AIA-360 Assay Specifications
ST cTnI2 Test Code 093

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	0.5 ng/mL (example)
4	Cal 3	5 ng/mL (example)
5	Cal 4	15 ng/mL (example)
6	Cal 5	45 ng/mL (example)
7	Cal 6	120 ng/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	50
12	Dil. Vol	100
13	Assay L	0.06
14	Assay H	115
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	cTnI2
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	3
22	Calib. CV	30
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	1.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-900 Assay Specifications
ST cTnI2 Test Code 093

No.	Item	Data
1	Code	93
2	ACT	1
3	Analyte	cTnI2
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/mL
6	CAL 2	0.5 ng/mL (example)
7	CAL 3	5 ng/mL (example)
8	CAL 4	15 ng/mL (example)
9	CAL 5	45 ng/mL (example)
10	CAL 6	120 ng/mL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	2
15	Assay Low	0.06
16	Assay High	115
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.06
20	Reschedule High	115
21	Factor A	1
22	Factor B	0
23	Sample Volume	50
24	Diluent Volume	100
25	2 Step reagent dispensing volume	0
26	Calibration code	93
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	3
30	CAL. CV	30
31	DIL. SP1	1
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	1
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	93
39	DIL. NAME	cTnI2
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	0
42	PRE. 1VOL (Vol. of pretreatment sol-1)	0
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	0
44	PRE. CODE (pretreated sol. code)	0
45	PRE. NAME (pretreated sol. name)	0
46	Protocol	1
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST cTnI2 Test Code 093

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.06
6	Reschedule high	115
7	Assay range low	0.06
8	Assay range high	115
9	Specimen diluent code	93
10	Specimen diluent name	cTnI2
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	100
15	Default multiplier for >H	10
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	3
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	0.5 ng/mL (example)
24	Cal - 03	5 ng/mL (example)
25	Cal - 04	15 ng/mL (example)
26	Cal - 05	45 ng/mL (example)
27	Cal - 06	120 ng/mL (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	30
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	50
39	Diluent volume	100
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1
49	Calibration Code Check	093
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Cardiac Troponin I
ST AIA-PACK cTnI 2nd Gen

Name and Intended Use

ST AIA-PACK cTnI 2nd-Gen is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of cardiac troponin I (cTnI) in human heparinized plasma on specific Tosoh AIA System analyzers. Cardiac troponin I measurements are used as an aid in the diagnosis of acute myocardial infarction (AMI).

Summary and Explanation of Test

Troponin exists as a complex of three distinct proteins: troponin T, troponin C, and troponin I.[1] The troponins regulate the calcium-modulated interaction of actin and myosin in striated (skeletal and cardiac) muscle with troponin I acting as the actinomyosin ATPase inhibitor.

Three isoforms of troponin I have been identified[2] which are products of different genes with unique amino acid sequences.[3] Cardiac troponin I (cTnI) is found exclusively in cardiac tissue.[4] Though the three forms of troponin I share some homologous structure, cTnI has a sequence of 31 amino acid residues at the amino terminus of the molecule which renders cTnI distinctly antigenically different from the other isoforms of troponin I, as well as troponin C and troponin T.[5] Monoclonal antibodies developed to react with epitopes in this unique portion of the cTnI molecule have made possible the development of in vitro diagnostic assays with great specificity for cTnI.

Biochemical markers of myocardial injury are considered the "gold standard" for the diagnosis of AMI and are particularly important in the nondiagnostic EKG patients.[6, 7] Clinical investigations have shown elevated cTnI concentrations to be extraordinarily useful in aiding the diagnosis of cardiac specific injury in patients with acute myocardial infarction,8 myocardial contusion,[9] and unstable angina.[10] Following an AMI, cTnI is released into the bloodstream from the damaged heart muscle and within 4 - 8 hours, levels of cTnI increase above levels established for non-AMI specimens. Peak concentrations are generally reached 12 - 18 hours after the AMI onset and cTnI concentrations remain elevated for 5 - 10 days.[11, 12, 13]

Troponin I shows a temporal rise and fall in AMI patients similar to that seen with CKMB mass. However, recent studies indicate that because of its cardiac specificity, cTnI is much more useful than CKMB in detecting myocardial injury especially in the presence of skeletal muscle damage and lesions (rhabdomyolysis, polytraumatism).[3, 10, 14, 15]

Principle of the Assay

The ST AIA-PACK cTnI 2nd-Gen is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. Troponin I present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the troponin I concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve. ST AIA-PACK cTnI 2nd-Gen replaces the original AIA-PACK cTnI product and has been labeled as second generation to avoid any possible confusion.

Material Provided (ST AIA-PACK cTnI 2nd-Gen, Cat. No. 0025205)

5 trays x 20 test cups (ST AIA-PACK cTnI 2nd-Gen Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti- troponin I mouse monoclonal antibody and mouse monoclonal antibody (to human troponin I) conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform cardiac troponin I analysis using the ST AIA-PACK cTnI 2nd-Gen (Cat. No. 0025205) on specific Tosoh AIA Systems. They are available separately from Tosoh.

Materials		Cat. No.
AIA-SYSTEMS:		
AIA-360		0019945
AIA-900		0022930
AIA-900 9tray Sorter		0022931
AIA-900 19tray Sorter		0022932
AIA-2000 ST		0022100
AIA-2000 LA		0022101
AIA-PACK:		
AIA-PACK Substrate Set II		0020968
AIA-PACK Substrate/Reconstituent		
ST AIA-PACK cTnI2 Calibrator Set		0025305
Calibrator #1	0 ng/mL	
Calibrator #2	0.5 ng/mL (approx.)	
Calibrator #3	5 ng/mL (approx.)	
Calibrator #4	15 ng/mL (approx.)	
Calibrator #5	45 ng/mL (approx.)	
Calibrator #6	120 ng/mL (approx.)	
ST AIA-PACK cTnI2 Sample Diluting Solution		0025505
AIA-PACK Wash Concentrate Set		0020955
AIA-PACK Diluent Concentrate Set		0020956
AIA-PACK Detector Standardization Test Cups		0020970
AIA-PACK Sample Treatment Cups		0020971
Sample Cups		0018581
ADDITIONAL REQUIREMENTS: (Except AIA-360)		
Pipette Tips (1000/Pkg)		019215
Tip Rack (Empty)		019216
Preloaded Pipette Tips (96 Tips X 50 Racks)		996010
Preloaded Pipette Tips (96 Tips X 5 Racks)		996005

Except for QC materials, only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The ST AIA-PACK cTnI 2nd-Gen is intended for in vitro diagnostic use only.
- ℞ only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots should not be mixed within a tray.
- The ST AIA-PACK cTnI 2nd-Gen contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human blood is not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- Do not use beyond the expiration date.
- The ST AIA-PACK cTnI 2nd-Gen has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with troponin I concentrations between approximately 115 and 24,800 ng/mL will read > 115 ng/mL. The "hook effect" phenomenon may occur at troponin I concentrations > 24,800 ng/mL.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
Refrigerator Temperature (2 - 8 °C):	
ST AIA-PACK cTnI 2nd-Gen	0025205
AIA-PACK cTnI2 Calibrator Set	0025305
ST AIA-PACK cTnI2 Sample Diluting Solution	0025505
AIA-PACK Substrate Set II	0020968
AIA-PACK Wash Concentrate	0020955
AIA-PACK Diluent Concentrate	0020956

Room Temperature (18 - 25 °C):

AIA-PACK Detector Standardization Test Cups	0020970
AIA-PACK Sample Treatment Cups	0020971

ST AIA-PACK cTnI 2nd-Gen test cups may be stored for 24 hours at a room temperature of 18 - 25 °C. Calibrators must be kept tightly sealed and refrigerated at 2 - 8 °C. After opening, calibrators should be used within 24 hours. After opening, Sample Diluting Solution is stable for up to 30 days refrigerated at 2 - 8 °C. Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 2 - 8 °C. Working diluent and wash solutions are stable for 30 days at room temperature (18 - 25 °C). Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Heparinized plasma is required for the assay. Citrated plasma SHOULD NOT BE USED. No special patient preparation is necessary.

To use heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Samples may be stored at 2 - 8 °C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20 °C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18 - 25 °C) and mix gently.

Due to clinical procedures such as the administration of anti-thrombolytic agents to patients for whom this test may be requested, and the fact that this test is often ordered on a STAT basis, the possible presence of fibrin in the samples is a real concern. Since fibrin can cause either a false negative result by decreasing the actual sample used in the assay or a false positive result when fibrin clots formed during the assay prevent proper washing before final measurement, extreme care must be taken to assure that samples are free from fibrin. Sample filters are suggested.

The sample required for analysis is 50 µL.

Procedure

1) Reagent Preparation

1a) Substrate Solution

Bring all reagents to room temperature (18 - 25 °C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate; mix thoroughly to dissolve.

1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L. Prepare Wash Solution at least 24 hours prior to use with this assay.

1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L. Prepare Diluent Solution at least 24 hours prior to use with this assay.

2) Calibration

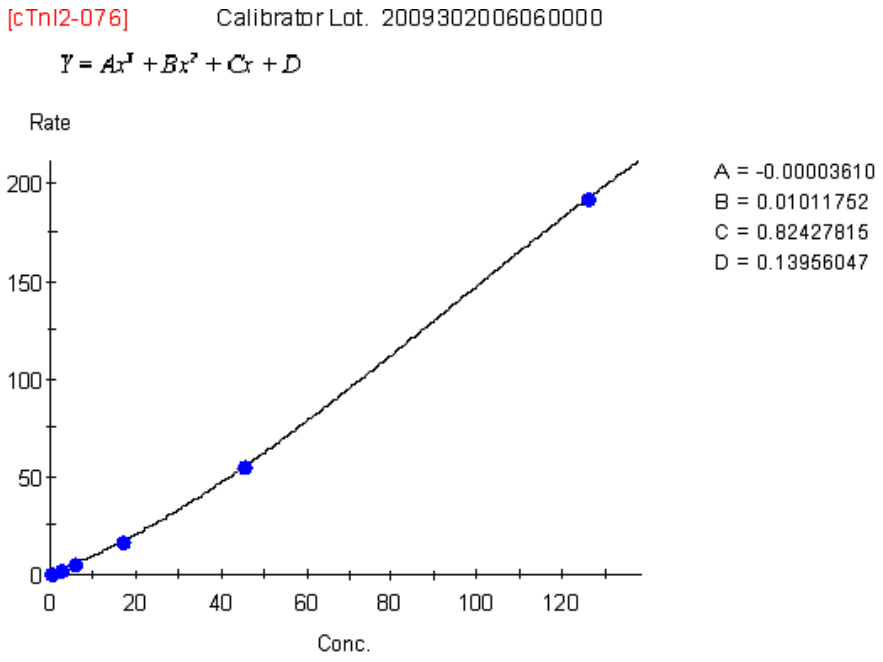
2a) Calibration Curve

The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

The calibration curve for the ST AIA-PACK cTnI 2nd-Gen is stable for up to 30 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change). For further information regarding instrument operation, consult the AIA System Operator's Manual.

The sample calibration curve below shows the algorithm used to calculate the results. This is an example only. Actual results will vary depending on the Instrument type and lot number used.



2b) Calibration Procedure

Refer to the appropriate AIA System Operator's Manual for procedural instructions.

i) Verify that the calibrator concentrations listed on the vial labels and the calibrator lot numbers have been correctly entered.

ii) The ST AIA-PACK cTnI 2nd-Gen CALIBRATOR (1) is provided ready for use.

iii) The ST AIA-PACK cTnI 2nd-Gen CALIBRATOR (2) - (6) are lyophilized. Lyophilized calibrators should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.

iv) Tosoh recommends that all calibrators be run in triplicate.

2c) Calibration Acceptability criteria

i) The mean rate for the zero calibrator should be $<$ 3.0 nM/sec.

ii) Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.

iii) The replicate values should be within a 10% range.

2d) Calibration Review and Acceptance

i) Using the criteria above, review the calibration curve carefully.

ii) Edit the calibration if necessary, then accept the calibration.

iii) For further information regarding calibration, consult the specific AIA System Operator's Manual.

3) Quality Control

3a) Commercially Available Controls

Commercially available controls should be run at least once per day. Tosoh does not provide quality control material for this assay. It is recommended that at least two (2) levels of controls, one near the AMI cutoff and one clearly elevated, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

3b) Quality Control Procedure

i) Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control material to be run with this assay is defined by individual laboratory policy.

4) Specimen Processing

4a) Preparation

Following specific instructions in the Operator's Manual for the analyzer, place samples on the instrument appropriately.

4b) Assay Procedure

i) Ensure a sufficient quantity of ST AIA-PACK cTnI 2nd-Gen test cups for the number of samples to be run.

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-900 and AIA-2000 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

Procedural Notes

1) Lyophilized Substrate must be completely dissolved.

2) Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.

3) If a specimen troponin I concentration is found to be greater than the linearity limit of the assay, 115 ng/mL; the specimen should be diluted with the ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for samples containing greater than 115 ng/mL is 1:5 or 1:10. It is desirable to dilute the sample so that the diluted sample reads between 2 and 115 ng/mL. The dilution factor should be entered into the software. For further information on specimen dilution, refer to AIA System Operator's Manual.

4) The AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK cTnI 2nd-Gen test cups can be used during the same run.

5) If the assay specifications for this test are not already in the system software, the specifications must be entered under Test Code 093.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to troponin I concentration in ng/mL.

For samples requiring dilution, the AIA instruments will automatically perform dilutions and calculate results if the dilution factors are entered into the software.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

- After calibration, two levels of controls are run in order to accept the calibration curve.
- The two levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve and the assay results before reporting patient values.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, therapy, etc.). Troponin I results should never be used as the single determining factor in treatment of the patient.

Physicians should be made aware that published literature indicates possible interference in troponin I assays - i.e. both false positive and false negative results.[16, 17] Studies on patients diagnosed with myocarditis and dilated cardiomyopathy indicate that disease specific cardiac autoantibodies may be present in the serum and plasma of these and other patients.18-20 Autoantibodies are generated against cardiac proteins and this could potentially cause false negative results if the autoantibodies produced interfere with the epitopes utilized in a particular manufacturer's assay.

Patient samples may also contain human anti-mouse antibody (HAMA) due to either natural antibody production or antibodies produced in response to therapy regimens. HAMA may cause either false positive or false negative results in assays using mouse monoclonal antibodies. Other heterophile antibodies are also known to interfere in assays of this type. ST AIA-PACK cTnI 2nd-Gen has been designed to minimize the effects of heterophile antibodies, but interference from high titers cannot be ruled out.

Results from this or any other in vitro diagnostic procedure which do not correlate with the clinical presentation of the patient should be interpreted with extreme caution.

The use of a single cTnI result on a patient should be discouraged. As with the other cardiac markers, a temporal pattern of rise and fall over time should be observed to aid in accurate interpretation of the results. Users are reminded that elevations in cTnI can occur for reasons other than myocardial infarction, and lack of a cTnI value over a certain value does not preclude AMI or serious cardiac injury. Assays from various manufacturers may yield different results.

Reactivity of the epitopes used in the assay vary with the form of cTnI present in a specific specimen. Also, assay calibration between manufacturers is not standardized.

Using ST AIA-PACK cTnI 2nd-Gen, the highest concentration of troponin I measurable without dilution is approximately 115 ng/mL, and the lowest measurable concentration is 0.06 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 115 ng/mL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 115 ng/mL.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Samples containing fibrin may exhibit either falsely elevated or falsely decreased results. Fibrin must be eliminated in the sample before assay begins.

No patients with skeletal muscle disorders were studied.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval corresponding to the characteristics of the population being tested and the sample type being used. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.[21] Troponin I results should be used in conjunction with the total clinical presentation of the patient. Protocol used for specimen collection intervals will have an effect on cut-off values since measurable Troponin I may not be detected until 4 – 6 hours after onset of symptoms in an AMI. Interpretation should be made in light of the time interval between symptoms and specimen collection.

Reference Ranges

To determine the expected values of the ST AIA-PACK cTnI 2nd-Gen in a healthy population, 40 apparently healthy male and female volunteers were tested. All samples tested gave results below the sensitivity limit of the assay.

In light of literature reports concerning false positive results due to rheumatoid factor, heparinized plasma from 10 patients with abnormal elevations in rheumatoid factor were tested. All results were less than the sensitivity limit of the assay.

Thirty-five hospitalized patients whose diagnosis did not include a cardiac component and who had elevated BUN and creatinine results (BUN 36 – 120 mg/dL; Creatinine 1.5 – 5.4 ng/mL) were evaluated with the ST AIA-PACK cTnI 2nd-Gen assay. All results were less than the minimal detectable concentration of the assay.

Each laboratory should establish its own diagnostic cut-off value based on the patient population and the collection protocol.

In our study, a group of hospitalized patients were divided into two cohorts, one (35 patients) having a clinical diagnosis of AMI and the other cohort (41 patients) a clinical diagnosis which excluded AMI. ROC curve analysis with the associated distribution of results in tabulated form are shown below in Figure 1 and Table 1 respectively. These data indicate that clinical sensitivity exceeds 90% with clinical specificity equal to 100% using 0.31 – 0.64 ng/mL as a range of values for AMI cutoff. These data represent one study and should only be used as a guide.

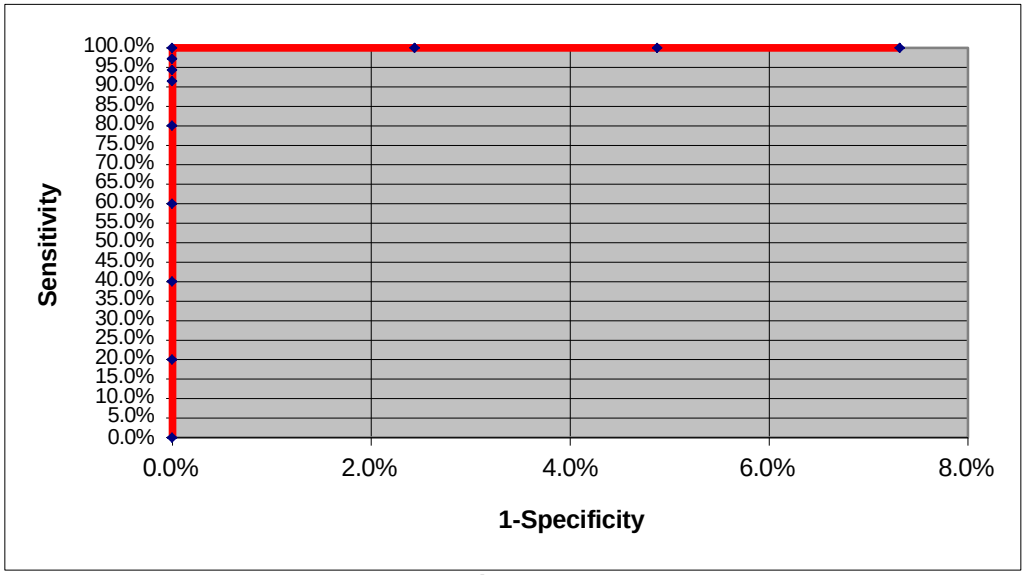


Figure 1

ng/mL	Sensitivity	Specificity	TP	TN	FP	FN
0	100.0%	92.7%	35	38	3	0
0.01	100.0%	95.1%	35	39	2	0
0.05	100.0%	97.6%	35	40	1	0
0.31	100.0%	100.0%	35	41	0	0
0.38	97.1%	100.0%	34	41	0	1
0.6	94.3%	100.0%	33	41	0	2
0.64	91.4%	100.0%	32	41	0	3
0.88	80.0%	100.0%	28	41	0	7
2.27	60.0%	100.0%	21	41	0	14
4.59	40.0%	100.0%	14	41	0	21
9.32	20.0%	100.0%	7	41	0	28
176.9	0.0%	100.0%	0	41	0	35

Table 1

Any injury causing myocardial cell damage can elevate the cTnI concentration above expected limits. Such an increase even above the diagnostic cut-off value does not necessarily indicate AMI. Patients presenting early in the AMI process may not demonstrate abnormal cTnI elevations for several hours.

Please review the Limitations of the Procedure before interpreting results from any cTnI assay.

Performance Characteristics

The following performance characteristics for recovery and dilution were determined using the Tosoh AIA NexIA Automated Immunoassay Analyzer.

1) Accuracy

1a) Recovery:

Three heparinized plasma samples were spiked with three different levels of troponin I and assayed before and after spiking.

Sample	Initial Value (ng/mL)	Troponin I Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Plasma A	0.00	2.98	2.98	3.11	104.5
	0.00	5.95	5.95	6.36	106.9
	0.00	11.90	11.90	12.70	106.7
Plasma B	0.00	5.73	5.73	5.50	96.1
	0.00	11.50	11.45	11.10	96.9
	0.00	22.90	22.90	22.40	97.8
Plasma C	0.00	9.40	9.40	9.05	96.3
	0.00	18.80	18.80	18.80	100.0
	0.00	37.60	37.60	38.60	102.7

1b) Dilution:

Three heparinized plasma samples with high concentrations of troponin I were serially diluted with AIA-PACK cTnI2 Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Plasma A	none		66.90	
	7.5/10	50.20	50.50	100.7
	5.0/10	33.50	33.90	101.2
	2.5/10	16.70	16.40	98.0
	1.0/10	6.69	6.43	96.0
Plasma B	none		51.20	
	7.5/10	38.40	38.50	100.3
	5.0/10	25.60	28.00	109.4
	2.5/10	12.80	13.60	105.9
	1.0/10	5.12	5.51	107.7
Plasma C	none		53.00	
	7.5/10	39.80	38.20	96.0
	5.0/10	26.50	24.80	93.5
	2.5/10	13.30	11.70	88.4
	1.0/10	5.30	4.75	89.5

1c) Comparative Analysis:

Seventy-six heparinized plasma samples were collected from hospitalized patients with a clinical diagnosis which either specifically ruled in or ruled out acute myocardial infarction based on WHO criteria and any other known cardiac involvement. Seventy-two other specimens from patients with a variety of conditions not specified were also tested. Results from the ST AIA-PACK cTnI 2nd-Gen assay (y) as measured on the AIA-600 II were compared to those obtained using an FDA cleared assay for Troponin I (x) on all 148 samples. One hundred and fifteen (115) samples were below the minimum detectable concentration of 0.06 ng/mL. Other samples ranged from 0.06 – 58.77 ng/mL.

Slope	**0.9129**
y-intercept	-0.2636 ng/mL
Correlation Coefficient	0.98
Range of Samples (y)	<0.06 – 58.77 ng/mL
Number of Samples (n)	148

Cut-off Comparison: A total of 148 sample pairs (126 different patients) were used to compare results judged as above or below the stated assay cut-off for AMI using the ST AIA-PACK cTnI 2nd-Gen and another FDA cleared cardiac Troponin I. To determine whether a result was positive or negative, a cut-off value of 0.6 ng/mL was used for the comparative assay according to its product labeling. Any result greater than 0.4 ng/mL on the ST AIA-PACK cTnI 2nd-Gen assay was considered above the cut-off.

	Dade		
Tosoh	**<=0.6**	**>0.6**	**Total**
<=0.4	116	4	120
>0.4	0	28	28
Total	116	32	148

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision coefficient of variation was evaluated in two plasma samples by 10 replicate determinations in five different lots of reagent on the AIA NexIA.

Rgt. Lot	Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
#9770	Plasma A Plasma B	10 10	4.63 26.54	0.07 0.58	1.56 2.18
#9772	Plasma A Plasma B	10 10	4.65 26.77	0.08 0.20	1.83 0.74
#9773	Plasma A Plasma B	10 10	4.55 25.72	0.07 0.64	1.47 2.49
#9774	Plasma A Plasma B	10 10	4.53 26.83	0.04 0.42	0.98 1.58
#9775	Plasma A Plasma B	10 10	4.55 26.54	0.05 0.54	1.17 2.03

2b) Inter-assay precision

The inter-assay (between run) precision coefficient of variation was evaluated at two different concentrations by analyzing control samples in 20 separate runs on the AIA NexIA.

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Plasma A Plasma B	20 20	6.42 32.48	0.18 0.67	2.86 2.06

Serial dilutions were assayed on the AIA-NexIA with ten replicates per dilution to determine the precision at the level of sensitivity (0.06 ng/mL) and at both extremes of the AMI cut-off interval.

Sample	Number of Replicates	Mean (ng/mL)	Coefficient of Variation (%)
Dilution A Dilution B Dilution C	10 10 10	0.062 0.302 0.630	8.5 5.2 2.4

Specificity

The following substances were tested for potential positive and negative interference by the in vitro addition of these drugs at a concentration greater than 10 times the upper therapeutic level to a human pool containing approximately 9 ng/mL troponin I. Studies were performed on the AIA-600 II. All values obtained were within 10% of the expected results indicating no significant interference from any of the drugs tested.

Acetylsalicylic acid (ASA)	Methyldopa
Allopurinol	Phenytoin
Atenolol	Propranolol
Captopril	Quinidine sulfate
Digoxin	Sodium Naprosyn
Furosemide	Thyroxine
Gemfibrozil	Zocor
Acetaminophen	Ibuprofen

Sensitivity

The minimal detectable concentration (MDC) of troponin I is estimated to be 0.06 ng/mL. The MDC is defined as that concentration of troponin I which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 20 replicate determinations of a zero calibrator. These studies were run on both the AIA NexIA and the AIA-600 II on multiple lots of reagent.

Interference

Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration. These studies were performed on the AIA NexIA.

- Added hemoglobin (up to 436 mg/dL), conjugated bilirubin (up to 18.6 mg/dL) and free bilirubin (up to 16.5 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 2000 mg/dL) does not interfere with the assay.
- Added albumin (up to 50 mg/dL) does not interfere with the assay.
- Added ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Added rheumatoid factor (up to 20 IU/mL) does not interfere with the assay.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

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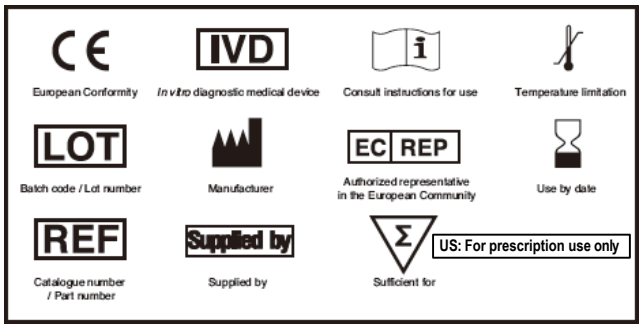


ST AIA-PACK cTnI 2nd-Gen Troponin I Calibrator Set

Intended Use

The ST AIA-PACK cTnI 2nd-Gen Calibrator Set is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK cTnI 2nd-Gen Assay.

Summary and Explanation

The ST AIA-PACK cTnI 2nd-Gen Calibrator Set contains buffered bovine serum albumin with assigned levels of cardiac troponin I. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Material Provided (Cat. No. 0025305)

2 x 1 mL	Calibrator No. 1	0	ng/mL

Buffered bovine serum albumin containing no detectable concentration of cardiac troponin I (0 ng cTnI/mL), and 0.1% sodium azide as preservative. (Ready to Use)

2 x 1 mL	Calibrator No. 2	0.5	ng/mL (approx.)
	No. 3	5.0	ng/mL (approx.)
	No. 4	15	ng/mL (approx.)
	No. 5	45	ng/mL (approx.)
	No. 6	120	ng/mL (approx.)

Buffered bovine serum albumin containing the assigned concentration of cardiac troponin I (described on each vial), and 0.1% sodium azide as a preservative. (Lyophilized)

Warnings and Precautions

- The ST AIA-PACK cTnI 2nd-Gen Calibrator Set is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human blood is not used for these calibrators, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

Using a volumetric pipette or a calibrated adjustable pipette reconstitute the lyophilized calibrators accurately with 1.0 mL of CAP Class I water or the clinical laboratory reagent water. Allow the lyophilized material to fully dissolve, then mix the calibrators gently but thoroughly prior to performing the calibration.

Bring calibrator to room temperature (18 - 25 °C) for use.

Always store the Calibrator Set in an upright position at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK cTnI 2nd-Gen Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours.

Procedure

Refer to the CALIBRATION PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The ST AIA-PACK cTnI 2nd-Gen Calibrator Set contains assigned concentrations of troponin I. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0 to 115 ng/mL of troponin I. The calibrators in this set are prepared gravimetrically and compared to internal reference standards.

Results

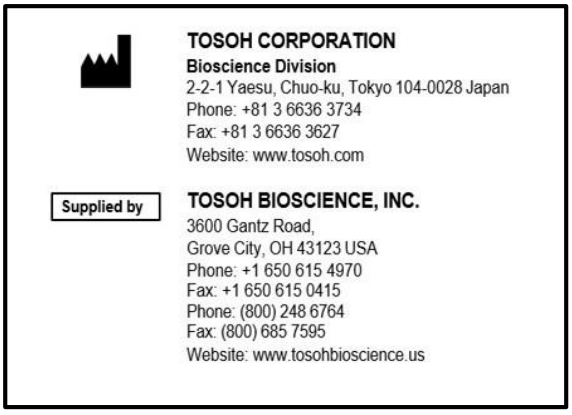
- The mean rate for the zero calibrator should be $<$ 3.0 nM/sec.
- Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
- The replicate values should be within a 10% range.

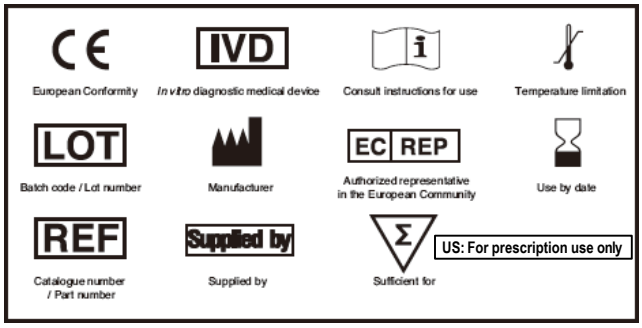
Limitations

The ST AIA-PACK cTnI 2nd-Gen Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.





AIA-PACK
cTnI 2nd-Gen Sample Diluting Solution

Intended Use

The ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of cardiac troponin I above the linear range of the assay.

Summary and Explanation

The ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution contains buffered bovine serum albumin with no detectable concentration of cardiac troponin I. This Sample Diluting Solution is to be used only with samples that are being tested for troponin I concentrations using the ST AIA-PACK cTnI 2nd-Gen assay.

Material Provided (Cat. No. 0025505)

4 x 4 mL	Sample Diluting Solution Buffered bovine serum albumin containing no detectable concentration of cardiac troponin I (0 ng cTnI /mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human blood is not used for ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution is provided ready to use.
- Always store in an upright position at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for up to 30 days when refrigerated at 2 - 8 °C.

Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen is found to contain greater than the linearity limit of approximately 115 ng/mL, the specimen should be diluted with the Sample Diluting Solution and assayed according to the Procedure in the AIA-PACK section of the analyte application.
2. The AIA 900 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for specimen containing greater than the concentration of the highest calibrator is 1:5 or 1:10. However, it is desirable to dilute so that the diluted sample reads between 2 and 115 ng/mL.

Results

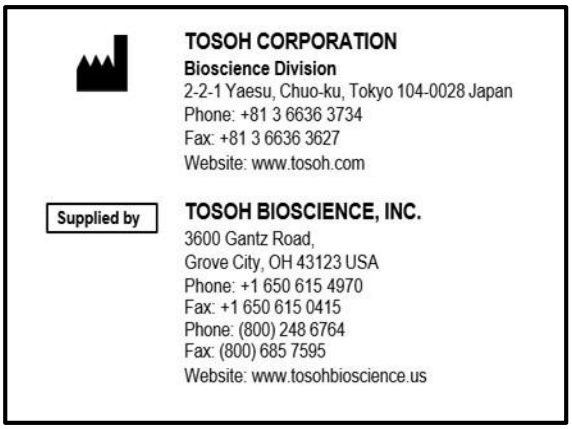
When an auto-dilution is performed, the AIA instrument will calculate the final result.

Limitations

The ST AIA-PACK cTnI 2nd-Gen Sample Diluting Solution is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.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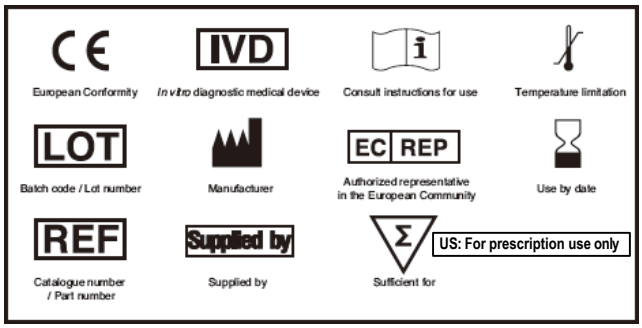
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