



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

I Background Information:

A 510(k) Number

K212648

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products CK-MB Reagent Pack

D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|-----------------|----------------|--|-------------------------|
| JHX | Class II | 21 CFR 862.1215 - Creatine Phosphokinase/Creatine Kinase Or Isoenzymes Test System | CH - Clinical Chemistry |

II Submission/Device Overview:

A Purpose for Submission:

This 510(k) is for modifications to the previously cleared VITROS Immunodiagnostic Products CK-MB Reagent Pack (K993068) including changes to mitigate the risk of biotin interference.

B Measurand:

Creatine kinase isoform MB (CK-MB)

C Type of Test:

Quantitative solid phase immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

For the quantitative measurement of CK-MB in human serum and plasma (EDTA or heparin) using the VITROS 3600 Immunodiagnostic System.

Measurements of creatine phosphokinase and its isoenzymes are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only, For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

VITROS 3600 Immunodiagnostic System (K083173)

IV Device/System Characteristics:**A Device Description:**

The VITROS CK-MB Reagent Pack contains:

- 100 coated wells (antibody, mouse monoclonal anti-CK-BB binds ≥ 344.9 ng CK-MB/mL).
- 8.4 mL assay reagent (buffer with bovine serum albumin, bovine alpha globulins, bovine gamma globulins and antimicrobial agent).
- 8.4 mL conjugate reagent (HRP-mouse monoclonal anti-CK-MB binds ≥ 344.9 ng CK-MB/mL) in buffer with bovine serum albumin and antimicrobial agent.

Testing is performed using the VITROS CK-MB Reagent Pack and the VITROS CK-MB Calibrators on the VITROS 3600 Immunodiagnostic System. The current VITROS Immunodiagnostic Products CK-MB assay is susceptible to interference from biotin. Ortho has made a modification to the manufacturing process to allow the biotinylated antibody capture conjugate to be pre-bound to the well, thus mitigating the risk of biotin interference. The modified product utilizes all the same antibodies and raw materials with the exception of the addition of 0.7% Tween 20 and an increase in EDTA concentration from 0.001M to 0.030M, both of these modifications are to improve serum/plasma agreement which required a conversion factor in the previously cleared product.

B Principle of Operation:

An immunometric immunoassay technique is used, which involves the reaction of CK-MB present in the sample with a microwell coated with biotinylated antibody (mouse monoclonal anti-CK-BB bound to Streptavidin), and a Horseradish Peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-CK-MB). Unbound (HRP)-labeled anti-CK-MB antibody conjugate is 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 system. The amount of CK-MB conjugate bound is directly proportional to the concentration of CK-MB present in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Vitros Immunodiagnostic Products CK-MB Reagent Pack

B Predicate 510(k) Number(s):

K993068

C Comparison with Predicate(s):

| Device & Predicate Device(s): | <u>K212648</u> | <u>K993068</u> |
|---|---|--|
| Device Trade Name | VITROS Immunodiagnostic Products CK-MB Reagent Pack | VITROS Immunodiagnostic Products CK-MB Reagent Pack |
| General Device Characteristic Similarities | | |
| Intended Use/Indications For Use | For the quantitative measurement of CK-MB in human serum and plasma (EDTA or heparin) | Same |
| Antibody | Monoclonal anti-CK-MB and anti-CK-BB | Same |
| General Device Characteristic Differences | | |
| Assay Architecture | Biotinylated antibody capture conjugate is pre-bound to the well | Biotinylated antibody capture conjugate is not pre-bound to the well |

VI Standards/Guidance Documents Referenced:

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

CLSI EP06-A2: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline- Second Edition

CLSI EP07-A3: Interference Testing in Clinical Chemistry; Approved Guidelines- Third Edition

CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples: Approved Guidelines- Third Edition

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision was evaluated using four serum pools tested on one VITROS 3600 Immunodiagnostic System using three reagent lots. The precision pools were prepared by selecting serum samples of a desired concentration (sample 2) or by adjusting the CK-MB concentration levels of the serum samples by spiking with either a blend of patient samples (sample 1) or by spiking with exogenous CK-MB (samples 3 and 4). Samples were measured for 20 days, twice a day in duplicate for a total of 80 measurements per sample in accordance with the recommendations in the CLSI EP05-A3 guideline. The results of a representative lot are shown below.

| Sample | Mean ng/mL | Repeatability | | Total (Within Lab) | |
|--------|---------------|---------------|------|-----------------------|------|
| | | SD | CV% | SD | CV% |
| 1 | 1.8 | 0.049 | 2.7% | 0.129 | 7.1% |
| 2 | 16.90 | 0.397 | 2.4% | 0.843 | 5.0% |
| 3 | 46.3 | 0.793 | 1.7% | 2.529 | 5.5% |
| 4 | 256 | 4.238 | 1.7% | 12.694 | 5.0% |

Lot-to-Lot precision was calculated by pooling the data for the three reagent lots and analyzing the precision of the study. Results are shown below.

| Sample | Mean ng/mL | SD | Between Lot CV (%) |
|--------|---------------|-------|-----------------------|
| 1 | 1.9 | 0.016 | 0.9% |
| 2 | 16.8 | 0.191 | 1.1% |
| 3 | 46.0 | 0.695 | 1.5% |
| 4 | 254 | 5.76 | 2.3% |

2. Linearity:

Linearity studies were performed following the recommendations in the CLSI EP06-A2 guideline. In the first study, a series of nineteen proportionally related admixtures of low and high concentration samples were tested. The samples used to prepare the mixtures were a low serum sample and a high serum sample spiked with exogenous CK-MB antigen. Five replicates were measured for each mid-level sample in the linearity panel and ten replicates were measured for high and low pools in the linearity panel. All linearity levels were tested

on three reagent lots and one VITROS 3600 System. The sample concentrations ranged from 0.08 to 448 ng/mL. The deviation from linearity was < 10%. A second linearity study was performed using native serum patient samples. The results of the linearity studies support that the assay is linear over the claimed measuring range of 0.22- 400 ng/mL.

Auto Dilution Study

Studies were performed to analyze the recovery of samples containing high levels of CK-MB outside the claimed measuring range of 0.22 - 400 ng/mL using 5 human serum sample spiked with human derived CK-MB material. The results support a sample dilution ratio of 1:5 (1-part sample to 4-parts diluent) for the VITROS Immunodiagnostic Products CK-MB Reagent Pack.

3. Analytical Specificity/Interference:

The VITROS CK-MB Reagent Pack was tested for interfering substances at CK-MB concentrations of approximately 3.00 ng/mL and 50.0 ng/mL following the recommendations in the CLSI EP07 and EP37 guidelines. Each test and control sample were run in replicates of 5 on 3 reagent lots. The sponsor defined interference as a bias >10% when compared to the control sample.

The following substances did not interfere with the VITROS CK-MB assay at the concentrations shown below.

| Substance | Concentration | Substance | Concentration |
|---|----------------------|------------------------------------|----------------------|
| Acetaminophen | 15.6 mg/dL | Ibuprofen | 71 mg/dL |
| N-acetylcysteine | 15 mg/dL | Levothyroxine | 0.0429 mg/dL |
| Amoxicillin | 5.40 mg/dL | Loratadine | 0.0087 mg/dL |
| Ascorbic acid | 5.25 mg/dL | Naproxen | 36 mg/dL |
| Bilirubin, conjugated | 40 mg/dL | Nifedipine | 0.0588 mg/dL |
| Bilirubin, unconjugated | 40 mg/dL | Oleic acid | 40 mg/dL |
| Biotin | 3510 ng/mL | Omeprazole | 0.840 mg/dL |
| Carbamazepine | 4.50 mg/dL | Phenytoin | 6.00 mg/dL |
| Carvedilol | 5 mg/dL | Prednisone | 0.010 mg/dL |
| Captopril | 0.264 mg/dL | Propranolol HCl | 0.115 mg/dL |
| Cholesterol | 400 mg/dL | Rheumatoid factor | 900 IU/mL |
| Clopidogrel | 30 mg/dL | Rivaroxaban | 0.270 mg/dL |
| Codeine | 0.141 mg/dL | Salicylic acid | 2.86 mg/dL |
| Cotinine | 0.24 mg/dL | Streptokinase | 150,000 U/dL |
| Dextromethorphan | 0.00156 mg/dL | Theophylline | 6 mg/dL |
| Digoxin | 0.0039 mg/dL | Total protein | 15 g/dL |
| Enoxaparin (Low Molecular Weight Heparin) | 360 U/dL | Tissue Plasminogen Activator (TPA) | 1.2 mg/dL |
| Ethanol | 600 mg/dL | Triglycerides | 1500 mg/dL |
| Furosemide | 1.59 mg/dL | Triolein | 3000 mg/dL |
| Hemoglobin | 1000 mg/dL | Vancomycin hydrochloride | 12.3 mg/dL |
| Heparin (sodium) | 330 U/dL | Verapamil | 0.160 mg/dL |

| Substance | Concentration | Substance | Concentration |
|-----------------------------|---------------|-----------------|---------------|
| Hydralazine (hydrochloride) | 1.44 mg/dL | Warfarin sodium | 8.0 mg/dL |
| Hydrocodone | 0.0072 mg/dL | | |

The substances listed in the table below caused significant interference at the original test concentration and titration testing was conducted to define the highest concentration where no interference was observed. The results are summarized below:

| Interferent | CK-MB Conc. (ng/mL) | Interferent Concentration | % Bias |
|-------------|---------------------|---------------------------|--------|
| Cefoxitin | 3.00 | 521 mg/dL | -27.7 |
| Cefoxitin | 3.00 | 348 mg/dL | -8.1 |
| Dextran 40 | 3.00 | 2400 mg/dL | -15.0 |
| Dextran 40 | 3.00 | 1800 mg/dL | -8.9 |
| Dextran 40 | 50.00 | 2400 mg/dL | -44.9 |
| Dextran 40 | 50.00 | 1800 mg/dL | 9.0 |

The interference with Cefoxitin and Dextran 40 were observed at levels above therapeutic concentrations.

Cross-reactivity:

Each potential cross-reactant was spiked into a buffer matrix containing no CK-MB at the concentrations listed in the table below. Testing was performed using five (5) replicates of each test sample on each of three (3) reagent lots. A negative control was also tested. Cross-reactivity was expressed as the mean result obtained for the cross-reactant sample divided by the cross-reactant concentration in percentage terms.

Results of cross-reactivity testing are summarized below:

| Test Substance | Conc. (µg/mL) | % Cross- reactivity |
|----------------|---------------|---------------------|
| CK-BB | 50 µg/dL | ND* |
| CK-MM | 4 mg/dL | ND* |

*Not Detectable. Concentration was below the measuring interval of the test, 0.22 to 400 ng/mL

Hook Effect

The sponsor provided data supporting the claim that there is no hook effect up to 44,200 ng/mL.

4. Assay Reportable Range:

The claimed assay reportable range of is 0.22- 400 ng/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Calibration of the VITROS CK-MB test is traceable to in-house reference calibrators which have been value-assigned to correlate to another commercially available test. The traceability of the modified device is the same as the predicate, K993068.

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined for the VITROS CK-MB Reagent Pack based upon recommendations in the CLSI EP17-A2 guideline. The detection limit studies were conducted using three VITROS CK-MB assay reagent lots on one VITROS 3600 System over five working days. Each reagent lot was calibrated on the first day of testing.

The LoB testing consisted of 20 replicates of four fluids containing no measurable CK-MB. LoB was determined as the 95th percentile of the measurements of the blank samples. The LoD testing consisted of 60 replicates of five neat serum samples (low levels of endogenous CK-MB) spiked with purified CK-MB sourced from human heart tissue. The LoD was calculated using the classical approach outlined in CLSI EP17-A2. The LoQ was determined using a precision profile approach with a %CV limit of 20%.

The results of the studies support the claims summarized in the following table:

| Analyte | LoB | LoD | LoQ |
|-----------------|------|------|------|
| CK-MB (ng/mL) | 0.07 | 0.22 | 0.22 |

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were conducted by testing 149 serum samples in singlicate using the modified VITROS CK-MB Reagent Pack (candidate device) using 3 reagent lots and the unmodified VITROS CK-MB assay (predicate device) in duplicate using 2 reagent lots on one VITROS 3600 System. For the modified device, each sample was tested using each reagent lot. For the unmodified device, each sample was tested using each lot and the mean for each sample was used in the comparison. The concentrations in the samples ranged from 0.61-378 ng/mL, as determined by the predicate device. Each sample was run in singleton. Weighted Deming regression analysis was performed. The regression analysis summary of one representative lot is shown below:

| N | Concentration Range (ng/mL) | Intercept (95% CI) (ng/mL) | Slope (95% CI) | Correlation Coefficient (r) |
|-----|-----------------------------|----------------------------|-----------------------|-----------------------------|
| 149 | 0.61-378 | 0.112 (0.051 to 0.172) | 0.99 (0.981 to 0.995) | 0.999 |

2. Matrix Comparison:

The study included samples from 49 individual whole blood donors. The following matrices were evaluated: serum, lithium heparin plasma and K2-EDTA plasma. Samples were unaltered, spiked using endogenous human serum samples with elevated CK-MB values, and spiked with concentrated human CK-MB to ensure the full measuring interval of the assay was tested.

Samples from each matrix were tested in singlicate using one reagent lot on one VITROS 3600 System. Weighted Deming regression analysis was performed using the serum data as the reference. Results are shown in the table below.

| Matrix | n | Slope | Intercept | Correlation Coefficient |
|----------------------------------|----------|------------------------|---------------------------|--------------------------------|
| Serum vs. Lithium heparin plasma | 49 | 1.01 (0.991, 0.098) | -0.045 (-0.188, 0.098) | 0.999 |
| Serum vs. K2-EDTA plasma | 49 | 1.00 (0.979, 1.015) | -0.047 (-0.178, 0.084) | 0.999 |

The results of the matrix comparison studies support the sponsor’s claim that lithium heparin plasma and K2-EDTA plasma are suitable specimen matrices for use with the assay.

C Clinical Studies:

1. Clinical Sensitivity:
Not applicable
2. Clinical Specificity:
Not applicable
3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The labeling states, “It is recommended that each laboratory establish its own expected values for the population it serves. These reference intervals are the upper 97.5 percentile of a panel of 269 apparently healthy individuals”. The existing claim, established based on the predicate, was verified following the recommendations CLSI EP28-A3.

| Specimen Type | Units = ng/mL (µg/L) |
|----------------------|-----------------------------|
| Serum | 3.38 |

The sponsor also provides the following information in the labeling that was also included in the predicate label.

| Description | Units = ng/mL (µg/L) | | | |
|---|-----------------------------|-------------|--------------------------------------|-----------------------|
| | N | Mean | Upper Limit (97.5 Percentile) | Observed Range |
| Hospitalized patients with noncardiac related disorders | 107* | 1.04 | 4.55 | – |

| Description | Units = ng/mL (μ g/L) | | | |
|--|----------------------------|---------|----------------------------------|-------------------|
| | N | Mean | Upper Limit (97.5 Percentile) | Observed Range |
| Hospitalized cardiac patients not currently experiencing a myocardial infarction | 59* | 1.42 | 5.31 | – |
| Hospitalized patients with confirmed myocardial infarction | 19** | 81.4*** | – | 12.8–176 |

*Serum samples.

** A minimum of two serial serum specimens per patient, taken over at least a 15 hour period.

*** Peak specimens.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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