


Abbott i-STAT 1™ Troponin I METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The i-STAT Troponin I test is an in vitro diagnostic test for the quantitative measurement of Troponin I (TnI) in whole blood or plasma samples using heparin as the anticoagulant. TnI measurements can be used as an aid in the diagnosis and assessment of the severity of congestive heart failure.

The cartridge is to be used with the i-STAT 1 Analyser bearing the  symbol, but not with the i-STAT Portable Clinical Analyser or the Philips Medical Systems (formerly Agilent Technologies) Blood Analysis Module (BAM). As part of the i-STAT System, the TnI test is to be used by trained health care professionals in accordance with a facility's policies and procedures.

2. HAZARDS

Patient Samples

All patient samples should be treated as potentially infectious and handled appropriately. Some quality control solutions contain human source material. Personal Protective Equipment should be worn when processing samples, quality control testing and maintenance procedures.

3. CLINICAL SIGNIFICANCE

Acute Coronary Syndrome (ACS) is a term used to describe a group of conditions resulting from insufficient blood flow to the heart muscle.¹ These conditions range from atypical chest discomfort and non specific electrocardiographic changes to a large ST-segment elevation, myocardial infarction and cardiogenic shock.² Symptoms can include chest pain including tightness and heaviness in the chest, discomfort in the arms and upper body, shortness of breath and other constitutional symptoms including sweating, nausea and light-headedness.¹

Diagnosis of ACS is based on a complete medical history, physical examination, electrocardiogram to evaluate the electrical activity of the heart and blood tests to evaluate the presence of biological markers resulting from cardiac cell injury.¹

Troponin T & I are members of a group of cardiac regulatory proteins which function to regulate the calcium mediated interaction of muscle filaments actin and myosin resulting in contraction and relaxation of striated muscle.⁴ Troponin T is almost exclusive to the myocardium, with small amounts expressed in skeletal muscle not detectable in current Troponin T assays.⁴ Insufficient blood flow and oxygen supply to the heart muscle causes necrosis of the myocardium and subsequent release of Troponin T & I into the bloodstream.⁵

Troponin T in the bloodstream rises to detectable levels after 4-6 hours, peaks at 10-12 hours and can be detected for up to 14 days post infarction.^{5,6} Troponin I is released from necrotic cardiac myocytes into the bloodstream within hours (~4-8

hours) after the onset of chest pain. The peak TnI concentration is generally reached in 12-48 hours.⁷ Troponin I serum levels can remain elevated for up to 4–7 days.⁵

The diagnostic utility of Troponin T & I to detect myocardial necrosis and to enable risk stratification in patients with ACS is well established.^{5,8} Furthermore, the use of Troponin T as a prognostic indicator for recurrence of ischaemic events and death in ACS patients is increasing.^{5,9}

Results from PoCT devices measuring Troponin T & I should always be used in conjunction with clinical presentation, history and other diagnostic information.

4. TEST PRINCIPLE

The i-STAT cardiac Troponin I (cTnI) test cartridge uses a two-site enzyme-linked immunosorbant assay (ELISA) method. Antibodies specific for cTnI are located on an electrochemical sensor fabricated on a silicon chip. Also deposited in another location on the sensor silicon chip is an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the cTnI molecule. The whole blood or plasma sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The cTnI within the sample becomes labeled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during an incubation period of approximately seven minutes. The sample is washed off the sensors, as well as excess enzyme conjugate. Within the wash fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate releasing an electrochemically detectable product. The electrochemical (amperometric) sensor measures this enzyme product, which is proportional to the concentration of cTnI within the sample.

4.1 Analytical specificity

The cTnI method is specific for cardiac troponin I. The following muscle proteins were tested and found to have an insignificant effect on the measured cTnI.

Crossreactant	Concentration	Percent Crossreactivity
Troponin C (cardiac)	1000 ng/mL	<0.002%
Troponin T (cardiac)	1000 ng/mL	0.65%
Troponin I (skeletal)	1000 ng/mL	<0.002%
Troponin T (skeletal)	1000 ng/mL	<0.002%

4.2 Analytical sensitivity

The analytical sensitivity of the cTnI method is 0.02 ng/mL, which is the lowest cTnI level that can be distinguished from zero.

4.3 Interference

The following substances were found to have no significant effect (less than 10%) on the cTnI method, when added to a plasma pool containing approximately 2 ng/mL of cardiac troponin I, at the concentrations indicated

Compound	Test Level ($\mu\text{mol/L}$ unless otherwise indicated)
Acetaminophen	1660
Allopurinol	294
Ascorbic Acid	227
Acetyl Salicylic Acid	3330
Atenolol	37.6
Caffeine	308
Captopril	23
Chloramphenicol	155
Diclofenac	169
Digoxin	6.15
Dopamine	5.87
Enalaprilat	0.86
Erythromycin	81.6
Furosemide	181
Sodium Heparin*	36 U/mL
Ibuprofen	2425
Isosorbide dinitrate	636
Methyldopa	71
Nicotine	6.2
Nifedipine	1.156
Phenytoin	198
Propranolol	7.71
Salicylic Acid	4340
Theophylline	222
Verapamil	4.4
Warfarin	64.9

4.4 Limitations of the test

- Samples from patients who have been exposed to animals or who have received therapeutic or diagnostic procedures employing immunoglobulin's or reagents derived from immunoglobulin's may contain antibodies, e.g. HAMA or other heterophile antibodies, which may interfere with immunoassays and produce erroneous results.
- The generation of potentially interfering antibodies in response to bacterial infections has been reported. While this product contains reagents that minimize the effect of these interferents, and QC algorithms designed to detect their effects, the possibility of interference causing erroneous results should be evaluated carefully in cases where there are inconsistencies in the clinical information.
- Partially clotted samples can result in elevated cTnI results above the reference range, as well as quality check codes. To prevent this from occurring, upon drawing the whole blood sample into a heparinised collection tube, the sample should be inverted gently at least 10 times.
- Grossly hemolyzed samples can cause a decreased alkaline phosphatase activity, resulting in decreased detection of cTnI, increased assay backgrounds, and/or quality check codes.
- Heparin at 90U/mL was found to decrease the cTnI level by approximately 20%.
- Hematocrits in the range of 0-65 %PCV have been demonstrated not to affect

results. Samples with hematocrit levels above this range have demonstrated increases in the test imprecision and quality check codes.

4.5 Accuracy

Method comparison data were collected using CLSI guideline EP9-A2. Venous blood samples were collected in heparinized evacuated tubes and analyzed in duplicate on the i-STAT System. A portion of the specimen was centrifuged and the separated plasma was analyzed in duplicate on the comparative method within 1 hour of collection. Deming regression analysis was performed on the first replicate of each sample. In the method comparison table, n is the number of specimens in the first data set, Sxx and Syy refer to estimates of imprecision based on the duplicates of the comparative and the i-STAT methods respectively. Sy.x is the standard error of the estimate, and r is the correlation coefficient.

	Dade Behring Stratus® CS
n	189
Sxx	0.28
Syy	0.31
Slope	0.883
Int't	0.029
Sy.x	1.40
Xmin	0.00
Xmax	46.27
r	0.975

4.6 Precision

Precision data were collected in multiple sites as follows: Duplicates of each control were tested daily for a period of 20 days, resulting in a total of 40 replicates.

Precision Data (ng/mL)

Control	Mean	SD	%CV
Level 1	0.53	0.04	7.8
Level 2	2.17	0.18	8.5
Level 3	31.82	2.42	7.6

5. INSTRUMENT



Product specifications

5.1 Operating Conditions and Technical Data

Temperature range	16° – 30°C
Relative humidity	Up to 90 % (non-condensing)
Maximum altitude	Not stated
Position	Place the meter on a level, vibration-free surface with display facing up including when it is in downloader. Place the meter away from devices that gives off heat and away from direct sunlight
Reportable Range	0.00 – 50.00 ng/mL. Samples above the reportable range will yield >50.00 ng/mL” on the analyser display screen.
Sample size	Minimum 17µL
Test time	10 minutes
Memory	4000 patient results and 1000 control results stored on analyser
Barcode scanner	Yes - Laser Diode 650 nm Maximum Output 1.0 mW.
Connectivity	Yes
Battery operation	2 x 9V lithium batteries or Rechargeable power pack
Mains connection	Yes - via downloader
Number of tests with fully charged battery	Not stated
Safely class	Not stated
Automatic power-off	Yes
Dimensions	230 x 80 x 70 mm
Weight	650g

5.2 Storage and transport conditions

Temperature range Meter (In original container)	-10° to +46°C
Relative humidity	Up to 90% (non-condensing)

6. SPECIMEN REQUIREMENTS


6.1 Sample Material

Venous or arterial whole blood collected in a plastic syringe containing sodium or lithium heparin or without anticoagulant if tested within 1 minute of patient draw. Plastic evacuated tube containing sodium or lithium heparin, without clot activators or serum separators is acceptable. Device used to transfer sample to cartridge must be plastic. Blood collection tubes containing EDTA, oxalate, citrate, or other additives are not recommended. Performance characteristics have not been established for samples taken from capillary tubes and direct skin punctures (e.g. fingersticks) so these sample types should not be used with the TnI cartridge.

Venepuncture (see suitable anticoagulants above)

- Skin surface must be cleaned with an alcohol swab and dried well prior to collection to ensure there are no substances on the skin surface
- Collection tubes must be filled at least half full.
- Ensure sample is properly mixed and discard the first two drops of blood from a syringe before testing
- Test the sample within 30 minutes after collection

7. CARTRIDGES / REAGENTS

A single-use disposable cartridge contains microfabricated sensors, a calibrant solution, fluidics system, and a waste chamber. A whole blood sample of approximately 1 to 3 drops is dispensed into the cartridge sample well, and the sample well is sealed before inserting it into the analyser. The cTnI cartridges can only be used with the i-STAT 1 analyser bearing the  Symbol.

7.1 Storage and handling

- Cartridges are sealed in individual pouches or portion packs.
- Store the main supply of cartridges in the refrigerator at a temperature between 2 to 8°C. Do not allow cartridges to freeze.
- May be stored at room temperature (18 to 30°C) for up to 2 weeks (the time frame indicated on the cartridge box).
- Should not be returned to the refrigerator once they have been at room temperature, and should not be exposed to temperatures above 30°C. Should not be used if the pouch has been punctured.
- Write the date on the cartridge box or individual cartridge pouches to indicate the room temperature expiration date.
- Cartridges should remain in pouches until time of use.
- Do not use after the labeled expiration date.
- The cartridge must sit at room temperature for 5 minutes before use.
- An entire box of cartridges should stand at room temperature for 1 hour before use.
- Handle the cartridge from its edges and avoid touching the contact pads or exerting pressure over the center of the cartridge.

8. CALIBRATION

Calibration is set at the factory during manufacturing and may be updated as necessary as part of regular software upgrades.

9. QUALITY CONTROL

Quality control material (perform as per your organisation protocol)

Accurately testing known levels of cTnI ensures that the system and your technique used in testing give accurate results on patient tests. The control solutions have defined (known) values. The results for these solutions must first fall within a certain acceptable range in order to allow valid patient testing.

A quality control test should be performed every time a new shipment of test cartridges are received, when a new lot number of cartridges are used, if the clinical picture does not correlate with the patient test results, after major maintenance, and at a minimum of once a month.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparison is encouraged.

The Abbott iSTAT 1 Analyser uses the following methods for quality:

- Electronic Stimulator Check (Electronic QC)
- Control solutions

9.1 Electronic Control

Perform electronic simulator check on analyser once per day. The Electronic Simulator is inserted into the cartridge port of the analyser to verify electrical measurement. A PASS/FAIL message indicates whether the analyser's measurements are within specification.

If a FAIL message appears in the display window on the analyser, repeat the test. If the instrument still fails, document the problem on the Instrument QC/Event Log and contact the Manufacturer for assistance. Do not use the device.

The Electronic Simulator should be stored at room temperature and protect contact pads from contamination by replacing the plastic cap and placing the Electronic Simulator in its protective case after use. To perform an electronic simulator check:

- Turn the analyser on.
- Press the Menu key to access the Administration Menu.
- Press the 3 key for Quality Tests
- Press the 4 key for Simulator.
- Scan or enter Operator ID.
- Enter the Simulator ID (serial number).
- Insert the simulator into the cartridge port.
- View results on analyser's screen.
- If PASS is displayed, continue to use the analyser.

- If FAIL is displayed for the external simulator, reinsert the simulator.
- If FAIL is displayed a second time, do not use the analyser and contact your Support Services representative.

9.2 Running control solutions

The control solutions has three levels:

- iSTAT cTnI quality control, level 1
- iSTAT cTnI quality control, level 2
- iSTAT cTnI quality control, level 3

The controls are ready-to-use liquid controls requiring no reconstitution or frozen storage. The controls are stable until the expiration date on the vial label when stored unopened at 2-8°C. Once opened, these controls are stable for 30 days when stored tightly capped at 2-8°C.

Preparing the meter

- Turn on the meter by pressing the 'Power' button once
- Press the Menu key to access the Administration Menu
- Press the 3 key for Quality Tests.
- Press the 1 key for Control.
- Press the 1 key for i-STAT Cartridge. Scan or Enter Operator ID. Repeat if prompted.
- Scan or Enter Patient ID. Repeat if prompted.
- Scan the Cartridge Lot number from the cartridge pouch by pressing and holding the "Scan" button.
- To scan a barcode, align the red laser light so it covers the entire barcode. The device will beep when it reads the barcode successfully
- The meter will now prompt you to insert the cartridge.

Applying the control solution

- The individual unopened cartridge pouch should sit at room temperature for 5 minutes before use
- Remove cartridge from the pouch. Handle the cartridge from its edges. Avoid touching the contact pads or exerting pressure over the center of the cartridge.
- Discard the first drop from the syringe to clear unseen bubbles.
- Touch the next drop to the well allowing cartridge to draw sample in. DO NOT use a needle. Fill only to the "RED FILL LINE" diagram.
- Close the cTnI cartridge by using the thumb and index finger of one hand to grasp the cartridge from its side edges away from the sample inlet. Use the thumb of the other hand to slide the plastic closure clip to the right until it locks into place over the sample well.

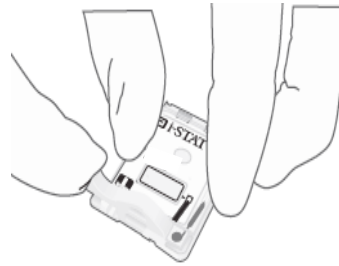
NOTE: When sliding the closure clip, do not place fingers near the sample well.

Inserting the cartridge into the meter

- To insert the cartridge into the cartridge port, grasp the cartridge by the thumb recess.
- Hold the device by the other hand and gently guide the cartridge into the analyser until fully inserted and it clicks into place.
- The analyser must remain on a level surface with the display facing up during

testing.

NOTE: Do not remove the cartridge while 'Cartridge Locked' message is displayed on the screen.



Results

- The countdown will begin as the sample is analysed.
- The results will then be displayed on the screen.
- Compare results to the value assignment sheet ranges. If results are within the expected ranges, use the cartridges as needed. Record your results.
- Remove the cartridge after the 'Cartridge Locked' message disappears. The analyser is ready for the next test immediately.

Remedial Action

If any results are outside the published expected ranges: DO NOT USE cartridges from the suspect lot. Quarantine the suspect lot. Notify the i-STAT System Coordinator immediately. Record the QC failure in the i-STAT QC Action Log along with the action taken.




10. TEST PROCEDURE

Before testing cTnI cartridges on the i-STAT 1 Analyser, the analyser must be customized. See full i-STAT instructions for procedure.

10.1 Performing the Test

Allow the individual unopened cartridge pouch to sit at room temperature for 5 minutes before use

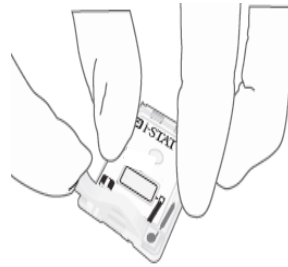
Preparing the meter

- Press  to turn on the device
- The analyser performs a self-check
- Press  to select 'iSTAT Cartridge'
- Scan or Enter Operator ID. Repeat if prompted.
- Scan or Enter Patient ID. Repeat if prompted.
- Scan the lot number on the cartridge pouch
- Position barcode 10-15 cm from the scanner window
- Press and hold  to activate the scanner
- Align the red laser light so it covers the entire barcode. The device will beep when it reads the barcode successfully
- The meter will now prompt you to insert the cartridge.

Applying the sample

- Handle the cartridge from its edges and avoid touching the contact pads or exerting pressure over the center of the cartridge.
- Following thorough mixing of the sample, discard 1 drop from the delivery device to clear unseen bubbles.
- Touch the next drop to the well allowing cartridge to draw sample in. DO NOT use a needle. Fill only to the "RED FILL LINE" diagram.
- Close the cartridge by using the thumb and index finger of one hand to grasp the cartridge from its side edges away from the sample inlet. Use the thumb of the other hand to slide the plastic closure clip to the right until it locks into place over the sample well.

NOTE: When sliding the closure clip, do not place fingers near the sample well.



Inserting the cartridge

- To insert the cartridge into the cartridge port, grasp the cartridge by the thumb recess. Hold the device by the other hand and gently guide the cartridge into the analyser until fully inserted and clicks into place.
- The analyser must remain on a level surface with the display facing up during testing.

NOTE: Do not remove the cartridge while 'Cartridge Locked' message is displayed on the screen.



Results

- The countdown will begin as the sample is analysed.
- The results will then be displayed on the screen.
- Record your results.
- Remove the cartridge after the 'Cartridge Locked' message disappears. The analyser is ready for the next test immediately.

11. RESULTS

- The i-STAT analyser contains a microprocessor that performs all calculations required for reporting results.
- Results are displayed numerically with their units.
- Results can be printed to the HP Portable Printer or the Martel Portable Printer with or without Downloader / Recharger. See printer manufacturers instructions
NOTE: Results printed on thermal paper will fade with time and are therefore not acceptable as a permanent chartable record.
- Results can be transmitted from the i-STAT Portable Clinical Analyser to the Central Data Station/Data Manager. See manufacturer's instructions

11.1 Interpretation of results

Collectively, the diagnosis of myocardial infarction should include measurement of cardiac related proteins and other clinical information including patient history and electrocardiographic data. Other conditions that may result in elevated cardiac proteins are: cardiac contusions, myocarditis, invasive examination of the heart, coronary artery bypass surgery, congestive heart failure and unstable angina. Therefore, these data must be considered when interpreting the results. Tnl is platform dependent so each site should establish a reference range that is representative of the patient population to be evaluated.

12. MAINTENANCE

- Clean the display and case with a gauze pad moistened with a mild non-abrasive cleaner, detergent, soap and water, alcohol or 10% bleach solution. Rinse with another pad moistened with water and dry.
- Use the electronic simulator daily.

13. REFERENCES

This method has been adapted from the iSTAT System – Abbott Point of Care Procedure Manual, cTnl test cartridge and control solution package inserts.

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