

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

K033300

B. Analyte:

Myoglobin

C. Type of Test:

Quantitative immunometric assay

D. Applicant:

Ortho-Clinical Diagnostics

E. Proprietary and Established Names:

Vitros Immunodiagnostic Products Myoglobin Reagent Pack

Vitros Immunodiagnostic Products Myoglobin Calibrators

Vitros Immunodiagnostic Products Myoglobin Range Verifiers

F. Regulatory Information:

1. Regulation section:

21 CFR § 866.5680, Myoglobin immunological test system

§ 862.1215, Creatine phosphokinase/creatin kinase or isoenzymes
test system

§862.1150, Calibrator

§862.1660, Quality Control Material

2. Classification:

Class II, Class II, Class I

3. Product Code:

DDR, JIS, JJX

4. Panel:

Clinical Chemistry (75)

Immunology (82)

G. Intended Use:

1. Indication(s) for use:

The Vitros Immunodiagnostic Products Myoglobin Reagent Pack is for the in vitro quantitative measurement of myoglobin concentration in human serum or plasma (EDTA or heparin) to aid in the diagnosis of myocardial infarction.

The Vitros Immunodiagnostic Products Myoglobin Calibrators are for in vitro use in calibration of the Vitros Immunodiagnostic system for the quantitative measurement of myoglobin in human serum and plasma (EDTA or heparin).

The Vitros Immunodiagnostic Products Myoglobin Range Verifiers are assayed for use in verifying the calibration range of the Vitros Immunodiagnostic system when used for the in vitro diagnostic measurement of myoglobin.

2. Special condition for use statement(s):

Myoglobin may be elevated in many conditions, such as skeletal muscle trauma, skeletal muscle or neuromuscular disorders, cardiac bypass surgery, renal failure, and strenuous exercise. An elevated myoglobin result must be used in addition to other clinical information such as other cardiac marker test results, ECG, symptoms, and clinical observations in order to aid in the diagnosis of acute myocardial infarction.

Not for use with frozen plasma samples.

For prescription use only.

3. Special instrument Requirements:

Vitros Immunodiagnostic system (cleared K962919)

H. Device Description:

The Vitros Immunodiagnostic products Myoglobin Reagent Pack is designed to measure myoglobin concentration in heparin or EDTA plasma using the Vitros Immunodiagnostic System. The device consists of streptavidin-coated plates and ready-to-use wet reagents including antibodies (biotinylated mouse monoclonal anti-myoglobin antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-myoglobin antibody) and a light-producing indicator.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Dade Dimension RxL Myoglobin (MYO) Method
Vitros Troponin I Range Verifiers
2. Predicate K number(s):
K984191
K992349
3. Comparison with predicate:

The device and the predicate share the same intended use and basic underlying scientific principle (both are sandwich-type solid-phase immunoassays that use an enzymatically-labeled tracer).

Similarities		
Item	Device	Predicate
Sample Type	Serum or plasma (EDTA or heparin)	Serum or plasma (heparin)
Antibody	Mouse monoclonal anti-myoglobin	Mouse monoclonal anti-myoglobin
Sample Volume	10 μ L	20 μ L
Incubation time and temperature	16 minutes at 37°C	14 minutes at 37°C
Range Verifier levels	Low and high	Low and high
Differences		
Item	Device	Predicate
Tracer Antibody	HRP-labeled anti-myoglobin	β -galactosidase labeled anti-myoglobin
Calibration Range	0 – 2000 ng/mL	0 – 1000 ng/mL
Instrumentation	Vitros Immunodiagnostic System	DADE Dimension Myoglobin Immunoassay System
Matrix of calibrators and/or range verifiers	Freeze dried horse serum spiked with human cardiac myoglobin	Freeze dried human serum spiked with human myoglobin

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

NCCLS EP9-A2 – Method Comparison and Bias Estimation Using Patient Samples
 NCCLS EP5-A – Evaluation of Precision Performance of Clinical Chemistry Devices
 NCCLS EP6-A – Evaluation of the Linearity of Quantitative Measurement Procedures, A Statistical Approach
 NCCLS EP7-P – Interference Testing in Clinical Chemistry

K. Test Principle:

Myoglobin present in the sample reacts simultaneously with biotinylated mouse monoclonal anti-myoglobin antibody and horseradish peroxidase (HRP)-labeled mouse monoclonal anti-myoglobin antibody. The myoglobin-antibody complex is captured by streptavidin which coats the sample wells and unbound material is washed away. Signal reagent is added which contains a luminol derivative and a peracid salt as well as a signal enhancer (substituted acetanilide). HRP catalyzes the oxidation of the luminol derivative producing light (the signal enhancer increases the level and duration of the light). This signal can be detected by the VITROS Immunodiagnostic System, allowing the determination of the concentration of HRP in the well. The amount of bound HRP is directly proportional to the concentration of myoglobin in the sample.

L. Performance Characteristics (if/when applicable):1. Analytical performance:*a. Precision/Reproducibility:*

Precision was assessed according to NCCLS EP5. Three frozen control sera and 2 frozen spiked human serum pools were assayed in duplicate, twice a day for at least 20 days. Experiments were done using 3 reagent lots and on 3 different systems (number of observations = 84). The results are summarized below (units = ng/mL):

	Mean [myo]	Within-run*		Within-calibration**		Within-lab***		Days
		SD	%CV	SD	%CV	SD	%CV	
System 1	72.7	1.00	1.4	2.19	3.0	2.27	3.1	30
	145	2.05	1.4	4.36	3.0	4.84	3.3	30
	354	4.45	1.3	10.8	3.0	11.6	3.3	30
	110	1.94	1.8	4.05	3.7	4.13	3.7	30
	944	15.7	1.7	26.5	2.8	27.0	2.9	30
System 2	74.1	0.871	1.2	1.92	2.6	1.79	2.4	31
	146	1.96	1.3	5.24	3.6	4.84	3.3	31
	35	3.57	1.0	12.1	3.4	12.1	3.4	31
	111	1.78	1.6	5.47	4.9	5.38	4.9	31
	923	17.7	1.9	42.4	4.6	46.0	5.0	31
System 3	75.6	0.639	0.9	2.17	2.9	2.66	3.5	31
	151	1.43	1.0	5.29	3.5	6.36	4.2	31
	368	4.28	1.2	10.6	2.9	13.3	3.6	31
	112	1.33	1.2	5.62	5.1	6.13	5.4	31
	940	12.3	1.3	31.6	3.4	43.8	4.6	31

*within run precision was determined using duplicate determinations in a single run

**total (within-calibration) precision was determined using a single lot of reagents over a single calibration interval

***within-lab precision was estimated using a single reagent lot calibrated weekly (4 calibrations total)

b. Linearity/assay reportable range:

The sponsor identifies the reportable range as 0 – 2000 ng/mL. The sponsor instructs the user to dilute samples with myoglobin concentrations > 2000 ng/mL up to 1:20 (sample:diluent) without loss of performance. This was tested using 11 samples on each of 3 lots of reagents. The overall mean % neat value was 98.7% with a range of 94.4% - 102.7%.

Extreme low and high pools of spiked serum (covering the reportable range) were mixed in different ratios to produce 8 pools of intermediate concentration. The High and Low pools were quantitated using 30 replicate measurements, and expected values for the intermediate pools were calculated. The intermediate pools were

quantitated and compared to the expected values. The following correlation was obtained:

$$\text{Observed} = 0.987(\text{Expected}) - 2.97; R^2 = 0.999$$

c. Traceability (controls, calibrators, or method):

The system uses a stored calibration curve that is generated for each lot and is encoded on the lot calibration card (a magnetic card with the encoded data). The card is scanned with the calibration of each new lot and the calibrators are processed by the user. The system compares the light signals expected from the Master Calibration to the light signals generated from processing the calibrators. The Master Calibration is rescaled to reflect the differences between actual and expected values.

The master calibrators are prepared by spiking purified human cardiac myoglobin into a horse serum based matrix. They are traceable to frozen stored samples that are themselves traceable to a commercially available method. The master calibrators cover the approximate range of 0 – 2450 ng/mL and are stored freeze dried.

Vitros Myoglobin Calibrators contain a horse serum based matrix plus antimicrobial agent spiked with human cardiac myoglobin. The calibrators (which are traceable to the master calibrators which are used to generate the Master Calibration) are provided freeze dried and have targeted values of 0, 25, and 350 ng/mL (the exact values are encoded on the lot calibration card).

The Vitros Myoglobin Range Verifiers contain two levels of material and are used to confirm the calibration range of the system. The Range Verifiers are packaged and sold separately. The target values are 0 and 1900 ng/mL, and are prepared by spiking human cardiac myoglobin into a horse serum based matrix. The range verifiers are traceable to reference calibrators that were value assigned using a commercially available assay.

Real time stability studies are summarized and acceptance criteria are identified.

d. Detection limit:

The analytical sensitivity, determined as 2 standard deviations above 20 measurements of a sample containing no analyte, was measured at <2 ng/mL.

e. Analytical specificity:

Potentially interfering substances were tested according to NCCLS EP7. Twenty-two substances were tested using a clinically relevant myoglobin concentration (approximately 95 – 120 ng/mL). No substances tested were shown to interfere (defined as bias <10%). (See product insert for list of substances and concentrations tested).

10 mg/mL hemoglobin results in cross-reactivity of < 0.001%.

Turbidity may affect assay results.

Citrated plasma samples are not recommended.

Not for use with frozen plasma samples.

Heterophilic antibodies in the sample may possibly interfere with immunoassays.

The assay shows no high dose hook effect with concentrations up to 100,000 ng/mL.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison was performed according to NCCLS EP9. A group of 197 patient samples (frozen stored specimens from hospital laboratories) were analyzed on the Vitros Immunodiagnostic system and with the predicate device. Samples that fell above the top of the range of the predicate device were diluted on board as recommended in the manufacturer's package insert prior to re-measurement in that assay. Linear regression determined using the Passing-Bablok method yielded the following relationship:

Device = 0.990(Predicate) + 0.81

95% CI of slope = 0.978 to 1.00

95% CI of intercept = 0.00 to 2.31

Correlation coefficient (Spearman Rank): 0.997

95% CI of Correlation Coefficient = 0.996 to 0.997

Range (as measured by the Vitros system): 16.1 – 1913 ng/mL

b. Matrix comparison:

Blood from 10 donors was spiked with myoglobin to concentrations between 30 and 2000 ng/mL. The blood was then processed to provide matched samples of serum, heparin plasma, and EDTA plasma. These samples were assayed and compared to the serum values. The results are summarized below (units = ng/mL):

Sample	Serum	Heparin Plasma		EDTA Plasma	
	Mean	Mean	% Diff.	Mean	% Diff.
1	59.8	58.1	-2.8	57.7	-3.5
2	237	233	-1.7	227	-4.2
3	95.9	117	22	114	18.9
4	52.4	51.6	-1.5	51.6	-1.5
5	914	903	-1.2	868	-5
6	1499	1477	-1.5	1468	-2.1
7	2129	2104	-1.2	2017	-5.3

8	27.8	28.9	4.0	27.6	-0.7
9	30.9	30.9	0.0	31.0	0.3
10	86.7	87.3	0.7	85.7	-1.2
Mean % Difference			1.68 %		-0.43 %
Range of % Differences		-2.8 to 22 %		-5.3 to 18.9 %	

3. Clinical studies:

a. *Clinical sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

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

4. Clinical cut-off:

Not applicable. See expected values for URL of normals.

5. Expected values/Reference range:

The value for the upper reference limit for normals (URL) was calculated based on 1745 samples from normal blood donors with no known heart disease. The donor ages ranged from 17 to 82 years of age (53% male, 47% female). The mean was 37.9 ng/mL with an SD of 26.5 ng/mL. The reference interval was calculated using a non-parametric estimate and represents the 97.5 percentile of the tested population (table below).

	n	97.5 percentile URL
All individuals	1745	101 ng/mL
Male	919	121 ng/mL
Female	826	61.5 ng/mL

The sponsor cautions that each laboratory should confirm the validity of the expected values for the population it serves.

M. Conclusion:

I recommend that the *Vitros* Immunodiagnostic Products Myoglobin Reagent Pack, the *Vitros* Immunodiagnostic Products Myoglobin Calibrators, and the *Vitros* Immunodiagnostic Products Myoglobin Range Verifiers are substantially equivalent to the legally marketed predicate devices.