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

A. 510(k) Number:

k062838

B. Purpose for Submission:

New device

C. Measurand:

Troponin I

D. Type of Test:

Quantitative

E. Applicant:

Ortho-Clinical Diagnostics, Inc.

F. Proprietary and Established Names:

VITROS Immunodiagnostic Products Troponin I ES Reagent Pack

VITROS Immunodiagnostic Products Troponin I ES Calibrators

VITROS Immunodiagnostic Products Troponin I ES Range Verifiers

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MMI	Class II	21 CFR 862.1215, Immunoassay method, troponin subunit	75, Clinical Chemistry (CH)
JIT	Class II	21 CFR 862.1150, Calibrator, Secondary	75, Clinical Chemistry (CH)
JJX	Class I	21 CFR 862.1660, Single (specified) analyte controls (assayed and unassayed)	75, Clinical Chemistry (CH)

H. Intended Use:

1. Intended use(s):

   VITROS Immunodiagnostic Products Troponin I ES Reagent Pack

   For *in vitro* diagnostic use only.
   For the quantitative measurement of cardiac Troponin I (cTnI) in human serum and plasma (heparin and EDTA) using the VITROS Immunodiagnostic System, to aid in the assessment of myocardial damage and risk stratification.

   Cardiac Troponin I measurement aids in the diagnosis of acute myocardial infarction and in the risk stratification of patients with non-ST-segment elevation acute coronary syndromes with respect to relative risk of mortality, myocardial infarction (MI) or increased probability of ischemic events requiring urgent revascularization procedures.

   VITROS Immunodiagnostic Products Troponin I ES Calibrators

   For *in vitro* diagnostic use only.
   For use in the calibration of the VITROS Immunodiagnostic System for the quantitative measurement of cardiac Troponin I (cTnI) in human serum and plasma (heparin and EDTA).

   VITROS Immunodiagnostic Products Troponin I ES Range Verifiers

   For in vitro diagnostic use only.
   For in vitro use in verifying the calibration range of the VITROS Immunodiagnostic System when used for the quantitative measurement of cardiac Troponin I (cTnI).

2. Indication(s) for use:

   See Intended Use section above

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   This device is intended for use with the VITROS Immunodiagnostic System which includes the ECi and ECiQ model analyzers.

I. Device Description:

One VITROS Troponin I ES Reagent Pack contains:

- 100 coated wells (streptavidin, binds ≥ 2 ng biotin/well)
- 7.0 mL conjugate reagent (HRP-mouse monoclonal anti-cTnI, binds ≥ 205 ng cTnI/mL) in buffer with bovine serum albumin and antimicrobial agent.
- 7.0 mL biotinylated antibody reagent (biotin-mouse monoclonal anti-cTnI, binds ≥ 205 ng cTnI/mL) in buffer with horse serum, bovine serum albumin, bovine gamma globulin and antimicrobial agent.

VITROS Troponin I ES Calibrators contain: 2 sets of Troponin I ES calibrators 1, 2 and 3 (2 mL liquid, human cTnI in serum with antimicrobial agent); nominal values 0, 0.08, and 15 ng/mL (exact values encoded on the lot calibration card), lot calibration card, protocol card and 24 calibrator bar code labels (8 for each calibrator).

Human blood products provided as components of the VITROS Troponin I ES Calibrators have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using approved methods (enzyme immunoassays).

VITROS Troponin I ES Range Verifiers contain: 2 sets of VITROS Troponin I ES Range Verifiers, low and high (2 mL liquid, human cTnI in serum with antimicrobial agent); nominal values 0, 76 ng/mL.

Human blood products provided as components of the VITROS Troponin I ES Range Verifiers have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using approved methods (enzyme immunoassays, EIA).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Beckman Access® AccuTnI Troponin I
2. Predicate K number(s):
   k974075
3. Comparison with predicate

Similarities		
Item	Device	Predicate
Principle	Chemiluminescence	Chemiluminescence
Antibody	Mouse monoclonal	Mouse monoclonal
Sample Type	serum and plasma	serum and plasma
Intended Use	For the quantitative measurement of cardiac Troponin I (cTnI) in human serum and plasma to aid in the assessment of myocardial damage and risk stratification. Cardiac Troponin I measurement aids in the diagnosis of acute myocardial infarction and in the risk stratification of patients with non-ST-segment elevation acute coronary syndromes with respect to relative risk of mortality, myocardial infarction (MI) or increased probability of ischemic events requiring urgent revascularization procedures.	For the quantitative determination of cardiac Troponin I (cTnI) levels in human serum and plasma to aid in the diagnosis and treatment of myocardial infarction and cardiac muscle damage. Cardiac troponin I determination aids in the risk stratification of patients with unstable angina or non-ST segment elevation acute coronary syndromes with respect to relative risk of mortality, myocardial infarction or increased probability of ischemic events requiring urgent revascularization procedures.

Differences		
Item	Device	Predicate
Measuring range	0.012 - 80.0 ng/mL	0.01 – 100 ng/mL
Hook Effect	No high dose hook up to 14,000 ng/mL	No high dose hook up to 1,920 ng/mL

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

STANDARDS
Title and Reference Number
CLSI - Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP9-A 1995)
CLSI - Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)
CLSI - Interference Testing in Clinical Chemistry; Approved Guideline (EP7-A)
CLSI – Evaluation of Linearity; Approved Guideline (EP6-A)
CLSI – Limits of Detection and Quantitation; Approved Guideline (EP17-A)
CLSI – Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver

Operating Characteristic (ROC) Plots; Approved Guideline (GP10-A)
CLSI – How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (C28-A2)

L. Test Principle:

The VITROS Troponin I ES assay is performed using the VITROS Troponin I ES Reagent Pack and the VITROS Troponin I ES Calibrators on the VITROS Immunodiagnostic System using Intellicheck® Technology. An immunometric immunoassay technique is used, which involves the simultaneous reaction of cardiac Troponin I present in the sample with a biotinylated antibody (mouse monoclonal anti-cTnI) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-cTnI). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the VITROS Immunodiagnostic System. The amount of HRP conjugate bound is directly proportional to the concentration of cTnI present.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Precision was evaluated consistent with CLSI document EP5. Two replicates of each of 2 frozen control sera and 4 frozen human sample pools were assayed on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 3 different VITROS Immunodiagnostic Systems.

	Units = ng/mL (µg/mL)								
		Within-run*		Within-calibration**		Within-lab***		No. of Observations	No. of Days
	Mean Troponin I Conc.	SD	CV (%)	SD	CV (%)	SD	CV (%)		
System 1	0.024	0.001	4.2	0.003	12.5	0.002	8.3	84	29
	0.063	0.001	1.6	0.003	4.8	0.003	4.7	84	29
	0.091	0.001	1.1	0.003	3.3	0.003	3.3	84	29
	0.413	0.016	4.0	0.017	4.2	0.018	4.3	84	29
	5.23	0.050	1.0	0.112	2.2	0.106	2.0	84	29
	56.3	0.810	1.5	1.75	3.2	1.49	2.6	84	29
System 2	0.028	0.001	3.7	0.003	11.1	0.003	10.7	84	29
	0.064	0.001	1.6	0.004	6.3	0.004	6.2	84	29
	0.092	0.001	1.1	0.004	4.4	0.004	4.3	84	29
	0.433	0.005	1.2	0.011	2.6	0.015	3.4	84	29
	5.58	0.047	0.9	0.117	2.1	0.139	2.5	84	29
	61.4	0.618	1.0	1.82	3.0	1.76	2.8	84	29
System 3	0.027	0.001	3.7	0.002	7.4	0.003	11.1	84	29
	0.059	0.002	3.4	0.004	6.8	0.005	8.5	84	29
	0.086	0.001	1.2	0.005	5.9	0.006	7.0	84	29
	0.393	0.005	1.3	0.011	2.8	0.016	4.0	84	29
	5.05	0.065	1.3	0.101	2.0	0.183	3.6	84	29
	62.8	0.823	1.3	1.75	2.8	2.96	4.7	84	29

* Within-run (Repeatability): Between-duplicate precision was determined using duplicate determinations.
** Within-calibration: Total within calibration precision was determined using a single lot of reagent over a single calibration interval.
***Within-lab: Total within-lab precision was estimated using a single reagent lot calibrated weekly.

To assess the low-end precision, eight low troponin I serum sample pools were tested in singleton, twice a day on 20 occasions over 28 days using 2 lots of reagent. From the resulting imprecision profiles, the coefficient of variation (CV) at the 99th percentile URL of 0.034 ng/mL was 10%. This % CV was determined using a combined mean value using both lots tested. This demonstrates that the VITROS Troponin I ES assay meets the recommendations as described by the ESC/ACC that the assay should have the imprecision (total CV) at the 99th percentile cutoff of 10% or less.

b. Linearity/assay reportable range:

Two buffer based pools with mean analyte concentrations of 0.011 ng/mL and 83.9 ng/mL respectively, were mixed in varying proportions to give a total of 11 pools. A total of 30 singleton determinations of pools 1 and 11, and 9 singleton determinations of pools 2 to 10 were made using 3 Master Lots. Calculated concentrations of pools 2 to 10 were determined from the mean measured concentrations of pools 1 and 11. These calculated concentrations were then compared with the mean measured concentrations of pools 2 to 10. The mean measured concentrations of pools 2 to 10, when expressed as a percentage of the calculated concentrations, gave a mean result of 97.5%, with a range of 96.4% to 99.6%. Analysis by linear regression indicated that the

assay is linear across the range tested (0.011 to 83.9 ng/mL) with an R^2 of 0.9996.

To assess low end linearity, two buffer based pools with mean analyte concentrations of 0.002 ng/mL and 0.155 ng/mL respectively, were mixed in varying proportions to give a total of 11 pools. A total of 30 singleton determinations of pools 1 and 11, and 9 singleton determinations of pools 2 to 10 were made using 3 Master Lots. Calculated concentrations of pools 2 to 10 were determined from the mean measured concentrations of pools 1 and 11. These calculated concentrations were then compared with the mean measured concentrations of pools 2 to 10. The mean measured concentrations of pools 2 to 10, when expressed as a percentage of the calculated concentrations, gave a mean result of 103%. The mean measured concentrations of pools 3 to 10, when expressed as a percentage of the calculated concentrations, gave a mean result of 106%. Analysis by linear regression also indicated that the assay is linear across the range of 0.015 to 0.155 ng/mL with an R^2 of 0.9955.

Hook Effect:
A series of samples having Troponin I concentrations ranging from 1.44 to 14,442 ng/mL were assayed in the VITROS Troponin I ES assay. Singleton determinations of each sample were made with each of 3 Master Lots. The high dose hook of the VITROS Troponin I ES assay was shown to be greater than 14,442 ng/mL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
The calibration for the VITROS Troponin I ES assay is defined by a set of Reference Calibrators. The Reference Calibrators are calibrated against a commercially available Troponin I ES assay. The Reference Calibrators are used to construct a number of Reference Calibration Curves (RCCs) from which a Master Curve may be established. This was used to determine the Troponin I ES concentration in the Calibrators for each Master Lot, as well as providing the Master Curve data for the Lot Calibration card.

To establish the open-stability of the VITROS Troponin I ES Range Verifiers, the verifiers were pooled and stored in sample cups at 2-8°C. The pooled Range Verifiers were tested at the initial time point (day 0) and days 4 and 7 of the trial using three Master Kit Lots and three lots of VITROS Troponin I ES Range Verifiers. The data supports the storage of the VITROS Troponin I ES Range Verifiers at 2-8°C for up to 7 days.

To establish the open-stability of the VITROS Troponin I ES Calibrators, the calibrators were opened, pooled and stored in sample cups at 2-8°C. The pooled calibrators were tested at the initial time point. The calibrators stored at 2-8°C were tested at weeks 2, 4, 8 and 10. The data supports the storage of the calibrators at 2-8°C after opening for up to 10 weeks.

d. Detection limit:

The limit of detection (LoD) for the VITROS Troponin I ES assay using human serum pools is 0.012 ng/mL, determined consistent with CLSI document EP17 and with proportions of false positives less than 1% and false negatives less than 1%; based on 600 determinations, with 150 blank and 450 low-level samples. The limit of quantitation (LoQ) is 0.012 ng/mL as determined by the lowest concentration at which precision and accuracy design requirements are still met and are within the linear range of the assay.

e. Analytical specificity:

The VITROS Troponin I ES assay was evaluated for interference consistent with CLSI document EP7. Commonly encountered substances were tested on 3 lots of reagents. Of the compounds tested, hemoglobin may interfere with the VITROS Troponin I ES assay resulting in a positive bias as shown in the table below.

			Units = ng/mL (µg/L)	
Interferent	**Interferent Concentration**		**Analyte Conc***	**Bias****
Hemoglobin	0.062 mmol/L	100 mg/dL	0.006	0.003
Hemoglobin	0.124 mmol/L	200 mg/dL	0.006	0.030
Hemoglobin	0.186 mmol/L	300 mg/dL	0.006	0.031
Hemoglobin	0.248 mmol/L	400 mg/dL	0.006	0.033
Hemoglobin	0.310 mmol/L	500 mg/dL	0.006	0.034

* Average test concentration of replicate determinations using three different lots of reagent, concentration below the measuring range.
** Estimate of the average difference observed.

The VITROS Troponin I ES assay was evaluated for interference consistent with CLSI document EP7. Of the compounds tested, none was found to cause a bias of >10% with the assay at the concentrations indicated at clinically relevant cTnI concentrations of 0.400 ng/mL

Compound	Concentration	Tested
Acetaminophen	1324 µmol/L	200 µg/mL
Albumin	na	8 g/dL
Allopurinol	2740 µmol/L	400 µg/mL
Ambroxol	1050 µmol/L	400 µg/mL
Ampicillin	144 µmol/L	50 µg/mL
Ascorbic acid	342 µmol/L	60 µg/mL
Aspirin	3.62 mmol/L	650 µg/mL
Atenolol	38 µmol/L	10 µg/mL
Bilirubin	342 µmol/L	20 mg/dL
Biotin	10.2 nmol/L	0.25 µg/dL
Caffeine	515 µmol/L	100 µg/mL
Captopril	230 µmol/L	50 µg/mL
Cinnarizine	1090 µmol/L	400 µg/mL
Cocaine	33 µmol/L	10 µg/mL
Diclofenac	169 µmol/L	50 µg/mL
Digoxin	7.8 nmol/L	0.61 µg/mL
Dipyrone	30 mmol/L	1000 mg/dL
Dopamine	4250 µmol/L	650 µg/mL
Erythromycin	273 µmol/L	200 µg/mL
Fibrinogen	na	1000 mg/dL
na = not applicable		

Compound	Concentration	Tested
Furosemide	1210 µmol/L	400 µg/mL
Ibuprofen	2425 µmol/L	500 µg/mL
Low MW Heparin	na	5 U/mL
Methyldopa	105 µmol/L	25 µg/mL
Nifedipine	173 µmol/L	60 µg/mL
Nitrofurantoin	269 µmol/L	64 µg/mL
Nystatin	na	7.0 µg/mL
Oxytetracycline	10.9 µmol/L	5.0 µg/mL
Phenytoin	365 µmol/L	100 µg/mL
Propranolol	24 µmol/L	5.0 µg/mL
Quinidine	62 µmol/L	20 µg/mL
Sodium Azide	3.08 mmol/L	200 µg/mL
Sodium Heparin	na	8 U/mL
Streptokinase	na	1.96U/mL
Theophylline	1380 µmol/L	250 µg/mL
t-PA	na	2.3 µg/mL
Trimethoprim	258 µmol/L	75 µg/mL
Triolein	33.9 mmol/L	3000 mg/dL
Verapamil	353 µmol/L	160 µg/mL
Warfarin	32.4 µmol/L	10 µg/mL

The cross-reactivity of the VITROS Troponin I ES assay was evaluated by adding the following substances to samples containing no analyte.

Cross-reactant Tested	Concentration Tested	Mean cTnI Result of Cross-reactant Pool Units=ng/mL	% Cross-reactivity
Skeletal Troponin I	1000 ng/mL	0.082	0.008
Cardiac Troponin C (Recombinant)	1000 ng/mL	0.017	0.002
Cardiac Troponin T (Recombinant)	1000 ng/mL	ND*	ND*
Actin (from Rabbit Muscle)	1000 ng/mL	ND*	ND*
Myosin (Recombinant)	1000 ng/mL	ND*	ND*
Tropomyosin (from porcine muscle)	1000 ng/mL	ND*	ND*
CK-MB (Recombinant)	1000 ng/mL	ND*	ND*
Myoglobin (Recombinant)	1000 ng/mL	ND*	ND*

*ND = Not Detectable. Concentration was below the measuring range of the assay.

Cross-reactivity was expressed as the mean result obtained for the cross-reactant pool divided by the cross-reactant concentration in percentage term.

f. Assay cut-off:

The 99th Percentile Upper reference Limit (URL) is 0.034 ng/mL.

The VITROS Troponin I ES assay AMI diagnostic cutoff is 0.120 ng/mL. See Clinical Cut-off section below for more details.

2. **Comparison studies:**

   a. ***Method comparison with predicate device:***
   A method comparison was performed based on CLSI document EP9 using samples analyzed on the VITROS Immunodiagnostic System with those analyzed using the Beckman Unicel™ DxI 800 Access Immunoassay System. The relationship between the two methods was determined by Deming regression. VITROS Troponin I ES = 0.9790 x Beckman Access AccuTnI + 0.2191 ng/mL with samples ranging from 0.015 to 73.5 ng/mL (n = 260).

   b. ***Matrix comparison:***
   Individual results from 20 donors using three determinations of each sample type (serum, EDTA plasma, and heparin plasma) and after storage at 2-8º C for 5 and 7 days and after 4 weeks at -20 º C were tabulated. The mean value for each specimen type within each storage condition was calculated from the three determinations. The overall mean, SD and CV (%) for each sample type across storage conditions were also calculated. For each condition (sample type or storage), % differences were calculated from the baseline (serum for specimen type and fresh for storage condition). The mean and range of the % differences, across all samples, for each condition were calculated. The results indicated that all sample types are suitable (< 10% mean percent difference) for use in the VITROS Troponin I ES assay and that their suitability is not affected by storage.

3. **Clinical studies:**

   a. ***Clinical Sensitivity:***
   Samples were collected and analyzed at two independent clinical sites for sensitivity and specificity. Samples were collected at the time intervals indicated in the chart below, determined from the time of admission. Sample results were generated using the VITROS Troponin I ES assay. Troponin I concentrations were evaluated in serial samples collected from a combined total of 506 prospectively enrolled individuals presenting with symptoms of Acute Coronary Syndrome. This patient population consisted of 289 males and 217 females ranging in age from 23 to 95. A total of 73 patients were diagnosed as AMI positive and 433 were diagnosed as non-AMI according to ACC/ESC/AHA criteria. A total of 1696 plasma and serum samples were collected from the 506 patients from the two clinical sites. Site 1 provided a total of 1393 samples. Site 2 provided a total of 303 samples. The data are presented in the table below:

		Hours Post Admission		
		0-6 hrs	6-12 hrs	12-24 hrs
VITROS Troponin I ES Assay (AMI cutoff = 0.120 ng/mL)	% Sensitivity	70 (86/123)	89 (78/88)	90 (43/48)
	% Specificity	96 (683/711)	94 (420/447)	94 (206/220)

Sensitivity/Specificity
(Combined Data using Peak Sample from each Serial Draw)
Sites 1 and 2: VITROS Troponin I ES

N	506		
Cut-Off value	> 0.12		
	With AMI	Without AMI	Total
Positive Result	69	31	100
Negative Result	4	402	406
Total	73	433	506
Sensitivity	94.52 %		
Specificity	93.08 %		

b. Clinical specificity:

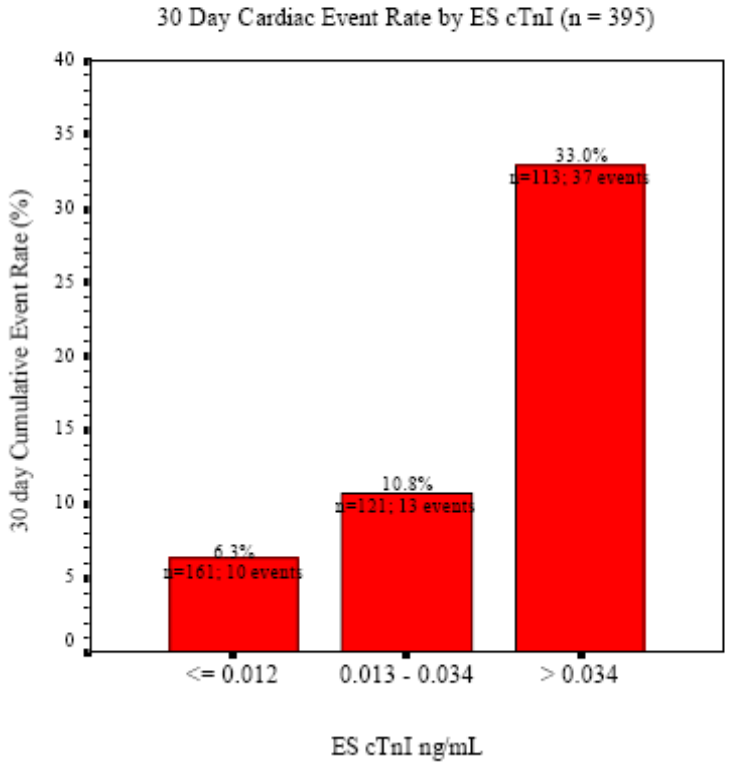
See Clinical Sensitivity section

c. Other clinical supportive data (when a. and b. are not applicable):

Risk Stratification
In a prospective study, 395 patients with myocardial ischemia symptoms suggestive of Acute Coronary Syndrome (ACS) were hospitalized in one medical center to rule in or rule out myocardial infarction (MI). Serial blood samples were obtained from each patient and tested using the VITROS Troponin I ES assay. These patients were followed up after baseline cTnI testing and monitored for short-term adverse cardiac events, which include re-hospitalization due to recurring ischemia, MI and all cause death.
The results were analyzed using the 99th percentile upper reference limit (0.034 ng/mL), as recommended by the ESC/ACC/AHA consensus document for the redefinition of MI.

The findings from this study verified previous clinical studies that showed patients with minor increases in cTnI values provide prognostic information about the long and short-term risk of death for patients with ACS. The data collected from this study indicate that patients with baseline cardiac troponin I values above the 99th percentile upper reference limit had a significantly higher short-term risk of death or recurrent ischemic events after presentation.

30 Day Cardiac Event including Cardiac Death (n = 395)				
	N	# Events	Cumulative Event Rate	P-Value
Troponin I ES				$p < 0.0001$
≤ 0.012	161	10	6.3%	
0.013 - 0.034	121	13	10.8%	
> 0.034	113	37	33.0%	



4. <u>Clinical cut-off:</u>

The 99th Percentile Upper Reference Limit (URL) is 0.034 ng/mL. This value was based on 21 estimates of the URL using over 10,000 serum, heparin and EDTA plasma samples from individual donors and included variation from raw materials, analyzer, operator, manufacturing processes and reagent age. The URL of 0.034 ng/mL was selected as the highest value determined from the multiple determinations of the 99th percentile. The 99th percentile values from the multiple studies ranged from 0.012 to 0.034 ng/mL.

The VITROS Troponin I ES assay AMI diagnostic cutoff is 0.120 ng/mL. A clinical study was performed consistent with CLSI document GP10. The study was conducted at two external clinical sites using prospectively collected, serially drawn specimens. A total of 506 patients with symptoms of acute coronary syndrome (ACS) were diagnosed as AMI or non-AMI according to the ESC/ACC/AHA criteria. 73 patients were diagnosed as AMI and 433 patients

were diagnosed as non-AMI. The peak sample from each patient's serial draw was obtained and used in the analysis. The peak cTnI result is defined as the highest cTnI concentration observed in the serial draw obtained from each patient.
The Receiver Operator Curve (ROC) compares clinical sensitivity and specificity at various decision thresholds. The optimum decision threshold maximizes the area under the curve (AUC) and represents the highest sensitivity and specificity for the assay. The AUC for the VITROS Troponin I ES assay was 0.963. Using the cutoff of 0.120 ng/mL cTnI, the sensitivity of the VITROS Troponin I ES assay was 95% (95% CI of 87%–99%) and the specificity was 93% (95% CI of 90%–95%).

5. Expected values/Reference range:

See Clinical cut-off section

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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