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

A. 510(k) Number:

k123930

B. Purpose for Submission:

Modifications of a previously cleared homocysteine reagent (k061588)

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative, homogeneous enzymatic assay

E. Applicant:

Ortho-Clinical Diagnostics, Inc.

F. Proprietary and Established Names:

VITROS Chemistry Products HCY 2 Reagent
VITROS Chemistry Products HCY 2 Performance Verifiers I, II, and III

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Urinary Homocysteine (Nonquantitative) Test System (LPS)	Class II	21 CFR 862.1377, Urinary homocysteine (non-quantitative) test	75 Clinical Chemistry (CH)
Single (Specified) analyte controls (assayed and unassayed) (JJX)	Class I, reserved	21 CFR 862.1660, Quality Control Material (assayed)	75 Clinical Chemistry (CH)

H. Intended Use:

1. Intended use(s):

   See indications for use (below).

2. Indication(s) for use:

   VITROS Chemistry Products HCY 2 Reagent:
   For *in vitro* diagnostic use only. VITROS Chemistry Products HCY 2 Reagent is used on VITROS Systems to quantitatively measure total homocysteine concentration in human serum and plasma. Serum and plasma homocysteine levels can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

   VITROS Chemistry Products HCY 2 Performance Verifiers I, II & III:
   For *in vitro* diagnostic use only. VITROS Chemistry Products HCY 2 Performance Verifiers are assayed controls used to monitor performance of VITROS Chemistry Products HCY and VITROS Chemistry Products HCY 2 Reagents on VITROS 5,1 FS Chemistry Systems, VITROS 4600 Chemistry Systems and VITROS 5600 Integrated Systems.

3. Special conditions for use statement(s):

   Prescription use only

   The labeling contains a prominent black-box warning:

IMPORTANT: Results should be interpreted with caution. Certain drugs and clinical conditions are known to alter HCY concentration in vivo. For example, patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate, may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism.

4. Special instrument requirements:

   VITROS 5,1 FS Chemistry System, VITROS 4600 Chemistry Systems and VITROS 5600 Integrated Systems.

I. Device Description:

The VITROS Chemistry Products HCY 2 Reagent consists of 1 dual chambered reagent pack containing 2 ready-to-use liquid reagents, 1 in each chamber. Reagent 1 is composed of the following active ingredients: Lactate Dehydrogenase 37.9 kU/L, Serine 0.8 mmol/L, NADH 0.5 mmol/L and Tris(2-Carboxyethyl)phosphine hydrochloride (TCEP) 2.9 mmol/L. Reagent 2 is composed of the following active ingredients: Cystathionine ß-synthase 0.748 kU/L and Cystathionine ß-lyase 16.4 kU/L.

The VITROS Chemistry Products HCY 2 Performance Verifiers I, II and III are prepared from processed human serum to which amino acid and preservative have been added. The HCY 2 Performance Verifiers are provided, separately, as (3) 1.5 mL vial for each control level, and are composed of the following active ingredient: homocysteine.

The HCY 2 Verifiers have the identical ingredients as the HCY Verifiers, which has been previously cleared in k061588. The HCY 2 verifiers have value assignment for both HCY reagent and HCY 2 reagent.

The VITROS Chemistry Products Calibrator Kit 27, required for the HCY 2 test, has been previously cleared in k061588.

- All human source materials were tested by FDA-approved methods and found to be negative for hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HBc), HCV antibody, HTLV-1/2 antibody, and HIV-1/2 antibody.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VITROS Chemistry Products HCY Reagent and VITROS HCY Performance Verifiers I, II, and III

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

   k061588

3. Comparisonwithpredicate:

Table 1	**Similarities and differences**	
Device Characteristic	**VITROS® HCY 2 assay (New device)**	**VITROS® HCY assay (Predicate device)**
Intended Use/Indications for Use	It is intended for use on VITROS Systems to quantitatively measure total homocysteine concentration in human serum and plasma. Serum and plasma homocysteine levels can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.	Same
Reagent Packs	One dual chamber reagent pack containing one reagent in each chamber	Two dual chamber reagent packs containing three reagents, one reagent in each of three chambers

Tests per pack/set	50 tests/reagent pack/ 6 reagent packs/carton=300 tests/carton	50 tests/ 2 reagent packs/ 6 reagent packs/carton=150 tests/carton
Reactive ingredients	Lactate Dehydrogenase (LDH)= 37.9 KU/L Serine=0.8 mmol/L (0.08% w/v) Nicotinamide Adenine Dinucleotide (NADH)=0.5 mmol/L (0.03% w/v) Tris (2-Carboxyethyl) phosphine hydrochloride (TCEP)=2.9 mmol/L (0.08% w/v) Cystathionine β-lyase=16.4 KU/L Cystathionine β-synthase=0.748 KU/L	Lactate Dehydrogenase (LDH)= 65 KU/L Serine=1.3 mmol/L (0.01% w/v) Nicotinamide Adenine Dinucleotide (NADH)=0.56 mmol/L (0.04% w/v) Tris (2-Carboxyethyl) phosphine hydrochloride (TCEP)=26.3 mmol/L (0.8% w/v) Cystathionine β-lyase=16 KU/L Cystathionine β-synthase=22 KU/L
Analyte measured	Homocysteine	Same
Sample Type	Serum and plasma	Same
Measurement Type	Quantitative	Same
Reportable Range	2.0 –50.0 µmol/L	1.0 –50.0 µmol/L
Instrumentation	VITROS 5,1 FS Chemistry Systems, VITROS 4600 Chemistry Systems and VITROS 5600 Integrated Systems.	Same
Reference Interval	Males: 6.6 – 14.8 µmol/L Females: 4.7 – 12.6 µmol/L	Same

Table 2	**Similarities and differences**	
Device Characteristic	**VITROS® HCY 2 Performance Verifiers (New Device)**	**VITROS® HCY Performance Verifiers (Predicate Device)**
Intended Use	Assayed controls used to monitor performance of VITROS homocysteine assay on the VITROS Systems.	Same
Vial Volume	1.5 mL/vial	5 mL/vial
Matrix	The performance verifiers are prepared from processed human serum with preservatives added.	Same
Format	Liquid	Same
Number of levels	Three	Same
Nominal Values	7.0, 12.5, and 25 µmol/L	7.0, 12, and 46 µmol/L

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

1. CLSI C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline-Second Edition
2. CLSI EP09-A2-IR Method Comparison and Bias Estimation Using Patient Samples; Appr Guideline- 2nd Edition Interim Rev
3. CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- 2nd Edition
4. CLSI EP-17-A Protocols for Determination of Limits of Detection and Limits of Quantitation
5. CLSI EP-6 Evaluation of the Linearity of Quantitative Measurement Procedures: Approved Guideline- First Edition
6. ISO 14971:2007, Medical devices - Application of risk management to medical devices
7. CLSI EP7-A2 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition

L. Test Principle:

The VITROS Chemistry Products HCY 2 assay utilizes a chemical reaction in which disulfide linked homocysteine (oxidized forms) in the sample is reduced by Tris (2-Carboxyethyl) phosphine hydrochloride (TCEP) to form reduced homocysteine. Reduced homocysteine reacts with serine in the presence of cystathionine b-synthase (CBS) to form L-cystathionine. L-cystathionine is broken down by cystathionine b-lyase (CBL) to produce homocysteine, pyruvate and ammonia. Pyruvate is reduced to lactate by lactate dehydrogenase (LDH) using NADH as coenzyme. The concentration of homocysteine is directly proportional to the amount of NADH converted to NAD^+ and is measured spectrophotometrically at 340 nm. Once a calibration has been performed, the homocysteine concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbance obtained in the assay of the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analyticalperformance:</u>

   a. Precision/Reproducibility:

   Precision studies were conducted by the sponsor on the VITROS 5,1 FS, VITROS 4600 and the VITROS 5600 Chemistry Systems following CLSI guidance document EP05-A2. Three levels of homocysteine-spiked, human serum-based samples were run 2 times per run, 2 runs per day, for a total of at least 21 days ($n \geq 80$ measurements/sample level). Results are summarized below.

System	Mean Conc. (µmol/L)	Within-Day SD (µmol/L)	Within-Day %CV	Within-Lab SD	Within-Lab %CV
VITROS 5,1 FS	6.1	0.16	2.6	0.18	3.0
	11.6	0.17	1.5	0.26	2.2
	24.6	0.22	0.9	0.43	1.7
VITROS 4600	6.2	0.16	2.6	0.22	3.5
	11.7	0.15	1.3	0.27	2.3
	24.4	0.24	1.0	0.47	1.9
VITROS 5600	6.3	0.18	2.9	0.19	3.0
	11.8	0.17	1.4	0.22	1.9
	24.4	0.26	1.1	0.42	1.7

b. Linearity/assay reportable range:

A linearity study was performed according to NCCLS EP6-A recommendations for evaluation of linearity. A series of 9 samples was prepared by combining various volumetric proportions of a spiked, high analyte human serum pool and a low analyte, human serum pool to produce samples with homocysteine values ranging from 0.3 to 74.9 µmol/L. Three determinations of each of these samples were made on each of three different VITROS HCY 2 Reagent reagent lots on the VITROS 5,1 FS, VITROS 4600, and VITROS 5600 Chemistry Systems.

The linear regression correlation between the expected values and the measured values is summarized below for a representative lot:

VITROS System	Regression equation	Correlation coefficient
5,1 FS	y = 1.05x + 0.075	0.9999
4600	y = 1.05x + 0.025	0.9999
5600	y = 1.05x + 0.028	0.9999

The linearity study provided by the sponsor supports a reportable range claim for the VITROS HCY 2 device of 2 – 50 µmol/L homocysteine.

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

Traceability

The VITROS HCY 2 assay is traceable to NIST 1955 materials. The VITROS Chemistry Products calibrator kit 27 was cleared in k061588.

HCY 2 Calibration Stability

HCY 2 assay calibration stability was assessed on all 3 VITROS systems, and the

sponsor claims that calibration is stable for 2 months. The protocol and acceptance criteria have been provided and found to be adequate to support the sponsor's claims.

HCY 2 Performance Verifier Value Assignment

The VITROS Chemistry Products HCY 2 Performance Verifiers I, II, and II were value assigned on 2 VITROS 5,1 FS Chemistry Systems using 2 lots of VITROS Chemistry Products HCY 2 Reagent, 2 lots of VITROS Chemistry Products HCY Reagent, and 1 run of 8 replicates per instrument (N=16 total replicates per assay).

Target values for HCY 2 Verifiers I, II and III are 7.0, 12.5, and 25 µmol/L, respectively, and can be used for both HCY and HCY 2 tests. Lot specific target ranges are provided in the value assignment sheet.

HCY 2 Performance Verifier Stability

The shelf life stability of the VITROS HCY 2 verifiers was tested on a VITROS 5,1 FS Chemistry System using a real time study extending at least one month beyond the selected expiration date. Testing is ongoing. The VITROS HCY 2 verifiers are stored refrigerated (2-8°C), and are stable for at least 8 months when stored at this storage temperature.

The opened vial stability of the VITROS HCY 2 verifiers was tested on a VITROS 5,1 FS Chemistry System. Testing is ongoing. The verifiers are stable for at least 14 days when stored at 2-8°C .

Real-time stability study protocol and acceptance criteria has been provided and found to be adequate to support the sponsor's claims.

d. Detection limit:

The sponsor performed a detection limit study for the VITROS HCY 2 assay on the VITROS 5,1 FS Chemistry System, the VITROS 4600 Chemistry System, and the VITROS 5600 Integrated System, according to CLSI guidance document EP17-A.

Limit of Blank (LoB) was determined for each of 3 reagent lots, based on One hundred (100) replicate homocysteine measurements of 1 blank sample (homocysteine stripped human serum) on each of the 3 VITROS chemistry systems. The limit of blank, as determined by adding the pooled blank standard deviation to the blank calculated concentration mean, was 0.39 µmol/L.

Limit of Detection (LoD) was determined for each of 3 reagent lots, based on One hundred (100) replicate homocysteine measurements of 5 low human serum-based samples on each of the 3 VITROS chemistry systems. The limits of detection were calculated using the determined LoB value and pooled SD values

from LoD samples. The sponsor claimed that the LoD for serum samples on VITROS chemistry systems was 0.79 µmol/L.

Limit of Quantitation (LoQ) determination was based on interassay precision of ≤20% for 12 replicate measurements of 4 low serum samples, per day for 3 days, on each of the 3 VITROS chemistry systems. The LoQ was determined to be 2.0 µmol/L for the VITROS HCY 2 assay on each of the 3 VITROS chemistry systems.

The HCY 2 test has a measuring range of 2 to 50 µmol/L.

e. Analytical specificity:

Interference studies were performed according to CLSI guidance document EP-7A to determine the effects from potential interferents on the VITROS HCY 2 assay. Various concentrations of interferents were spiked into serum pools containing homocysteine at low (~6 umol/L) and elevated (~25 umol/L) concentrations. All samples were tested in triplicate on each of the 3 VITROS Chemistry Systems. The sponsor states that interference is considered to be non-significant if the bias between the tested and control samples are within ±10% for HCY concentration of >12 umol/L and within 1.79 umol/L for HCY concentration of <12 umol/L.

Below is a summary table of the highest concentration of interferent tested and found not to interfere based on the stated acceptance criteria.

Compound	**Concentration** **Conventional units**
N-Acetyl L-Cysteine	326 µg/L
Adenosine	6.7 mg/dL
Ascorbic Acid (L)	10 mg/dL
Bilirubin	25 mg/dL
Creatinine	30 mg/dL
L-Glutathione	31 mg/dL
Intralipid (Turbidity)	400 mg/dL
L-Methionine	29.8 mg/dL
S-(5'-Adenosyl)-L-Methionine Chloride	8.7 mg/dL
Total Protein (Serum)	9.5 g/dL
Urea	500 mg/dL
Hemoglobin	500 mg/dL
L-Homocysteine thiolactone	50 µmol/L
Pyruvic Acid	2 mmol/L
Rheumatoid Factor	300 IU/mL
L-Cysteine	300 µmol/L

Based on their interference study results, the sponsor made the following limitations in their labeling:

- Patient samples containing cystathionine (reference interval: 0.065 to 0.301 µmol/L 13) show positive equimolar interference with the VITROS Chemistry Products HCY 2 assay. For example, a sample containing 10.0 µmol/L homocysteine and 0.3 µmol/L cystathionine will generate a VITROS Chemistry Products HCY 2 assay result of ~10.3 µmol/L. Elevated cystathionine levels can be observed in patients with certain conditions, such as renal disease, folate deficiency, and cystathionine beta-lyase deficiency.

- Patient samples containing elevated levels of L-cysteine (normal range: 178 to 196 µmol/L 15) can show positive interference with the VITROS Chemistry Products HCY 2 assay. For example, a sample containing 6.2 µmol/L homocysteine and an additional 600 µmol/L added L-cysteine could generate a VITROS Chemistry Products HCY 2 assay result of ~8.6 µmol/L (bias of +2.4 µmol/L).

- For samples that generate a Sample Integrity Turbidity-index flag, dilute the sample and re-analyze (refer to the Sample Dilution section of the product insert).

An additional study analyzed 10 paired sets of serum and 2x Li-Heparin and 2x EDTA plasma samples to determine the impact of short draws on HCY2 assay performance. Due to observed negative bias, the sponsor recommends that short draws not be used.

The sponsor has the following limitations in the labeling:

Whole blood samples should be centrifuged within 1 hour after collection or refrigerated for up to 8 hours prior to centrifugation. After blood is drawn, homocysteine is released from erythrocytes and the concentration will increase at a rate of approximately 1 µmol/L per hour at room temperature.

Plasma specimens must be collected in plasma tubes that are at least half full. Smaller volumes can result in negative biases.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed on at least 110 human serum samples, 10 of which were spiked with homocysteine, on the VITROS 5,1 FS, VITROS 4600 and the VITROS 5600 Chemistry Systems, versus the predicate method, following CLSI-EP9A2-IR. Three reagent lots were used for the testing. Samples were measured in singlicate and ranged in homocysteine concentration from 2.90 to 44.4 umol/L. Results are summarized below for the linear regression analyses resulting from a plot of homocysteine concentrations obtained on the subject (Y-axis) and predicate (X-axis) devices, for a representative lot.

Instrument System vs. predicate	**n**	**Slope**	**Intercept (µmol/L)**	**r**
VITROS 5,1 FS	110	1.002	0.424	0.997
VITROS 4600	123	0.984	0.598	0.997
VITROS 5600	111	0.991	0.459	0.993

b. Matrix comparison:

Matrix comparison studies were completed 40 paired serum and plasma samples (EDTA Plasma and Li-Heparin Plasma; 32 were native samples and 8 were spiked samples). Each sample was tested in duplicate on each of 3 lots of VITROS Chemistry Products HCY 2 Reagent on all 3 VITROS Chemistry Systems. Linear regression analysis using singlet set of data showed the following relationships between plasma and serum samples:

	n	**Slope**	**Correlation Coefficient**	**Range of Sample Conc. (µmol/L)**	**Intercept**
Li Heparin plasma vs. Serum	40	0.98	0.999	6.7 - 44.7	-0.19
EDTA plasma vs. Serum	40	1.01	0.999	6.2 - 43.0	-0.88

The sponsor concluded that Lithium heparin plasma and EDTA plasma are acceptable anti-coagulants to be used with the VITROS HCY 2 assay.

3. Clinicalstudies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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

Not applicable

4. Clinicalcut-off**:**

Not applicable

5. Expected values/Reference range:

Expected values are based on data collected using the predicate VITROS HCY device, where the homocysteine concentrations for the 2.5^{th} to 97.5^{th} reference interval were determined to be as follows for males and females:

Males: 6.6 to 14.8 µmol/L
Females: 4.7 to 12.6 µmol/L

These reference intervals were also validated for use with the VITROS HCY 2 device on the VITROS 5,1 FS, VITROS 4600 and the VITROS 5600 Chemistry Systems by reference range transference, following CLSI C28-A3. The sponsor performed a validation study with 40 healthy adults (20 male and 20 female) to confirm the reference interval.

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.