I. PURPOSE

Principles of the procedures
The Access AccuTnI assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with monoclonal anti-cTnI antibody conjugated to alkaline phosphatase and paramagnetic particles coated with monoclonal anti-cTnI antibody. 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 Lumi-Phos* 530 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.

Summary and Explanation
Coronary artery disease is not only a leading cause of death in men and women in the US, but is also associated with other life-threatening complications (1,2,3). The development of symptoms of coronary artery disease, which at times is unexpected and sudden, is associated with increased risk for adverse cardiac events such as death, myocardial infarction (MI), or hospitalization requiring urgent revascularization. Therefore, patients presenting with ischemic syndromes require prompt management. Specific and sensitive cardiac markers (troponin I and T) have been used in conjunction with other clinical findings and patient history information to better identify subjects with MI (1,2,4,5,6). These specific cardiac markers have also been used to identify those patients at higher risk for short- and long-term adverse cardiac events (endpoints/outcomes) (1,7,8,9,10).

Cardiac troponin I is a contractile protein exclusively present in the cardiac muscle (11,12). It is one of three subunits of the troponin complex (I, T, C), which with tropomyosin are bound to actin in the thin filament of the myofibril. cTnI is found as free troponin I (free TnI) and complexed with tropinin C (binary IC), with troponin T (binary IT) or with both troponin C and troponin T (ternary ITC). Its physiological role is to inhibit the ATPase activity of the actin-myosin complex in the absence of calcium, and therefore, to prevent muscular contraction (13). Three tissue isoforms have been identified:

- Fast troponin I and slow troponin I with molecular weights of 19,800 Da each, expressed in fast twitch and slow twitch skeletal muscle fibers, respectively.

- cTnI with a molecular weight of 24,000 Da contains an additional 31 amino acid residues in the N-terminal.

Sequencing of cTnI from mammals has shown important differences between the cardiac (14) and skeletal (15) forms. All three troponin I isoforms are encoded by different genes. The human cTnI exhibits only 52% and 54% amino acid sequence homology with the human fast and slow skeletal troponin I, respectively. The Access AccuTnI monoclonal antibody pair is selected to be cTnI specific. In addition, it has been well documented that skeletal muscle does not express cTnI, either during development or in response to stimuli (16). Therefore, the absolute cardiospecificity of cTnI allows distinction between cardiac and skeletal injuries, and allows diagnosis of myocardial infarction distinct from muscle lesions (rhabdomyolysis, polytraumatism) and non-cardiac surgery (16,17,18,19). Elevated troponin I levels have also been documented in cases of unstable angina (UA) (20) and congestive heart failure (CHF) (21).

cTnI levels in acute myocardial infarction (AMI) exhibit similar rise and fall patterns to those found in CK-MB. The collection of at least three blood samples during the early triage period has been recommended (22). cTnI is 13 times more abundant in the myocardium than CK-MB and does not normally circulate in the blood, so the signal to noise ratio is more favorable for the detection of myocardial necrosis (23). Cumulative data from several studies indicate troponin I levels are detectable (above quoted values for non-AMI samples) 3-6 hours after the onset of chest pain.
Troponin I levels peak at approximately 12-16 hours and can remain elevated for 4-9 days post-AMI. These same studies noted that the time to peak concentration of cTnI occurred later in patients who did not receive thrombolytic therapy (17,24,25).

Unstable angina comprises a broad spectrum of patients with varying levels of risk for suffering an adverse event such as death, MI, or other major cardiac complications requiring hospitalization and potentially urgent revascularization (UR). However, it has been reported that it is difficult to predict who will suffer an adverse event in those patients with UA and without evidence of ST segment elevation (NSTEMI) (1). The development and commercialization of the more specific and sensitive cardiac troponin I (cTnI) immunoassays have significantly contributed to the diagnosis of MI and to the risk stratification of patients with NSTEMI/UA. The 2000/2002 American College of Cardiology (ACC) and the American Heart Association (AHA) Guideline Update for the management of these patients strongly recommends to include cTnI measurements for the risk stratification of patients presenting with symptoms suggestive of acute coronary syndromes (2,6). In light of the potential adverse outcomes faced by these patients such as cardiac death or nonfatal ischemic events, an assessment of the prognosis should assist physicians in identifying and managing high risk patients. Ultimately, the assessment of the prognosis will be useful in both selecting the site of care and in identifying patients most likely to benefit from specific therapeutic interventions. The 2002 ACC and the European Society of Cardiology (ESC) guidelines recommend that individual laboratories define their own reference range and that an elevated value of cTnI be defined as a measurement above the 99th percentile of a normal control group (i.e. the 99th percentile upper reference limit) (4,5).

Recent studies have shown that the predominant cTnI form present in blood of patients after AMI is the binary troponin IC complex with smaller amounts of the ternary ITC complex, binary IT complex and free cTnI (26,27,28,29). The pattern of release of these forms over the course of AMI is still under investigation. The differential recognition of complexed and free cTnI forms is common for many commercial methods (26,30,31). For some assays, the relative responses to the various forms of cTnI are nearly equal, while other assays demonstrate a substantial difference. The latter may lead to over- and under-estimation of the true concentration of troponin I in a complex biological milieu. Equimolar binding, defined as the ability to recognize both the complexed and free cTnI forms equally, allows an unbiased determination of the total cTnI present in samples from the same subject over the course of AMI. The Access AccuTnI assay recognizes the binary troponin IC or IT or ternary troponin ITC complexes and free cTnI equally. In addition, the assay responds to both the phosphorylated and dephosphorylated forms of cTnI complex equally (32).

cTnI is highly susceptible to proteolysis and enzymatic modification. Substantial degradation occurs both in vivo and in vitro. The C-terminal of the molecule is preferentially cleaved then followed by the cleavage of the N-terminal (27,33,34). The Access AccuTnI assay utilizes monoclonal antibodies directed against the more stable region of the molecule, and is therefore less affected by degradation of cTnI.

II. POLICY/SCOPE
This procedure is intended for use by licensed CLS staff at Parnassus Chemistry section.

III. TEST AVAILABILITY
Test is available 24 hours a day, 7 days per week. Turnaround time: Stat: 1 hour, Routine: same or next day.

IV. SPECIMEN REQUIREMENTS
A. Lithium heparin plasma is the recommended sample. Serum and plasma (heparin) are acceptable samples. Heparin and serum samples should not be used interchangeably (22). The Access DxI System does not provide the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) are used in the AccuTnI assay. The AMI cutoff value presented in the Clinical Performance section applies to heparin...
plasma and serum samples. A study performed by Beckman Coulter, Inc. comparing EDTA plasma samples to heparin plasma samples produced the following correlation: \( y = 0.864x - 0.049, r = 0.999 \).

**B. Observe the following recommendations for handling, processing, and storing blood samples:**

1. Collect all blood samples observing routine precautions for venipuncture.
2. Allow samples to clot adequately before centrifugation.
3. Keep tubes stoppered at all times.
4. Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than two hours.
5. Samples should be centrifuged and refrigerated within two hours of blood draw.
6. Serum or plasma should be physically separated from contact with cells as soon as possible with a maximum time limit of two hours from the time of collection.
7. Remove any residual fibrin or cellular matter. Failure to do so can contribute to falsely elevated results.
8. 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.
9. 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.
10. If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C or colder.
11. Samples may be stored for six months at -20°C.

**C. Use the following guidelines when preparing specimens:**

1. Ensure residual fibrin and cellular matter has been removed prior to analysis.
2. Follow blood collection tube manufacturer’s recommendations for centrifugation.

**D. 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.**

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

**SAMPLE VOLUME**

The sample volume required to perform a single Troponin I (AccuTnI+3) test on the DxI 600 Immunoassay System varies depending on the type of sample container used. **When using the 0.5 mL pediatric insert cups (brown rack series), the minimum volume is 200 µl.** When using the aliquot tube from the power processor automation line (green rack series), the minimum volume is **300 µl** (600 µl including dead volume of the aliquot module).
V. EQUIPMENT, REAGENTS AND SUPPLIES for DxI 600 Immunoassay Analyzer

A. R1: Access AccuTnI Reagent Pack
Cat. No. A78803: 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.

1. 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.

2. R1b: 0.1 N NaOH.

3. R1c: TRIS buffered saline with surfactant, < 0.1% sodium azide and 0.1% ProClin 300.

4. 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.

B. Access AccuTnI Calibrators
Cat. No. 33345: S0-S5, 1 mL/vial

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 RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Provided ready to use. Freeze upon receipt at -20°C or colder. Mix contents thoroughly by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at -20°C or colder. After initial use, the thawed vials are stable at 2 to 10°C for 60 days. Label the vials with the date of thaw or the date of expiration. Return calibrators to 2 to 10°C after each use. Do not refreeze opened vials. Signs of possible deterioration are control values out of range. Refer to calibration card for exact concentrations.

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.

1. S0: Buffered bovine serum albumin (BSA) matrix with surfactant < 0.1% sodium azide, and 0.1% ProClin 300.

2. S1–S5: Recombinant troponin complex at cTnI levels of approximately 0.3, 1.2, 5.0, 25 and 100 ng/mL (µg/L) in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.

3. Calibration Card: 1

C. Access Substrate
Cat. No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability.
An increase in substrate background measurements may indicate instability.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unopened</td>
<td>2–8°C</td>
<td>Until expiration date stated on the label</td>
</tr>
<tr>
<td>Equilibration prior to use (unopened)</td>
<td>15–30°C (room temperature)</td>
<td>Minimum 18 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum 14 days</td>
</tr>
<tr>
<td>In use (opened)</td>
<td>External fluids tray</td>
<td>Maximum 14 days</td>
</tr>
<tr>
<td></td>
<td>substrate position</td>
<td></td>
</tr>
</tbody>
</table>

Refer to the appropriate system manuals and/or Help system detailed instructions.

R2 Access Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

D. UniCel DxI:
UniCel DxI Wash Buffer II, Cat. No. A16793, 1 x 10 L

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

Refer to the appropriate system manuals and/or Help system detailed instructions.

R3 Wash Buffer II: TRIS buffered saline, surfactant, < 0.1 % sodium azide, and 0.1% ProClin 300.

E. Quality Control

BioRad Liquichek Cardiac Markers Plus Control LT level 1 (REF 146)
BioRad Liquichek Cardiac Markers Plus Control level 2 (REF 182)
BioRad Liquichek Cardiac Markers Plus Control level 3 (REF 183)

Store frozen at -20 to -70°C. Stable until expiration date on bottle. Once thawed and opened, controls are stable for 8 days.

F. Access Sample Diluent A
Cat. No. 81908: 4 mL/vial

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the specific assay labeling under “Limitations of the Procedure” in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.
Access Sample Diluent A: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

G. Citranox and Contrad: Store at room temperature 15-30°C. Stable until expiration date on bottle. Diluted 1:5 Citranox solution is made using 1 part Citranox and 4 parts of DI water; stable for 21 days after preparation.

H. System Check Solution: Store refrigerated at 2-8 °C. Stable until expiration date on bottle at 2-8°C.

I. Reaction Vessels: Store at room temperature between 15 and 30°C. Stable until expiration date printed on box.

VI. WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use.

SAFETY PRECAUTIONS

1. CAUTION: Patient samples and blood-derived products may be routinely processed using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practice, regardless of their origin, treatment, or prior certification. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne pathogens. Biosafety level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

2. Citranox cleaning solution is acidic and may cause eye or skin irritation.

3. Contrad 70 cleaning solution is alkaline and may cause severe eye irritation.

4. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up (36).

5. Xi. Irritant: 0.5% ProClin 300.
   - R 43: May cause sensitization by skin contact.
   - S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

HANDLING PRECAUTIONS

1. Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate. Be especially careful not to contaminate the substrate when putting a new bottle into use.

VII. CALIBRATION/ CALIBRATION VERIFICATION

Run the Access AccuTnI S0 and S1 Calibrators in quadruplicate, and the S2–S5 calibrators in duplicate.

Note: TRPI assay should be calibrated every 30 days per UCSF protocol.
An active calibration curve is required for all tests. For the Access AccuTnI 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.
The Access AccuTnI Calibrators are provided at six levels - zero and approximately 0.3, 1.2, 5.0, 25 and 100 ng/mL. Assay calibration data are valid up to 56 days.

See the DxI Operations Guide for entering calibration test requests. Each level of calibrator is tested in duplicate. Once the Access DxI Total β-hCG (5th IS) calibration is accepted and stored, run all 3 levels of controls. Patient samples can be run if controls are within acceptable limits.

The Access DxI System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. A “Failed” message occurs when the calibration fails to meet a specification. Refer to the DxI Event Log and “Instructions for Use” manual for possible causes and corrective actions.

**AccuTnI Calibrators**: Provided ready to use. Freeze upon receipt at -20°C or colder. Mix contents **thoroughly** by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at -20°C or colder. After initial use, the thawed vials are stable at 2 to 10°C for 60 days. Label the vials with the date of thaw or the date of expiration. Return calibrators to 2 to 10°C after each use. Do not refreeze opened vials. Signs of possible deterioration are control values out of range. Refer to calibration card for exact concentrations.

**VIII. QUALITY CONTROL**

The recommended control requirement for an Access DxI AccuTnI+3 assay is a single sample of all Troponin I control levels tested on the day shift and one level of control once every 8 hours, thereafter. In addition, all 3 levels of controls are tested after calibration is performed:

- **Day Shift**: Levels 1, 2, 3
- **Evening Shift**: Level 2
- **Night Shift**: Level 3

**BioRad Liquichek Cardiac Markers Plus Control LT level 1** (REF 146)
**BioRad Liquichek Cardiac Markers Plus Control level 2** (REF 182)
**BioRad Liquichek Cardiac Markers Plus Control level 3** (REF 183)

Store frozen at -20 to -70°C. Stable until expiration date on bottle. Once thawed and opened, controls are stable for 8 days.

Verify that the controls are within acceptable ranges before testing patients. See QC chart or check in Sunquest.

When a control value is out of the specified range, it may indicate deterioration of the reagents, an instrument problem or errors in technique. Associated test results may be invalid and require retesting. Assay recalibration may be indicated. Refer to the DxI online Help or “Instructions for Use” for further troubleshooting information.

**IX. PROCEDURE**

**Important Note**: Samples will be run on aliquots. No direct tube sampling from the automation line. Inspect samples for clots, fibrin, particulate matter, and other debris prior to processing them on an analyzer. If any of these are present, centrifuge the specimen for a second time before testing.

A. Refer to the DxI General Operation Guide and “Instructions for Use” for Daily Setup and details on ordering and loading:
   - Daily Cleaning samples Contrad and 1:5 diluted Citranox
   - Calibrators (including new calibrator lots)
B. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

C. 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.

X. RESULTING/REPORTABLE RANGE

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data.

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.

Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 0.00 – 80.00 ng/mL [µg/L]).

REPORTING RESULTS

Report values less than 0.02 µg/L as < 0.02 µg/L.

- If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.02 ng/mL [µg/L]).

- If a sample contains more than the stated value of the highest Access AccuTnI Calibrator (S5), report the result as greater than that value [i.e., ~ >80.00 ng/mL (µg/L)].

Note: Dilution can only be done if requested by the ordering physician and approved by the Medical Director or Laboratory Medicine Resident.

- Alternatively, make a 1:10 dilution using the Sample Diluent A. 50 µl of the patient sample and 450 µl of the Sample Diluent A OR 100 µl of the patient sample and 900 µl of the Sample Diluent A. Type in the dilution factor in the Dilution box when entering the test request. Manually order the TnIDx test. The system will automatically multiply the result by the dilution factor and report that value. See Type Dilution column on Sample Report. DO NOT multiply the result again by the dilution factor.

Reportable range is <0.02 to ~ >80.00 µg/L depending on the most recent calibration S5 calibrator value.

Sunquest will automatically append the following messages to all positive results:

Effective December 16, 2014, due to a change in troponin assay and instrumentation, troponin values in the range of 1.0 micrograms/L and above will read approximately 10 to 35% lower than in the past. This assay change does not affect the normal range cutoff of <0.05 micrograms/L.

Normal range (99th percentile): < 0.05 µg/L. Values are NOT comparable to troponin I test performed at MtZ (troponin I POC).
XI. NORMAL RANGE

Expected Values

The reference range <0.05 µg/L was verified by in-house testing of 57 volunteers in the UCSF Chemistry laboratory.

Troponin I concentrations were measured in human plasma (lithium heparin) samples collected from apparently healthy 28 males and 29 females using the Access AccuTnI+3 assay.

The coefficient of variation of the Beckman Coulter DxI assay at a level of 0.03 µg/L is ~10% which has been confirmed by in-house testing.

1. Myocardial infarction has been redefined by the National Academy of Clinical Biochemistry (NACB) and the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee (4,5,22). These organizations recommend the use of biochemical markers in conjunction with the 97.5th percentile (NACB) or 99th percentile (ESC/ACC) reference ranges to aid in the diagnosis of myocardial infarction and cardiac damage.

2. The 97.5th and 99th percentiles (upper reference limit) as determined using lithium heparin plasma samples for a population of apparently healthy adults with no known cardiac disease are shown in the following table:

<table>
<thead>
<tr>
<th>n</th>
<th>Age Range</th>
<th>97.5th percentile</th>
<th>99th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>254</td>
<td>19–88</td>
<td>0.03 ng/mL (95% CI at this concentration is 0.02–0.04)</td>
<td>0.04 ng/mL (95% CI at this concentration is 0.03–0.05)</td>
</tr>
</tbody>
</table>

3. World Health Organization (WHO) (40) requires two of the following criteria for confirmation of AMI: evolutionary changes in the ECG or history of chest pain coupled with elevated cardiac enzymes. After a myocardial infarction, cTnI concentrations are detectable 3-6 hours after the onset of chest pain, peak at approximately 12-16 hours, and can remain elevated for 4-9 days post-AMI.

4. Any condition resulting in myocardial injury can potentially elevate cTnI levels above the expected normal range. Clinical studies have documented these conditions to include stable or unstable angina pectoris, congestive heart failure, myocarditis, and cardiac surgery or invasive testing (4,5,16,41,42).
5. Non-atherosclerotic mechanisms such as arteritis, coronary artery dissection, coronary embolism, and cocaine or amphetamine use may be responsible for acute ischemic syndrome potentially leading to cTnI levels above the reference range for healthy individuals (43,44,45).

Panic Range ≥ 0.05 µg/L  
Note: The first elevated troponin for a patient will be called and appended with an appropriate ETC “RPTC” Reported with read back confirmation with the name of the person taking the result, their phone number, your initials, the time and date of the call. Subsequent elevated troponins for the same patient in the next 72 hours after the initial report will not be called and ETC “PVNC” Panic Value not called per policy will be appended.

XII. LIMITATIONS OF PROCEDURE

1. The Access AccuTnI results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, clinical examination, electrocardiogram (ECG), data from additional tests, and other appropriate information. Medical decisions should not be based on a single AccuTnI determination at one time point (22).

XIII. SENSITIVITY/SPECIFICITY/INTERFERENCE

1. 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. (47,48)

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

2. 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.(38,39) Carefully evaluate the results of patients suspected of having these types of interferences.

3. Samples containing up to 40 mg/dL bilirubin (conjugated), 1000 mg/dL fibrinogen, 1000 mg/dL Triolein (triglycerides), 500 mg/dL hemoglobin, or 6000 mg/dL of human serum albumin do not affect the concentration of cTnI assayed. All cTnI values obtained in the presence of each interferent were ± 10% of the control.

4. The lowest detectable level of cTnI distinguishable from zero (Access AccuTnI Calibrator S0) with 95% confidence is 0.01 ng/mL (µg/L).

5. The Access AccuTnI assay does not demonstrate any "hook" effect up to 1,920 ng/mL (µg/L).

6. The following table describes the cross-reactivity of the assay with other myofibrillar proteins. Each of the potential cross reactants was added to a lithium heparin plasma pool containing approximately 2 ng/mL purified cTnI complex and assayed in replicates of 10. This study was based on CLSI EP7-P (49).
<table>
<thead>
<tr>
<th>Substance</th>
<th>Analyte Added (ng/mL)</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal troponin I</td>
<td>1000</td>
<td>0.034</td>
</tr>
<tr>
<td>Cardiac troponin C</td>
<td>1000</td>
<td>-0.002</td>
</tr>
<tr>
<td>Recombinant human cardiac troponin T</td>
<td>1000</td>
<td>-0.004</td>
</tr>
<tr>
<td>Actin (rabbit)</td>
<td>1000</td>
<td>-0.003</td>
</tr>
<tr>
<td>Myosin</td>
<td>1000</td>
<td>0.001</td>
</tr>
<tr>
<td>Tropomyosin (rabbit)</td>
<td>1000</td>
<td>-0.008</td>
</tr>
<tr>
<td>Human CK-MB</td>
<td>1000</td>
<td>-0.001</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1000</td>
<td>-0.002</td>
</tr>
</tbody>
</table>

XIV. ALTERNATE METHODS

Use the Backup Dxl 600.

XV. REFERENCES


