaline phosphatase conjugate diluted in ACES buffered saline, with surfactant, BSA matrix, protein (bovine, goat, mouse), < 0.1% sodium azide, and 0.25% ProClin 300.

*ProClin is a trademark of LANXESS Corp.

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

Paramagnetic Particles (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%
Ancillary Reagent (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%
Conjugate (Compartment R1d)	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.

Lithium Chloride 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%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access AccuTnI+3 Calibrators (for use on UniCel DxI Access Immunoassay Systems)
   Provided at zero and approximately 0.2, 0.9, 3.7, 20, and 80 ng/mL (µg/L).
   Cat. No. A98265
2. Quality Control (QC) materials: commercial control material.
3. Access Substrate
   Cat. No. 81906
4. UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1 Access AccuTnI+3 Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

Run the Access AccuTnI+3 Calibrator S0 and S1 in quadruplicate, and the Calibrator S2-S5 in duplicate.

An active calibration curve is required for all tests. For the Access AccuTnI+3 assay, calibration is required every 56 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 patient 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.[19] Include 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. Several options are commercially available that meet these criteria. 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 of the quality control material. The thermal profile of native human cardiac troponin I was used in development of the assay. Quality control materials containing troponin I not from this source (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. 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 ng/mL. To change sample reporting units to the International System of Units (SI units) µg/L, or alternate units such as pg/mL or ng/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System (µg/L), multiply ng/mL by multiplication factor 1. For manual conversion to pg/mL or ng/L, multiply ng/mL by a factor of 1,000.

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

Patient 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, and the application of mathematical adjustments. Patient 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

Apparently Healthy Adults

Beckman Coulter conducted a multicenter prospective study to establish the 99th percentile upper reference limit (URL) in a population of apparently healthy adults with no known diseases of the cardiovascular system or other serious acute or chronic diseases or infections. Lithium heparin plasma samples were evaluated. Five hundred twenty-seven (527) subjects were enrolled at seven geographically diverse locations. Both male and female subjects were included with approximately half above the age of 40 years and the other half between 18 and 40 years.

Subjects were excluded from the study if they met any of the following criteria:

- Disease(s) of/or affecting the cardiovascular system, including: hypertension, angina, coronary artery disease, history of MI, history of percutaneous transcoronary angiography/angioplasty (PTCA) or coronary artery bypass graft (CABG), peripheral artery disease including cerebrovascular disease/stroke or deep vein thrombosis, hemostatic disorders, and/or congestive heart failure.
- Currently taking a medication for cardiovascular disease, including: ACE inhibitors, angiotensin II receptor blockers, beta-blockers, calcium channel blockers, diuretics, blood thinners, and/or platelet inhibitors.
- Diabetes.
- Chronic kidney disease.
- Other serious chronic disease(s), including: cancer, leukemia, emphysema, chronic obstructive pulmonary disease (COPD), HIV, HBV, HCV, lupus erythematosus, rheumatoid arthritis, and/or scleroderma.

- Acute bacterial or viral infection, including: bronchitis, upper respiratory infection, strep throat, influenza, pneumonia, mononucleosis, oral/genital herpes outbreak, and/or urinary tract infection.
- Pregnancy.

All samples were analyzed using UniCel DxI 800 Access Immunoassay Systems. Results from this study demonstrated the 99th percentile upper reference limit (URL) to be < 0.03 ng/mL with a 97.5% upper confidence limit (UCL) of 0.04 ng/mL.

Table 1.0 99th Percentile Upper Reference Limit of a Healthy Population

n	Age Range (years)	99th Percentile (97.5% UCL)
527	18-94	< 0.03 ng/mL (0.04 ng/mL)

In addition to this Reference Interval study of healthy adults, a multicenter prospective study of 1929 patients presenting to the Emergency Department with chest pain was conducted in order to evaluate diagnostic cutoff values and establish clinical utility. See the following Clinical Performance Evaluation section for further information.

CLINICAL PERFORMANCE EVALUATION

Diagnosis of Myocardial Infarction

As described in the Summary and Explanation section of this document, the Universal Definition of Myocardial Infarction document has recommended the use of more sensitive cTn assays and lower cutoffs. To establish clinical performance of Access AccuTnI+3 to aid in the diagnosis of MI, a clinical study was conducted to evaluate sensitivity (% MI correctly identified) and specificity (% non-MI correctly identified) at cutoffs near the 99th percentile URL.

The multicenter prospective study enrolled 1,929 subjects from Emergency Department (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.

Study endpoints (final diagnoses) were adjudicated by an independent panel of expert physicians using criteria consistent with the 2007 Universal Definition of Myocardial Infarction.[11‡] Investigators and adjudicators were blinded to the Access AccuTnI+3 assay results. The adjudicators were also blinded to the site diagnoses. All results presented below were based on the adjudicated diagnoses. The MI incidence was 13% (253/1929).

To assess performance of the Access AccuTnI+3 assay, study samples were tested at four independent testing facilities on a UniCel DxI 800 Access Immunoassay System. Testing was performed using lithium heparin plasma samples.

‡The 2012 Third Universal Definition of Myocardial Infarction was published after completion of this prospective study.[12]

Clinical Sensitivity and Specificity

The American College of Emergency Physicians (ACEP) recommends serial troponin measurements for patients presenting early after symptom onset (< 8 hours).[20] However, patient estimates of symptom timing may not be totally reliable, prompting serial sampling recommendations after admission to the emergency department. Based on these guidelines and literature recommendations, study results are presented for the following time intervals:

- < 8 hours and ≥ 8 hours after symptom onset
- Baseline, ≥ 1 - 3 hours, ≥ 3 - 6 hours and ≥ 6 - 9 hours after admission

Receiver Operating Characteristic (ROC) curves and Areas Under the Curve (AUC) were generated for serial time intervals after symptom onset and admission. Study results showed the AccuTnI+3 assay to have significant diagnostic efficacy at all time intervals (AUCs 0.93-0.97, p < 0.0001).

Diagnostic sensitivity (% MI correctly diagnosed) and specificity (% Non-MI correctly diagnosed) were also determined as shown in the tables below. Estimates of sensitivity and specificity were determined by dividing the number of patients correctly diagnosed by the AccuTnI+3 assay (n) by the total number of patients with an adjudicated diagnosis (N). Cutoffs of 0.03 ng/mL and 0.04 ng/mL yielded 92% and 86% sensitivity respectively for cTnI measurements ≥ 8 hours after symptom onset. Specificity ranged from 89% to 93%.

A cutoff of 0.03 ng/mL may be used as an aid in the diagnosis of MI. The Access AccuTnI+3 LoQ is 0.04 ng/mL (µg/L) at 10% CV.

Table 2.0 Diagnostic Sensitivity and Specificity for Serial Time Intervals After Symptom Onset

TnI cutoff for Diagnosis of MI (≥) (ng/mL)	Hours After Symptom Onset	Sensitivity (MI patients correctly diagnosed)			Specificity (Non-MI patients correctly diagnosed)		
		%	n/N	95% CI	%	n/N	95% CI
0.03	< 8 hours	90	148/165	84 - 94	91	902/993	89 - 93
	≥ 8 hours	92	145/158	86 - 96	89	993/1112	87 - 91
0.04	< 8 hours	84	138/165	77 - 89	94	937/993	93 - 96
	≥ 8 hours	86	136/158	80 - 91	93	1038/1112	92 - 95

Table 3.0 Diagnostic Sensitivity and Specificity for Serial Time Intervals After Admission to the Emergency Department

TnI cutoff for Diagnosis of MI (≥) (ng/mL)	Hours After Admission to ED	Sensitivity (MI patients correctly diagnosed)			Specificity (Non-MI patients correctly diagnosed)		
		%	n/N	95% CI	%	n/N	95% CI
0.03	Baseline	84	213/253	79 - 89	90	1508/1675	89 - 91
	≥ 1 - 3	94	116/124	88 - 97	91	923/1014	89 - 93
	≥ 3 - 6	91	143/157	86 - 95	89	839/941	87 - 91
	≥ 6 - 9	93	40/43	90 - 99	90	222/246	86 - 94
0.04	Baseline	72	182/253	66 - 77	94	1577/1675	93 - 95
	≥ 1 - 3	89	110/124	82 - 94	95	960/1014	93 - 96
	≥ 3 - 6	83	131/157	77 - 89	93	875/941	91 - 95
	≥ 6 - 9	93	40/43	81 - 99	94	231/246	90 - 97

In clinical practice, even higher specificity is attained from detection of a rise and/or fall in cardiac biomarker values as described in the Universal Definition of Myocardial Infarction.[11,12] When serial samples are obtained and the marker is considered in the clinical context of each patient, acute events such as MI may be distinguished from other conditions causing myocardial injury. The AccuTnI+3 assay is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and clinical information.

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

Positive Predictive Values (PPV, probability of MI diagnosis in patients with elevated cTnI) and Negative Predictive Values (NPV, probability of non-MI diagnosis in patients with non-elevated cTnI) were calculated for the multicenter prospective study, per CLSI Guideline I/LA21-A2.[21] Predictive value analysis, unlike ROC analysis, is directly proportional 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. Non-representative study populations with high MI prevalence (35-50%) may overestimate apparent diagnostic accuracy, particularly PPVs (up to 80-90%).[22] Since predictive value analysis is prevalence dependent, results will vary by region and facility.

Study results are shown in the following tables. 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). NPVs indicate nearly all patients with cTnI values < 0.03 ng/mL were diagnosed with conditions other than myocardial infarction (non-MI). PPVs indicate that approximately 55-63% of patients with cTnI values ≥ 0.03 ng/mL were diagnosed with MI. The Access AccuTnI+3 LoQ is 0.04 ng/mL (µg/L) at 10% CV. NPVs indicate that 96-99% of patients with cTnI values < 0.04 ng/mL were diagnosed with conditions other than myocardial infarction (non-MI). PPVs indicate that approximately 65-73% of patients with cTnI values ≥ 0.04 ng/mL were diagnosed with MI.

Table 4.0 PPV and NPV for Serial Time Intervals After Symptom Onset

AccuTnI cutoff (ng/mL)	Hours After Symptom Onset	Positive Predictive Value (Patients above cutoff diagnosed as MI)			Negative Predictive Value (Patients below cutoff diagnosed as Non-MI)		
		%	n/N	95% CI	%	n/N	95% CI
0.03	< 8 hours	62	148/239	55 - 68	98	902/919	97 - 99
	≥ 8 hours	55	145/264	49 - 61	99	993/1006	98 - 99
0.04	< 8 hours	71	138/194	64 - 77	97	937/964	96 - 98
	≥ 8 hours	65	136/210	58 - 71	98	1038/1060	97 - 99

Table 5.0 PPV and NPV for Serial Time Intervals After Admission to the Emergency Department

AccuTnI cutoff (ng/mL)	Hours After Admission to ED	Positive Predictive Value (Patients above cutoff diagnosed as MI)			Negative Predictive Value (Patients below cutoff diagnosed as Non-MI)		
		%	n/N	95% CI	%	n/N	95% CI
0.03	Baseline	56	213/380	51 - 61	97	1508/1548	97 - 98
	≥ 1 to 3	56	116/207	49 - 63	99	923/931	98 - 100
	≥ 3 to 6	58	143/245	52 - 65	98	839/853	97 - 99
	≥ 6 to 9	63	40/64	50 - 74	99	222/225	96 - 100
0.04	Baseline	65	182/280	59 - 71	96	1577/1648	95 - 97
	≥ 1 to 3	67	110/164	59 - 74	99	960/974	98 - 99
	≥ 3 to 6	67	131/197	59 - 73	97	875/901	96 - 98
	≥ 6 to 9	73	40/55	59 - 84	99	231/234	96 - 100

Note: Since predictive value analysis is prevalence-dependent, results will vary by region and facility.

These results are representative of the use of low troponin cutoffs, emphasizing the importance of serial samples when low cutoffs are used. However, even a single elevated troponin value (≥ 0.03 ng/mL) increased the probability of MI from 13% to 55-63%, providing important information to the clinician.

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

Of the 1676 non-MI patients in the Beckman Coulter prospective multicenter pivotal trial, 188 (11%) had at least one cTnI value ≥ 0.03 ng/mL on one or more of the serial draws. Of these 188 patients, 98.4% (185/188) 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.[23,24] 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.

PROCEDURAL NOTES

LIMITATIONS

This product is for use on UniCel DxI Access Immunoassay Systems only. It is not compatible with Access 2 Immunoassay Systems.

1. Ambient laboratory temperature should be maintained between 18°C and 28°C (64.4°F and 82.4°F) while conducting patient sample testing. This assay employs an algorithm to correct for laboratory temperature fluctuations that could impact the accuracy of troponin test results. Up to 10% residual systematic bias may be observed when comparing patient results obtained at 18°C and 28°C (64.4°F and 82.4°F).
2. The reportable measuring range of the assay is defined as the range from 20% CV Limit of Quantitation (LoQ) to the S5 calibrator, 0.03 to ~80 ng/mL (µg/L). Values outside of this range should be reported as < 0.03 ng/mL or > S5 calibrator (~80 ng/mL), respectively. Do not dilute patient samples as this could lead to lower than expected troponin results.
3. 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 antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[25,26] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
4. 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.[27,28] Fibrinolytic agents activate proteases that may influence protein measurements, including troponin.[29] Carefully evaluate the results of patients suspected of having these types of interferences.
5. The thermal profile of 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.
6. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[16,17,18] Following blood collection tube manufacturers' specimen collection and handling recommendations are essential to reduce preanalytical errors.
7. The Access AccuTnI+3 assay is not intended to be used in isolation. Results should be interpreted in conjunction with other diagnostic tests and clinical information. When serial samples are obtained and troponin is considered in the clinical context of each patient, acute events such as MI may be distinguished from other conditions causing myocardial injury.[12]
8. The Access AccuTnI+3 assay does not demonstrate any “hook” effect up to 2,000 ng/mL (µg/L).

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY

Performance characteristics data listed in the studies below were generated using UniCel DxI 800 Access Immunoassay Systems.

The Access AccuTnI+3 assay demonstrates clinically acceptable linearity throughout the analytical measuring range. Twelve studies, based on CLSI EP6-A,[30] were performed to determine linearity of the Access AccuTnI+3 assay. For each study, one high sample at or above the highest calibrator level and one low sample approximately at the Limit of Detection (LoD) were mixed to make seven evenly distributed sample concentrations. Four replicates of the seven mixed samples, eight replicates of the low sample and eight replicates of the high sample were run on the UniCel DxI Access Immunoassay System. The Access AccuTnI+3 assay demonstrates linearity with a maximum deviation between a linear and non-linear fit of ≤ 15%.

IMPRECISION

This assay exhibits within laboratory imprecision of ≤ 8% at concentrations > 0.075 ng/mL (µg/L), and within laboratory Standard Deviation (SD) ≤ 0.006 ng/mL (µg/L) at concentrations ≤ 0.075 ng/mL (µg/L). Within laboratory imprecision includes both within run and between run imprecision. One study, based on CLSI EP5-A2[31] guidelines, provided the following data. This study used one UniCel DxI 800 Access Immunoassay System, one low spiked patient pool, three commercial controls and one high patient pool. These samples were tested in duplicate in two runs per shift, two shifts per day, over a total of 11 days, generating at least 40 independent runs.

Sample	Mean (ng/mL)	Within Run (%CV)	Between Run (%CV)	Within Laboratory Imprecision (%CV)
Low Spiked Patient Pool	0.05	5	7	8
Commercial Control 1	0.95	2	4	4
Commercial Control 2	3.19	2	6	6
Commercial Control 3	13.83	4	3	5
High Patient Pool	58.04	4	4	5

A separate study was performed to assess overall imprecision by incorporating additional variables than the study above. This overall imprecision study was conducted over 12 days, and included two UniCel DxI 800 Access Immunoassay Systems, calibrations, reagent lots, and fluctuations in laboratory temperature. Three patient pools were analyzed throughout this study.

Sample	Mean (ng/mL)	Within Laboratory Imprecision (SD, ng/mL)	Within Laboratory Imprecision (%CV)	Overall Imprecision (SD, ng/mL)	Overall imprecision (%CV)
Patient pool 1	0.04	0.003	6	0.005	12
Patient pool 2	0.42	0.017	4	0.029	7
Patient pool 3	0.98	0.036	4	0.067	7

ANALYTICAL SPECIFICITY / INTERFERENCES

The following potential interfering substances were added to lithium heparin plasma pools at three concentrations of cTnI (< 0.03 ng/mL, and approximately 0.05 ng/mL and 0.50 ng/mL). Additionally, five UniCel DxI 800 Access Immunoassay Systems were used in total and each substance was tested at two concentrations. Values were calculated as described in CLSI EP7-A2.[32] Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added) on a single instrument. For cTnI samples ~0.50 ng/mL, the difference between the control and test samples was ≤ 10%. For cTnI samples ~0.05 ng/mL, the difference between the control

and test samples was ≤ 0.006 ng/mL. For cTnI samples < 0.03 ng/mL, the control and test samples were ≤ 0.03 ng/mL. At the highest concentrations listed below, no interference was observed.

Substance	Highest Concentration Added		Substance	Highest Concentration Added
Acetaminophen	20 mg/dL		**Furosemide**	40 mg/dL
Acetylsalicylic Acid	65 mg/dL		**Hemoglobin**	5 mg/mL
Allopurinol	40 mg/dL		**Human Serum Albumin**	6,000 mg/dL
Ambroxol	40 mg/dL		**Ibuprofen**	50 mg/dL
Ampicillin	5 mg/dL		**Low MW Heparin**	28.8 U/mL
Ascorbic Acid	6 mg/dL		**Methyldopa**	2.5 mg/dL
Atenolol	1 mg/dL		**Nifedipine**	60 µg/mL
Bilirubin (conjugated)	40 mg/dL		**Nitrofurantoin**	6.4 mg/dL
Bilirubin (unconjugated)	40 mg/dL		**Nystatin**	2.15 mg/dL
Biotin	290 ng/mL		**Oxytetracycline**	24 mg/dL
Caffeine	10 mg/dL		**Phenytoin**	10 mg/dL
Captopril	5 mg/dL		**Propranolol**	500 µg/mL
Cinnarizine	40 mg/dL		**Quinidine**	2 mg/dL
Cocaine	2 mg/dL		**Simvastatin**	20 µg/mL
Diclofenac	5 mg/dL		**Theophylline**	25 mg/dL
Digoxin	200 ng/mL		**Triglycerides**	3,000 mg/dL
Dopamine	30 mg/dL		**Trimethoprim**	7.5 mg/dL
Erythromycin	20 mg/dL		**Verapamil**	16 mg/dL
Fibrinogen	1,000 mg/dL		**Warfarin**	30 µg/mL

To evaluate potential cross-reactivity of the assay with other myofibrillar proteins, the substances shown in the following table were added to two levels of cTnI human lithium heparin plasma samples and run on a UniCel DxI 800 Access Immunoassay System; five instruments were used in total. Values for cross-reactivity were calculated as described in CLSI EP7-A2.[32] No significant cross-reactivity was observed (< 1%).

Substance	Concentration Added (ng/mL)
Actin	1000
Cardiac troponin C	1000
Recombinant human CK-MB	1000
Myoglobin	1000
Myosin	1000
Recombinant human cTnT	250

Substance	Concentration Added (ng/mL)
Skeletal troponin I	1000
Tropomyosin	1000

LIMIT OF BLANK

The Access AccuTnI+3 assay has a Limit of Blank (LoB) of < 0.01 ng/mL (µg/L). One study using three UniCel DxI 800 Access Immunoassay Systems determined the LoB for Access AccuTnI+3 to be 0.004 ng/mL (µg/L). LoB was tested using a protocol based on CLSI EP17-A2.[33]

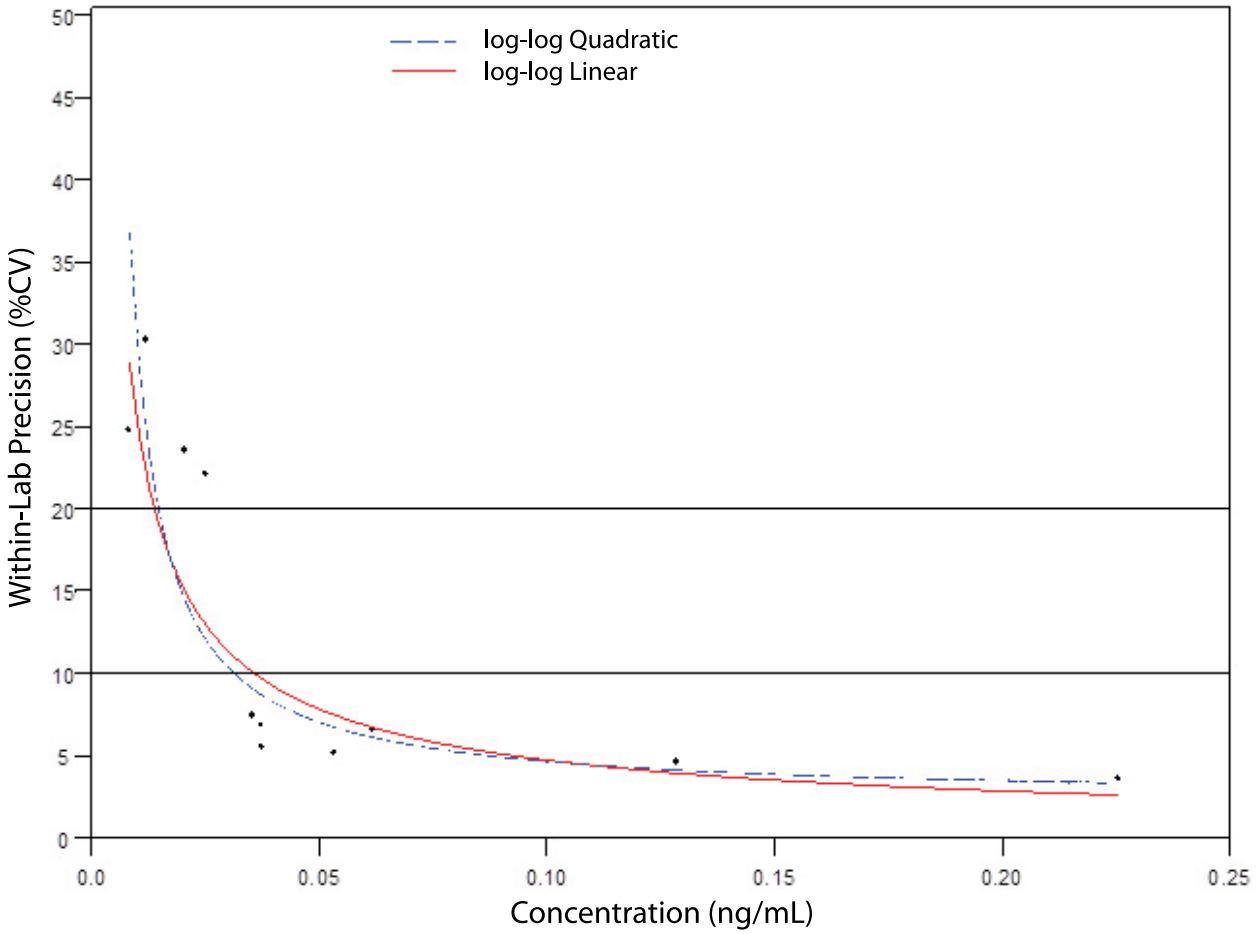
LIMIT OF DETECTION

The Access AccuTnI+3 assay has a Limit of Detection (LoD) of 0.01 ng/mL (µg/L). One study using three UniCel DxI 800 Access Immunoassay Systems determined the LoD for Access AccuTnI+3 to be 0.008 ng/mL (µg/L). LoD was tested using a protocol based on CLSI EP17-A2.[33]

LIMIT OF QUANTITATION

The Access AccuTnI+3 Limit of Quantitation (LoQ) is 0.04 ng/mL (µg/L) at 10%CV and 0.03 ng/mL (µg/L) at 20%CV. LoQ for Access AccuTnI+3 was determined using a protocol based on CLSI EP17-A2.[33] Multiple studies were completed using three UniCel DxI 800 Access Immunoassay Systems, and a minimum of 60 replicates of several low concentration native human cardiac troponin I samples were measured in each study. The expected imprecision in the clinically relevant concentration range was estimated by combining data from multiple studies to create a best fit regression describing the relationship of within laboratory %CV and troponin I concentration. LoQ regression estimates are measurements of central tendency as defined in CLSI EP17-A2.[33]

Limit of Quantitation (within laboratory imprecision estimate)



ADDITIONAL INFORMATION

REVISION HISTORY

Revision H

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

Revision J

Updated ProClin trademark statement.

SYMBOLS KEY

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

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