510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k172787

B. Purpose for Submission:

New device

C. Measurand:

Cardiac Troponin I

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

Access hsTnl

G. Regulatory Information:

1. Regulation section:

H. Intended Use:

Product Code	Classification	Regulation Section	Panel
MMI	Class II	21 CFR 862.1215 - Creatine phosphokinase/creatine kinase or isoenzymes test system	Clinical Chemistry (75)

1. Intended use(s):

See Indication(s) for use.

2. Indication(s) for use:

   Access hsTnI is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the Access 2 Immunoassay Systems to aid in the diagnosis of myocardial infarction (MI).

3. Special conditions for use statement(s):

   For prescription use; for *in vitro* diagnostic use.

4. Special instrument requirements:

   Performance data below was collected on the Access 2 Immunoassay System.

I. Device Description:

The Access hsTnI reagent packs contain specific reagents for the *in vitro* diagnostic measurement of cTnI including:

- R1a: Dynabeads paramagnetic particles coated with mouse monoclonal anti human cTnI antibody suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin 300.
- R1b: 0.1 N NaOH.
- R1c: TRIS buffered saline, surfactant, protein (mouse), < 0.1% sodium azide, and 0.1% ProClin 300.
- R1d: Sheep monoclonal anti human cTnI alkaline phosphatase conjugate diluted in ACES buffered saline, with surfactant, BSA matrix, protein (bovine, sheep, mouse), < 0.1% sodium azide, and 0.25% ProClin 300.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Access AccuTnI+3 Reagent and Access AccuTnI+3 Calibrators for use on the Access 2 Immunoassay System.

2. Predicate 510(k) number(s):

   k121214

3. Comparison with predicate:

Similarities		
Item	**Predicate Device: Access AccuTnI+3 (k121214)**	**Candidate Device: Access hsTnI**
Intended use/Indications for Use	Is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma to aid in the diagnosis of myocardial infarction.	Same.
Analyte	Cardiac troponin I	Same.
Assay Principle	Chemiluminescent sandwich assay	Same.
Specimen Type	Serum and heparinized plasma	Same.

Differences		
Item	**Predicate Device: Access AccuTnI+3 (k121214)**	**Candidate Device: Access hsTnI**
Primary reagent materials	Sold phase magnetic particles, sheep monoclonal anti-human cTnI antibodies	Dynabeads paramagnetic particles coated with mouse monoclonal anti-human cTnI antibody
Measuring Range	20 -100,000 pg/mL	2.0-27,027 pg/mL
Thermal susceptibility/Assay Protocol File (APF)	AccuTnI+3 reagent susceptible to thermal changes; APF includes thermal algorithm	hsTnI reagent not susceptible to thermal changes; hsTnI APF doesn't have thermal algorithm

K. Standard/Guidance Document Referenced (if applicable):

Clinical and Laboratory Standards Institute (CLSI) EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Third Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline—Second

Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

L. Test Principle:

The Access hsTnI assay is a sequential two-step immunoenzymatic ("sandwich") assay. Monoclonal anti cTnI antibody conjugated to alkaline phosphatase is added to a reaction vessel along with a surfactant containing buffer and sample. After a short incubation, paramagnetic particles coated with monoclonal anti cTnI antibody are added. The human cTnI binds to the anti cTnI antibody on the solid phase, while the anti cTnI antibody alkaline phosphatase conjugate reacts with different antigenic sites on the cTnI molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of cTnI in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The sponsor evaluated precision in several studies following the recommendations in CLSI EP5-A3.

   Multiple temperature (run and calibration) within laboratory study: This study evaluated precision of the proposed device under the following temperature conditions:

   Calibrating at 18°C and running samples at 18°C, 23°C and 28°C
   Calibrating at 23°C and running samples at 18°C, 23°C and 28°C
   Calibrating at 28°C and running samples at 18°C, 23°C and 28°C

   In total, nine different calibration temperature/run temperature conditions were evaluated for each sample.

For each set of temperature conditions, five serum and five lithium heparin plasma samples with TnI concentrations of approximately 10, 20, 100, 5,000, and 20,000 pg/mL were used in the determination of imprecision. The low sample concentrations (~10 and ~20 pg/mL) were chosen to represent patient samples with troponin levels near the 99th percentile cutoffs. The 10 and 20 pg/mL samples were prepared by pooling two to four individual patient samples with endogenous troponin concentrations near the targeted range. The higher concentration samples were prepared by spiking patient sample pools with natively sourced antigen.

Each sample was assayed in duplicate, in up to four runs per day, over at least 10 days for a total of 40 runs and 80 replicates for each sample at each of the nine combinations of temperature conditions noted above. This was repeated using three reagent lots and three instruments for each calibration/run temperature condition (instrument and lot were confounded in this study). One calibrator lot was used for the entire study.

The within-laboratory imprecision includes within-run, between-run, and between-day variance components. The total %CV estimates include the within-laboratory sources of variability (within-run, between-run, and between-day) as well as between-lot and between-instrument variability. The following results from one set of temperature conditions are representative of the results from this precision study:

Sample Type	N	Mean (pg/mL)	Within-run		Between-run		Between-Day		Within-Lab		Total	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Plasma 1	240	8.89	0.45	5.0%	0.29	3.3%	0.12	1.3%	0.70	7.8%	0.89	10.0%
Plasma 2	240	17.83	0.75	4.2%	0.12	0.7%	0.24	1.4%	0.81	4.6%	1.14	6.4%
Plasma 3	240	79.38	2.46	3.1%	1.04	1.3%	1.54	1.9%	2.77	3.5%	4.14	5.2%
Plasma 4	240	4540.39	165.26	3.6%	129.58	2.9%	143.6	3.2%	221.21	4.9%	337.16	7.4%
Plasma 5	240	16089.92	569.47	3.5%	302.64	1.9%	351.8	2.2%	733.54	4.6%	1038.14	6.5%
Serum 1	240	9.72	0.47	4.8%	0.27	2.8%	0.26	2.7%	0.73	7.5%	0.95	9.7%
Serum 2	240	11.96	0.48	4.0%	0.35	2.9%	0.02	0.1%	0.66	5.5%	0.89	7.4%
Serum 3	240	98.74	3.20	3.2%	1.28	1.3%	1.13	1.1%	2.69	2.7%	4.52	4.6%
Serum 4	240	3983.58	143.37	3.6%	68.53	1.7%	78.87	2.0%	180.36	4.5%	252.98	6.4%
Serum 5	240	18921.95	714.28	3.8%	247.92	1.3%	219.5	1.2%	1087.37	5.7%	1342.46	7.1%

A second within-laboratory precision study was performed at one external site under normal laboratory conditions. Four commercial controls (QC), five lithium heparin plasma sample pools, and five serum sample pools were run in duplicate, two runs per day for 20 days. This study was performed using one reagent lot and one instrument. Results for this study are shown below:

Sample	N	Mean (pg/mL)	Between-Day		Between-Run		Within-Run (Repeatability)		Within-Laboratory	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
Plasma 1	80	11.7	0.34	3.0	0.35	3.0	0.38	3.3	0.62	5.3
Plasma 2	80	29.8	0.00	0.0	0.56	1.9	0.93	3.1	1.09	3.6
Plasma 3	80	97.5	0.00	0.0	1.95	2.0	2.52	2.6	3.19	3.3
Plasma 4	80	19310.0	836.00	4.3	0.00	0.0	794.00	4.1	1153.00	6.0
Plasma 5	80	6.8	0.44	6.5	0.20	2.9	0.22	3.3	0.53	7.8
QC 1	79	19.2	0.75	3.9	0.37	1.9	0.70	3.6	1.09	5.7
QC 2	80	53.1	1.75	3.3	1.07	2.0	1.45	2.7	2.51	4.7
QC 3	80	1043.0	3.30	4.0	14.00	1.3	27.00	2.6	51.00	4.9
QC 4	80	14516.0	473.00	3.3	361.00	2.5	408.00	2.8	722.00	5.0
Serum 1	79*	12.0	0.29	2.4	0.36	3.0	0.24	2.0	0.52	4.4
Serum 2	80	27.9	0.00	0.0	0.61	2.2	0.87	3.1	1.06	3.8
Serum 3	80	100.7	0.43	0.4	0.85	0.8	3.18	3.2	3.32	3.3
Serum 4	80	16902.0	0.00	0.0	709.00	4.2	711.00	4.2	1004.00	5.9
Serum 5	80	7.3	0.44	6.1	0.40	5.5	0.32	4.3	0.68	9.2

*No result for one replicate due to insufficient quantity of sample

External Reproducibility Study: An external reproducibility study was performed at three sites as follows:

Four serum samples, four lithium heparin plasma samples and four quality control materials were run in duplicate, two runs per day. Two of the serum and two of the lithium heparin samples were native samples; the other two serum and lithium heparin samples were spiked with natively sourced troponin antigen. The daily runs were separated by a minimum of two hours. Calibration and run temperatures were kept at room temperature (20-25 °C) for the duration of these studies. The study was run at three external sites for five days. The instruments were calibrated prior to the start of testing and the testing was performed in its entirety on one calibration at each site. One reagent lot and one calibrator lot was used in the study. The results are summarized below:

Sample	N	Mean (pg/mL)	Total Standard Deviation	Between Site CV (SD)	Between Day CV (SD)	Between Run CV (SD)	Repeatability CV (SD)	Reproducibility CV
Plasma	60	11.3	0.41	1.0% (0.11)	0.7% (0.08)	2.0% (0.22)	2.8% (0.32)	3.6%
Plasma	60	29.7	1.05	0.0% (0.00)	1.3% (0.38)	1.1% (0.32)	3.1% (0.93)	3.6%
Plasma	60	98.4	2.88	1.6% (1.59)	0.0% (0.00)	1.5% (1.50)	1.9% (1.88)	2.9%
Plasma	60	18565.0	705.00	0.8% (157.00)	0.0% (0.00)	0.0% (0.00)	3.7% (687.00)	3.8%
Serum	60	11.80	0.52	0.4% (0.05)	0.5% (0.06)	1.8% (0.22)	2.5% (0.30)	3.2%
Serum	60	27.5	1.06	0.1% (0.04)	0.0% (0.00)	1.3% (0.36)	2.4% (0.67)	2.8%
Serum	60	99.7	3.32	0.0% (0.00)	0.0% (0.00)	1.6% (1.62)	2.9% (2.91)	3.3%
Serum	60	16374.0	1004.00	0.5% (85.00)	1.0% (170.00)	1.4% (229.00)	2.9% (477.00)	3.4%
QC	60	19.2	0.91	2.9% (0.56)	0.8% (0.16)	1.6% (0.31)	3.3% (0.63)	4.7%
QC	60	54.2	2.44	0.0% (0.00)	3.6% (1.95)	1.7% (0.93)	2.1% (1.12)	4.5%
Q	60	1088.0	40.00	0.3% (3.00)	2.9% (31.00)	0.9% (10.00)	2.1% (23.00)	3.7%
QC	60	14543.0	567.00	1.1% (154.00)	2.0% (290.00)	2.1% (310.00)	2.4% (344.00)	3.9%

Field Precision Study: The sponsor performed a field effectiveness imprecision study as follows:

The Access hsTnI assay and a panel of additional Access assays representing an example of a hospital test menu was exercised on three Access 2 instruments over three days. The ambient temperature of the laboratory, within which the instruments were operated, was cycled to reflect the worst case thermal profile for Access customers. The Access hsTnI assay was requested as part of a cardiac panel (hsTnI and CKMB), as a STAT test, and individually. Three lithium heparin plasma and three serum samples were prepared with concentrations across the measuring range of the assay. The lowest concentration lithium heparin plasma sample and the lowest concentration serum sample were prepared from pools of patient samples. The remaining two lithium heparin plasma and two serum samples were prepared by spiking patient sample pools with natively sourced antigen. Samples were assayed in

randomized singleton for a total of 15 replicates per sample across all temperature conditions during a single day. Additionally, a series of other assays were randomly executed throughout the study in order to mimic typical laboratory use of the instrument. During the course of a day, the ambient temperature in which the study was executed was cycled between 18°C and 28°C as described in the following table:

Time point	Ambient Temperature
1	23°C
2	20.5°C
3	18°C
4	23°C
5	25.5°C
6	28°C

A single calibration curve was generated at 23 +/- 2 °C on each instrument for this study. Differences in the number of data points in the table were due to experimental design (automatic flex testing of high samples) and some replicates inadvertently not tested or not yielding results. Representative results are summarized below:

Sample	N	Mean (pg/mL)	Within-day SD	Within-day CV	Between-day SD	Between-day CV	Total SD	Total CV
Plasma 1	44	9.76	0.82	8.4%	0.14	1.4%	0.83	8.5%
Plasma 2	45	80.01	3.74	4.7%	0.00	0.0%	3.74	4.7%
Plasma 3	88	12150.06	531.64	4.4%	0.03	0.0%	531.64	4.4%
Serum 1	45	7.01	0.66	9.4%	0.00	0.0%	0.66	9.4%
Serum 2	44	53.46	2.68	5.0%	2.06	3.8%	3.38	6.3%
Serum 3	88	11431.32	513.56	4.5%	0.14	0.0%	513.56	4.5%

b. *Linearity/assay reportable range:*

Two studies were performed to determine the linearity of the Access hsTnI assay following the recommendations in CLSI EP6-A.

The first study evaluated the low range of the assay. This study was done on one Access 2 instrument per temperature, using one reagent lot and one calibrator lot at each of the three temperatures evaluated (18°C, 23°C, and 28°C) so that a total of three reagent pack lots were evaluated (one calibrated and run at 18°C, the second calibrated and run at 23°C, and the third calibrated and run at 28°C). Samples were prepared using lithium heparin plasma as well as serum. The low sample used was a normal female lithium heparin and serum sample. The high sample was prepared by spiking native human antigen into a lithium heparin and serum samples to a concentration of approximately 100 pg/mL TnI. In addition to the high and low troponin concentration samples, eight mixtures were tested in this study (including

samples around the cut-off for each sample type). These samples were prepared independently by using incrementally larger proportions of the high sample mixed with the low sample, in order to achieve troponin concentrations that covered the low range of the assay. The low sample was run in replicates of eight, and all other samples were run in replicates of four.

In the second study, lithium heparin plasma and serum samples covering the range of the assay were evaluated. The low sample used was a normal female sample. The high sample was prepared by spiking native human antigen into a lithium heparin and a serum sample until a concentration at approximately the Access hsTnI S6 commercial calibrator was achieved. In addition to the high and low hsTnI concentration samples, seven mixtures were tested in this study for each sample type. These samples were prepared independently by using incrementally larger proportions of the high sample diluted with the low sample, in order to achieve troponin concentrations that covered the low end of the assay range. The low sample was run in replicates of eight, and all other samples were run in replicates of four.

The maximum deviation from linearity for these studies was 2.66%. Representative results for weighted linear regression are shown below:

Study one

Plasma:
y=0.987x+0.010 (range tested: 1.030 pg/mL to 84.66 pg.mL)

Serum:
y= 1.006x -0.006 (range tested: 0.977 pg/mL to 85.5 pg/mL)

Study two

Plasma:
y=0.976x + 0.17 (range tested: 0.701 pg/mL to 29,534 pg/mL)

Serum:
y=1.016x -0.008 (range tested: 0.465 pg/mL to 28,332 pg/mL)

These studies support the sponsor's measuring range claim of 2.0 to 27,027 pg/mL.

Hook Effect

The sponsor demonstrated that there was no high dose hook effect from the concentration of the Access hsTnI S6 calibrator (~ 27,027 pg/mL) to 2,500,000 pg/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The manufacturer's assay is traceable to a commercially available method via value transference of true patient sample results. The manufacturer's traceability process is based on EN ISO 17511, and has been reviewed and found acceptable.

Sample stability

To evaluate the stability of TnI in patient samples, at least 30 serum and 30 lithium heparin samples (ranging from 2 to 25,000 pg/mL) were tested using the Access hsTnI assay on the Access 2 instrument fresh (baseline) and at various time points after storage at room temperature (20-25°C), refrigerated (2-8°C), and frozen (-20°C). The studies support the following sample handling claims in the labeling:

- 4 hours at room temperature (15 to 25°C)
- 48 hours refrigerated (2-8°C)
- 3 days frozen (-20°C or colder)

d. *Detection limit:*

Limit of Blank (LoB) Test Protocol
Limit of Blank was determined for the Access hsTnI assay on the Access 2 Immunoassay System in accordance with CLSI EP17-A2. Four blank samples were evaluated in replicates of 5 in one run per day for three days on three Access 2 instruments for a total of 180 measurements per instrument/reagent lot combination. Three reagent lots were evaluated using one reagent lot at each of three temperatures (18°C, 23°C, and 28°C). LoB was calculated non-parametrically for each lot and the highest estimate was used. The sponsor determined the LoB to be 0.8 ng/mL.

A second LoB study was performed to confirm the insensitivity of the system to different ambient temperature conditions. The study took 144 replicates of three reagent lots measured on three separate instruments, performed under each of the following conditions:

Calibrating at 18°C and running samples at 18°C, 23°C and 28°C
Calibrating at 23°C and running samples at 18°C, 23°C and 28°C
Calibrating at 28°C and running samples at 18°C, 23°C and 28°C

The LoB was calculated for each of these conditions using the same non-parametric analysis as in the initial LoB determination. This study confirmed the results of the original study.

Limit of Detection (LoD) Test Protocol

Limit of Detection was determined for the Access hsTnI assay on the Access 2 Immunoassay System in accordance with CLSI EP17-A2. Five lithium heparin and five serum samples containing low levels of TnI were evaluated in replicates of five in one run per day for five days on three instruments for a total of 50 replicates for each sample on each instrument/reagent lot combination. Three reagent lots were evaluated using one reagent lot at each of three temperatures (18°C, 23°C, and 28°C).

The Limit of Detection was determined as the concentration at which 95% of the measurements would be greater than the LoB. LoD was calculated for each temperature condition (each using a unique reagent lot). The LoD was determined by the sponsor to be 2.0 pg/mL.

A second LoD study was performed to confirm the insensitivity of the system to different ambient temperature conditions. 50 replicates of five serum and lithium heparin samples were measured for each of the following conditions:

Calibrating at 18°C and running samples at 18°C, 23°C and 28°C
Calibrating at 23°C and running samples at 18°C, 23°C and 28°C
Calibrating at 28°C and running samples at 18°C, 23°C and 28°C

The study confirmed the results of the original study.

Limit of Quantitation (LoQ) Protocol

Native lithium heparin and native serum samples containing troponin I were evaluated in replicates of five in two runs per day for five days on three instruments, so that a total of 50 replicates for each sample on each instrument/reagent lot combination were obtained. Three reagent lots were evaluated using one reagent lot at each of three temperatures (18°C, 23°C, and 28°C).

A variance components model was used to estimate the within-run, between-day, and within-laboratory (total) %CV for each sample on each instrument and reagent lot combination. A precision profile was used to calculate the 20% CV LoQ value for each instrument and reagent lot combination.

To confirm that the system was insensitive to temperature, a second LoQ study was undertaken. 13 lithium heparin and thirteen serum samples containing low levels of troponin I were evaluated in replicates of five in two runs per day for five days on three instruments for a total of 50 replicates for each sample on each instrument/reagent lot combination. The fifty replicates of these 13 serum and 13 lithium heparin samples were measured for each of the following conditions:

Calibrating at 18°C and running samples at 18°C, 23°C and 28°C
Calibrating at 23°C and running samples at 18°C, 23°C and 28°C
Calibrating at 28°C and running samples at 18°C, 23°C and 28°C

A variance components model was used to estimate the within-run, between-day, between-run and within-laboratory (total) %CV for each sample on each instrument and reagent lot combination. A precision profile was used to calculate the 10% CV LoQ and the 20% CV LoQ value for each instrument and reagent lot combination.

These studies each indicated that the LoQ was equal to the LoD (2.0 pg/mL), and support the sponsor's claimed measuring range of 2.0 pg/mL to 27,027 pg/mL.

e. Analytical specificity:

Potential interference due to endogenous and exogenous substances (listed in the table below) was evaluated. The test protocol was designed following the recommendations in CLSI EP7-A2. Serum and lithium heparin troponin samples at low (~10 pg/mL) and high (~100 pg/mL) troponin concentrations were prepared by spiking pooled patient samples with natively sourced human troponin antigen. Known concentrations of potential interferents were added to these samples at concentrations based on the recommendations in EP7-A2. Results from these spiked samples were then evaluated against control samples. The study was run on one instrument, using three reagent lots and one lot of calibrators. 12 replicates were tested for each control sample and each spiked sample, and this was repeated across three reagent lots. The mean concentration of the replicates was calculated for the control patient sample and the spiked sample preparations for each lot. The variation added by the interferent was calculated as a difference in concentration from the control concentration.

The sponsor considered a percent difference between test samples and control samples of greater than ± 10% for samples containing more than 11.5 pg/mL TnI and greater than ± 2.3 pg/mL for samples containing equal or less than 11.5 pg/mL TnI to be significant interference.

The following table describes the interferents evaluated and testing results:

Substance	**Highest Concentration Tested Without Significant Interference**
Acetylsalicylic Acid	65 mg/dL
Acetaminophen	50 mg/dL
Atenolol	1 mg/dL
Atorvastatin (Lipitor)	20 µg/mL
Conjugated Bilirubin	40 mg/dL
Unconjugated Bilirubin	20 mg/dL
Bivalirudin	42 µg/mL
Caffeine	10 mg/dL
Captopril	5 mg/dL
Cinnarizine	40 mg/dL

Substance	Highest Concentration Tested Without Significant Interference
Clopidogrel	75 µg/mL
Cocaine	2 mg/dL
Cyclosporine	5 µg/mL
Digoxin	200 ng/mL
Dopamine	65 mg/dL
Fibrinogen	1000 mg/dL
Furosemide	40 mg/dL
Hemoglobin	400 mg/dL
Human Serum Albumin	6000 mg/dL
Ibuprofen	50 mg/dL
Intralipid	3000 mg/dL
Sodium Heparin	28.8 U/mL
Methyldopa	2.5 mg/dL
Nitrofurantoin	6.4 mg/dL
Nystatin	2 mg/dL
Phenobarbital	20 µg/mL
Rifampicin	60 µg/mL
Rosuvastatin (Crestor)	20 µg/mL
Tissue Plasminogen Activator (TPA)	2.5 µg/mL
Verapamil	16 mg/dL

In addition, the labeling includes the following limitations:

"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 and fibrin. Carefully evaluate results if the sample is suspected of having these types of interferences."

"Endogenous alkaline phosphatase (ALP), exogenous ALP and proteins capable of binding to ALP may cause interference. Elevated ALP levels are commonly observed in patients with hepatobiliary disease and bone disease associated with increased osteoblastic activity. Alkaline phosphatase levels above 400 U/L may cause false positive results. In one study, a sample with cTnI concentration of approximately 8 pg/mL demonstrated an increase of 4 pg/mL when spiked with 800 U/L of alkaline phosphatase."

"Access hsTnI should not be used for patients taking asfotase alfa (i.e. Strensiq)."

HAMA

The sponsor provided results demonstrating that their formulation reduces the effects of HAMA interferents.

The labeling contains the following limitations:

"For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g., HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples."

Troponin Autoantibodies

Troponin autoantibodies have been reported to be present in approximately 10-20% of patients presenting to the ER and may lead to falsely low troponin assay results and delay in treatment of acute coronary syndrome.[1,2] The Access hsTnI assay was designed to minimize interference from troponin autoantibodies and the sponsor conducted design verification activities to assess this.

[1]Park JY and Jaffe AS, Troponin Autoantibodies: From Assay Interferent to Mediator of Cardiotoxicity, *Clin Chem* 2017, 63(1):30-32.

[2]Nussinovitch U, Shoenfeld Y, Anti-troponin autoantibodies and the cardiovascular system, *Heart* 2010; 96(19):1518-1524.

Cross-Reactivity:

Potential cross-reactive substances were evaluated following the recommendations in CLSI EP7-A2 using serum and lithium heparin samples targeted to a low (~10 pg/mL) and high concentration (~100 pg/mL) of troponin. These samples were prepared by spiking pooled patient serum or plasma with natively sourced troponin antigen. Test samples were prepared by spiking each lithium heparin and serum sample with a potentially cross-reactive substance. A control sample without each substance was also prepared. Test and control samples were analyzed in replicates of 12 and the average concentration of each sample was calculated separately for three reagent lots.

For each substance tested, cross-reactivity was defined either as a difference in concentration between the control and test sample greater than 10% (for samples >11.5 pg/mL troponin), or by a change in concentration between diluent control and test sample ≤2.30 pg/mL (for sample concentrations ≤ 11.5 pg/mL troponin).

The following substances did not show cross-reactivity as defined above at the concentrations below:

Cross Reactant	Cross Reactant concentration (ng/mL)
Actin	1000
CK-MB	1000
Myoglobin	1000
Myosin	1000
Cardiac Troponin C	250
Skeletal Troponin I	250
Tropomyosin	1000
Cardiac Troponin T	125

f. Assay cut-off:

Not applicable. See Section 4: Clinical cutoff.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison with the predicate device was not performed. Instead, accuracy was assessed in a clinical study (see section 3, Clinical studies, below).

Thermal Method Comparison:

The Access 2 Immunoassay System requires a thermal algorithm for the Access AccuTnI+3 Reagent. However, the sponsor claims that the candidate device is not sensitive to ambient temperature conditions. To support this claim, a thermal method comparison study was completed to demonstrate the impact of temperature differences across the measuring range of the assay. A minimum of 192 patient samples (each in both lithium heparin plasma and serum) across the measuring range were tested using three reagent pack lots on four different Access 2 instruments at three temperature conditions: 18°C, 23°C and 28°C (for both calibration and testing for a total of 9 different temperature condition combinations). A Passing-Bablok regression analysis was performed comparing the concentrations from the three different run temperatures of the samples within a given calibration temperature. Results from a representative lot are summarized below:

		Plasma				Serum			
Cal Temp	Run Temperature (X vs Y)	n	Passing-Bablok Slope			n	Passing-Bablok Slope		
			Slope	Lower 95% Confidence	Upper 95% Confidence		Slope	Lower 95% Confidence	Upper 95% Confidence Limit
18 °C	18°C v 23°C	199	1.03	1.02	1.03	188	1.03	1.02	1.04
	23°C v 28°C	196	0.99	0.98	1.00	188	0.99	0.98	1.00
	18°C v 28°C	196	1.01	1.00	1.02	190	1.03	1.01	1.04
23 °C	18°C v 23°C	195	1.03	1.02	1.03	188	1.03	1.02	1.04
	23°C v 28°C	192	0.99	0.98	1.00	187	0.99	0.98	1.00
	18°C v 28°C	192	1.01	1.00	1.02	190	1.02	1.01	1.04
28 °C	18°C v 23°C	199	1.03	1.02	1.03	191	1.03	1.02	1.04
	23°C v 28°C	199	0.98	0.98	1.00	190	0.99	0.98	1.00
	18°C v 28°C	199	1.01	1.00	1.02	191	1.02	1.01	1.03

The sponsor concluded that operation of the instrument across the labeled operating range of 18°C – 28°C does not impact analyte recovery for both lithium heparin plasma and serum sample types.

The results summarized above are similar when the calibration temperatures are compared across different run temperatures.

b. Matrix comparison:

Not applicable. The sponsor demonstrated the analytical and clinical performance of lithium heparin plasma and serum for use with the Access hsTnI assay on the Access 2 instrument.

3. Clinical studies:

a. Clinical Sensitivity:

A multicenter prospective study was conducted to evaluate the diagnostic accuracy of the Access hsTnI assay using the established 99th percentile URLs (see Section 4, Clinical cutoff). The study was designed to establish the clinical performance of Access hsTnI as an aid in the diagnosis of MI.

A multicenter prospective study conducted in 2010-2011 enrolled 1929 patients from Emergency Departments presenting with chest pain or equivalent ischemic symptoms suggestive of Acute Coronary Syndromes. Lithium heparin and serum samples from 1851 patients were available for testing with the Access hsTnI assay on the Access 2 Immunoassay System.

True MI statuses of all subjects were adjudicated by an independent panel of expert physicians using criteria consistent with the Universal Definition of Myocardial

Infarction. Adjudicators were blinded to the assay results and the attending physicians' diagnosis. All results presented below were based on the adjudicated diagnoses. The MI incidence was 13% (238/1851). Serial samples were collected from patients within 9 hours of admission to the Emergency Department (i.e., triage). The sample collection times were at time of admission (baseline), 1 to 3 hours, 3 to 6 hours, and 6 to 9 hours after admission to the Emergency Department.

Samples were tested at three independent clinical laboratories on Access 2 systems. Testing was performed using serum and lithium heparin plasma samples. Study results are shown in the tables below. Results are presented for different time intervals between ED presentation and specimen collection: Clinical performance was estimated at overall (male and female combined), male- and female-specific 99th percentile upper reference limit (URL) cut-offs, calculated as described in Section 5, Expected values/Reference range, below. The results are summarized below:

Plasma samples:

Overall analysis (99th Percentile URL = 17.5 pg/mL)

	Sensitivity		**Specificity**	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	86.0% (86/100)	[77.6% - 92.1%]	90.4% (510/564)	[87.7% - 92.7%]
1-3 hours	94.8% (128/135)	[89.6% - 97.9%]	90.4% (985/1090)	[88.5% - 92.1%]
3-6 hours	92.7% (140/151)	[87.3% - 96.3%]	90.0% (1045/1161)	[88.1% - 91.7%]
6-9 hours	98.6% (70/71)	[92.4% - 100.0%]	85.8% (387/451)	[82.2% - 88.9%]

	Positive Predictive value (PPV)		**Negative Predictive Value (NPV)**		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	61.4% (86/140)	[52.8% - 69.5%]	97.3% (510/524)	[95.6% - 98.5%]	15.1% (100/664)
1-3 hours	54.9% (128/233)	[48.3% - 61.4%]	99.3% (985/992)	[98.6% - 99.7%]	11.0% (135/1225)
3-6 hours	54.7% (140/256)	[48.4% - 60.9%]	99.0% (1045/105	[98.1% - 99.5%]	11.5% (151/1312)
6-9 hours	52.2% (70/134)	[43.4% - 60.9%]	99.7% (387/388)	[98.6% - 100.0%]	13.6% (71/522)

Females (99th percentile URL = 11.6 pg/mL))

	Sensitivity		Specificity	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	90.0% (27/30)	[73.5% - 97.9%]	89.8% (228/254)	[85.4% - 93.2%]
1-3 hours	97.7% (42/43)	[87.7% - 99.9%]	90.4% (482/533)	[87.6% - 92.8%]
3-6 hours	98.0% (48/49)	[89.1% - 99.9%]	89.2% (497/557)	[86.4% - 91.7%]
6-9 hours	100.0% (22/22)	[84.6% - 100%]	83.6% (188/225)	[78.1% - 88.1%]

	Positive Predictive value (PPV)		Negative Predictive Value (NPV)		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	50.9% (27/53)	[36.8% - 64.9%]	98.7% (228/231)	[96.3% - 99.7%]	10.6% (30/284)
1-3 hours	45.2% (42/93)	[34.8% - 55.8%]	99.8% (482/483)	[98.9% - 100.0%]	7.5% (43/576)
3-6 hours	44.4% (48/108)	[34.9% - 54.3%]	99.8% (497/498)	[98.9% - 100.0%]	8.1% (49/606)
6-9 hours	37.3% (22/59)	[25.0% - 50.9%]	100.0% (188/188)	[98.1% - 100%]	8.9% (22/247)

Males (99th percentile URL = 19.8 pg/mL)

	Sensitivity		Specificity	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	87.1% (61/70)	[77.0% - 93.9%]	87.7% (272/310)	[83.6% - 91.2%]
1-3 hours	94.6% (87/92)	[87.8% - 98.2%]	88.7% (494/557)	[85.8% - 91.2%]
3-6 hours	92.2% (94/102)	[85.1% - 96.6%]	88.6% (535/604)	[85.8% - 91.0%]
6-9 hours	98.0% (48/49)	[89.1% - 99.9%]	83.2% (188/226)	[77.7% - 87.8%]

	Positive Predictive value (PPV)		Negative Predictive Value (NPV)		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	61.6% (61/99)	[51.3% - 71.2%]	96.8% (272/281)	[94.0% - 98.5%]	18.4% (70/380)
1-3 hours	58.0% (87/150)	[49.7% - 66.0%]	99.0% (494/499)	[97.7% - 99.7%]	14.2% (92/649)
3-6 hours	57.7% (94/163)	[49.7% - 65.4%]	98.5% (535/543)	[97.1% - 99.4%]	14.4% (102/706)
6-9 hours	55.8% (48/86)	[44.7% - 66.5%]	99.5% (188/189)	[97.1% - 100.0%]	17.8% (49/275)

Serum samples:

Overall analysis (99th Percentile URL =18.2 pg/mL)

	Sensitivity		Specificity	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	84.3% (91/108)	[76.0% - 90.6%]	90.9% (541/595)	[88.3% - 93.1%]
1-3 hours	93.6% (132/141)	[88.2% - 97.0%]	91.1% (1009/1108)	[89.2% - 92.7%]
3-6 hours	93.4% (142/152)	[88.2% - 96.8%]	90.0% (1082/1202)	[88.2% - 91.7%]
6-9 hours	95.5% (64/67)	[87.5% - 99.1%]	85.9% (403/469)	[82.4% - 88.9%]

	Positive Predictive value (PPV)		Negative Predictive Value (NPV)		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	62.8% (91/145)	[54.3% - 70.6%]	97.0% (541/558)	[95.2% - 98.2%]	15.4% (108/703)
1-3 hours	57.1% (132/231)	[50.5% - 63.6%]	99.1% (1009/1018)	[98.3% - 99.6%]	11.3% (141/1249)
3-6 hours	54.2% (142/262)	[48.0% - 60.3%]	99.1% (1082/1092)	[98.3% - 99.6%]	11.2% (152/1354)
6-9 hours	49.2% (64/130)	[40.4% - 58.1%]	99.3% (403/406)	[97.9% - 99.8%]	12.5% (67/536)

Females (99th percentile URL = 11.8 pg/mL)

	Sensitivity		Specificity	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	86.2% (25/29)	[68.3% - 96.1%]	89.7% (236/263)	[85.4% - 93.1%]
1-3 hours	97.7% (42/43)	[87.7% - 99.9%]	90.6% (491/542)	[87.8% - 92.9%]
3-6 hours	98.0% (49/50)	[89.4% - 99.9%]	88.4% (512/579)	[85.5% - 90.9%]
6-9 hours	100.0% (20/20)	[83.2% - 100%]	83.4% (196/235)	[78.0% - 87.9%]

	Positive Predictive value (PPV)		Negative Predictive Value (NPV)		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	48.1% (25/52)	[34.0% - 62.4%]	98.3% (236/240)	[95.8% - 99.5%]	9.9% (29/292)
1-3 hours	45.2% (42/93)	[34.8% - 55.8%]	99.8% (491/492)	[98.9% - 100.0%]	7.4% (43/585)
3-6 hours	42.2% (49/116)	[33.1% - 51.8%]	99.8% (512/513)	[98.9% - 100.0%]	7.9% (50/629)
6-9 hours	33.9% (20/59)	[22.1% - 47.4%]	100.0% (196/196)	[98.1% - 100%]	7.8% (20/255)

Males (99th percentile URL = 19.7 pg/mL

	Sensitivity		Specificity	
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Baseline	86.1% (68/79)	[76.5% - 92.8%]	88.0% (292/332)	[84.0% - 91.3%]
1-3 hours	93.9% (92/98)	[87.1% - 97.7%]	88.5% (501/566)	[85.6% - 91.0%]
3-6 hours	93.1% (95/102)	[86.4% - 97.2%]	88.8% (553/623)	[86.0% - 91.1%]
6-9 hours	95.7% (45/47)	[85.5% - 99.5%]	82.5% (193/234)	[77.0% - 87.1%]

	Positive Predictive value (PPV)		Negative Predictive Value (NPV)		
Interval	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**MI Prevalence**
Baseline	63.0% (68/108)	[53.1% - 72.1%]	96.4% (292/303)	[93.6% - 98.2%]	19.2% (79/411)
1-3 hours	58.6% (92/157)	[50.5% - 66.4%]	98.8% (501/507)	[97.4% - 99.6%]	14.8% (98/664)
3-6 hours	57.6% (95/165)	[49.7% - 65.2%]	98.8% (553/560)	[97.4% - 99.5%]	14.1% (102/725)
6-9 hours	52.3% (45/86)	[41.3% - 63.2%]	99.0% (193/195)	[96.3% - 99.9%]	16.7% (47/281)

The following limitation is included in the labeling:

"Positive predictive values (PPV) demonstrated for female subjects using the established female 99th percentile URL values were lower than the PPV values obtained using the overall 99th percentile URL values. Using the lower female 99th percentile URLs may result in a higher proportion of positive test results for females that are non-MI. Taking into consideration the lower bound of the 95% CI, in the worst-case scenario (serum drawn at 6-9 hours after presentation) up to 78% of positive test results for females may be non-MI."

b. *Clinical specificity:*

See clinical sensitivity above.

4. Clinical cut-off**:**

These clinical cutoffs are based on the reference range study in section 5, below. This assay has three cut-offs per sample matrix, as follows:

99th Percentile URL of a healthy population

Sample Matrix	Population	N	99th percentile URL pg/mL (ng/L)	95% CI pg/mL (ng/L)
Lithium heparin plasma	Females	595	11.6	8.4 - 18.3
	Males	494	19.8	14.0 - 42.9
	Overall	1,089	17.5	12.6 - 20.7
Serum	Females	595	11.8	8.7 - 18.7
	Males	493	19.7	14.3 - 44.3
	Overall	1,088	18.2	13.1 - 23.1

5. Expected values/Reference range:

To establish the 99th percentile upper reference limit, lithium heparin plasma and serum specimens were collected prospectively from apparently healthy individuals. To capture an apparently healthy population, subjects were surveyed and excluded if they met any of the following criteria, disease(s) of/or affecting the cardiovascular system, currently taking a medication for cardiovascular disease, diabetes, chronic kidney disease, other serious chronic disease(s) (e.g., cancer, COPD, HIV, lupus, erythematosus, etc.), acute bacterial or viral infection, or pregnancy. 1089 lithium heparin plasma samples and 1088 serum samples were collected from subjects enrolled at five geographically diverse locations.

Both male and female subjects were included in the reference range study to determine the 99th percentile URL using a non-parametric empirical, univariate distribution function following CLSI Guidelines C28-A3c. Results are shown below:

99th Percentile URL of a healthy population

Sample Matrix	Population	N	99th percentile URL pg/mL (ng/L)	95% CI pg/mL (ng/L)
Lithium heparin plasma	Females	595	11.6	8.4 - 18.3
	Males	494	19.8	14.0 - 42.9
	Overall	1,089	17.5	12.6 - 20.7
Serum	Females	595	11.8	8.7 - 18.7
	Males	493	19.7	14.3 - 44.3
	Overall	1,088	18.2	13.1 - 23.1

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.