510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231517

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products CEA Reagent Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DHX	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification of the previously cleared device to mitigate biotin interference

B Measurand:

Carcinoembryonic antigen (CEA)

C Type of Test:

Quantitative, immunochemiluminescent assay

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 carcinoembryonic antigen (CEA) concentration in human serum and plasma (EDTA or heparin) using the VITROS 5600 Integrated System, to aid in the prognosis and management of cancer patients in whom changing concentrations of CEA are observed.

For In Vitro Diagnostic Use Only

C Special Conditions for Use Statement(s):

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

The package insert of the devices contains the caution statement: "Different CEA test methods cannot be used interchangeably. CEA in a given patient sample determined with tests from different manufacturers can vary due to differences in test methods and reagent specificity. A change to the test manufacturer used during serial monitoring of a patient should be accompanied by additional sequential testing to confirm baseline values. The results reported by the laboratory to the physician must include the identity of the CEA test used."

D Special Instrument Requirements:

VITROS 5600 Integrated System (K081543)

IV Device/System Characteristics:

A Device Description:

VITROS Immunodiagnostic Products CEA Reagent Pack is supplied ready for use and contains:

- 100 coated wells (antibody, mouse monoclonal anti-CEA, binds ≥8 ng CEA/well)
- 9.7 mL assay reagent (buffer containing bovine serum albumin, bovine gamma globulin and antimicrobial agent)
- 9.7 mL conjugate reagent (Horseradish Peroxidase labeled mouse monoclonal anti-CEA, binds ≥123 ng CEA/mL) in buffer with bovine serum albumin and antimicrobial agent

VITROS CEA Calibrators are supplied ready to use and include:

- 1 set of VITROS CEA Calibrators 1 and 2 (human CEA in bovine serum with antimicrobial agent, 2 mL); nominal values 3 and 250 ng/mL
- Lot calibration card
- Protocol card
- 16 calibrator bar code labels (8 for each calibrator)

Materials Required but not Provided:

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- VITROS Immunodiagnostic Products High Sample Diluent B
- Quality control materials
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

The VITROS Immunodiagnostic Products CEA Reagent Pack has been modified from the previously cleared assay which is susceptible to interference from biotin. The modification was made to allow the biotinylated antibody capture conjugate to be pre-bound to the well, which eliminates the risk of biotin interference.

B Principle of Operation:

An immunometric immunoassay technique is used. CEA present in the sample reacts with a biotinylated antibody (mouse monoclonal anti-CEA) bound to streptavidin on a microwell. In the next step, a horseradish peroxidase (HRP)-labelled antibody conjugate (mouse monoclonal anti-CEA) binds to the immobilized CEA. 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 system. The amount of HRP conjugate bound and light produced is directly proportional to the concentration of CEA present in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

VITROS Immunodiagnostic Products CEA Reagent Pack/Calibrator/Range Verifiers

B Predicate 510(k) Number(s):

K041322

C Comparison with Predicate(s):

Device & Predicate Device(s):	K231517	K041322
Device Trade Name	VITROS Immunodiagnostic Products CEA Reagent Pack	VITROS Immunodiagnostic Products CEA Reagent Pack
General Device Characteristic Similarities		
Intended Use/ Indications For Use	For the quantitative measurement of carcinoembryonic antigen	For the quantitative measurement of carcinoembryonic antigen (CEA) concentration in human

	(CEA) concentration in human serum and plasma (EDTA or heparin) using the VITROS 5600 Integrated System, to aid in the prognosis and management of cancer patients in whom changing concentrations of CEA are observed.	serum and plasma (EDTA or heparin) using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems, to aid in the prognosis and management of cancer patients in whom changing concentrations of CEA are observed.
Type of Test	Quantitative	Same
Methodology	Chemiluminescent immunoassay	Same
Sample Matrix	Serum, plasma (EDTA, heparin)	Same
Sample Volume	20 µL	Same
Analyte	CEA	Same
Capture Antibody	Mouse monoclonal anti-CEA	Same
Detection Antibody	HRP-labelled mouse monoclonal anti-CEA antibody	Same
Detection	Luminescence	Same
Calibrators	Two user calibrators with master calibration	Same
Traceability	Calibrated against First International Reference Preparation 72/225	Same
Measuring Range	0.31– 400 ng/mL	Same
Detection Limit	LoB: 0.08 ng/mL LoD: 0.31 ng/mL LoQ: 0.31 ng/mL	LoB: 0.06 ng/mL LoD: Same LoQ: not reported
General Device Characteristic Differences		
Wells	Biotinylated antibody pre-coated on the well	No biotinylated antibody pre-coated on the wells
Instrument Platform	VITROS 5600 Integrated System	VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- 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
- CLSI EP35, Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures – First Edition
- CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition

VII Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the VITROS Immunodiagnostic Products CEA Reagent Pack was evaluated by testing a panel of four serum sample pools on one VITROS 5600 System using three reagent lots. The CEA concentration of the samples were at 6.44, 40.8, 223 and 381 ng/mL. Testing was performed with two replicates per run, two runs per day over 20 days, yielding a total of 80 measurements for each reagent lot. Imprecision was calculated by combining the data for three reagent lots (N=240 datapoint per sample) and the results are summarized in the table below:

Sample	N	Mean (ng/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP1	240	6.44	0.1	1.7%	0.1	1.5%	0.1	1.5%	0.1	2.1%	0.2	3.4%
PP2	240	40.8	0.6	1.5%	0.8	2.1%	0.6	1.5%	0.5	1.1%	1.3	3.1%
PP3	240	223	4.3	1.9%	3.4	1.5%	3.2	1.4%	4.5	2.0%	7.8	3.5%
PP4	240	381	5.6	1.5%	6.6	2.1%	6.0	1.6%	8.7	2.3%	13.7	3.6%

2. Linearity:

Linearity was evaluated per CLSI EP06, 2nd edition. A low pool of CEA stripped human serum sample and a high pool (CEA high patient sample), with a target dose above the top end of the measuring interval of 400 ng/mL, were mixed to create 13 levels ranging from

0.22 to 499.6 ng/mL. Each sample was tested in five replicates using one lot of the modified VITROS Immunodiagnostic Products CEA Reagent Pack on one VITROS 5600 System. For each level, the mean of the measured values, predicted value and the deviation from linearity were calculated. Predicted values were calculated using a best fitted straight line by a weighted least squares linear regression analysis. Percent deviations from linearity were calculated as differences between the observed values (mean values) and the predicted values divided by the predicted values. The study results are summarized in the following table:

Dilution Range (ng/mL)	Slope	Intercept	R2	% Deviation from Linearity
0.22 to 499.6	1.043	-0.067	0.999	-4.4% – 4.5%

Linearity was demonstrated from 0.22 ng/mL to 499.6 ng/mL with deviations from linearity within ±10%. The results support the linearity of the claimed analytical measuring range (AMR): 0.31 – 400 ng/mL.

Dilution Study:

To assess the automated dilution feature, samples were prepared with CEA concentrations targeted at 12,000 ng/mL, 18,000 ng/mL, 24,000 ng/mL, 30,000 ng/mL, 38,000 ng/mL, 55,000 ng/mL, and 75,000 ng/mL by spiking recombinant CEA analyte into seven unique native human serum samples. These samples were tested in duplicate using one lot of each predicate device and the modified VITROS Immunodiagnostic Products CEA Reagent Pack using the automatic 1:100 dilution performed by the analyzer. The 55,000 ng/mL and 75,000 ng/mL samples were diluted 1:5 manually before being further diluted 1:100 by the analyzer. The value of each sample obtained from the modified VITROS Immunodiagnostic Products CEA Reagent Pack was compared to the expected value and percent recovery was calculated. The results support an automatic sample dilution of 1:100 for the modified VITROS Immunodiagnostic Products CEA Reagent Pack.

Hook Effect

The hook effect was assessed using a high dose hook panel that was prepared with VITROS high sample diluent (HSDB) (containing no measurable CEA) spiked with endogenous CEA antigen to produce a sample with a concentration of approximately 546,000 ng/mL. This sample was diluted with VITROS HSDB to produce a set of 10 samples (the High Dose Hook Panel) with concentrations spanning between 273 and 546,000 ng/mL. Samples were tested in singlicate using the modified VITROS Immunodiagnostic Products CEA Reagent Pack on one VITROS 5600 System. The claim for the hook effect for the modified VITROS Immunodiagnostic Products CEA Reagent is up to 80,000 ng/mL.

3. Analytical Specificity/Interference:

Interference:

Potential interfering substances were tested for their ability to interfere with the modified VITROS Immunodiagnostic Products CEA Reagent Pack using procedures recommended in the CLSI EP07-A3. Each potential endogenous and exogenous interfering substance was

tested at two analyte concentrations: 3 ng/mL and 15 ng/mL. Test samples were spiked with the test substance and results were compared to matched control samples spiked with an equal volume of solvent (blank). The CEA in the test samples and control samples were measured in five replicates using each of three reagent lots on the modified VITROS Integrated 5600 System. The recovery was calculated by comparing measurements of the test and control samples. No interference (≤ ±10% difference of test from control) for the modified VITROS Immunodiagnostic Products CEA Reagent Pack was seen, up to the concentrations of the potential interfering substances tested as shown in the tables below.

Endogenous Substance	Concentration
Bilirubin, conjugated	100 mg/dL
Bilirubin, unconjugated	100 mg/dL
Biotin	0.351 mg/dL
Cholesterol, total	400 mg/dL
HAMA (Human Anti-Mouse Antibodies)	800 µg/L
Intralipid	2000 mg/dL
Rheumatoid Factor	900 IU/mL
Total Protein	15 g/dL
Triglycerides, total	1500 mg/dL

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Acetaminophen	20.3 mg/dL	5-Fluorouracil	34.8 mg/dL
N-Acetylcysteine	15.0 mg/dL	Furosemide	1.59 mg/dL
Acetylsalicylic acid	50 mg/dL	Gamma globulin	6 g/dL
Alpha-tocopherol	6.45 mg/dL	Hydralazine	1.44 mg/dL
Aminoglutethimide	39.8 mg/dL	Hydrocodone	0.0072 mg/dL
Amoxicillin	5.40 mg/dL	Ibuprofen	71 mg/dL
Ascorbic acid	6 mg/dL	Levothyroxine	0.0429 mg/dL
Bleomycin	300 mg/dL	Loratadine	0.0087 mg/dL
Carbamazepine	4.50 mg/dL	Methotrexate	136 mg/dL
Cefoxitin sodium	695 mg/dL	Mitomycin C	5.52 mg/dL
Cholecalciferol	19.2 µg/dL	Morphine	0.78 mg/dL
Cisplatin	1.3 mg/dL	Naproxen	36.0 mg/dL
Codeine	0.141 mg/dL	Omeprazole	0.840 mg/dL
Cotinine	0.24 mg/dL	Phenytoin	6.00 mg/dL
Cyclophosphamide	54.9 mg/dL	Prednisone	0.010 mg/dL
Dextran 40	1200 mg/dL	Tamoxifen	4.8 ug/dL
Dextromethorphan	0.00156 mg/dL	Theophylline	6.0 mg/dL
Doxorubicin	5.2 mg/dL	Vancomycin	12.3 mg/dL
Enoxaparin	360 U/dL	Vinblastine	138 mg/dL
Ethanol	600 mg/dL	Vincristine	140 mg/dL
Etoposide	83 mg/dL		

Cross-reactivity:

The cross-reactivity of the VITROS Immunodiagnostic Products CEA Reagent Pack was evaluated by adding Non-specific Cross-reacting Antigen 1 (NCA 1) at 500 ng/mL to a

sample containing no CEA and testing in three lots of reagent. No cross-reactivity was detected.

4. Assay Reportable Range:

The assay measuring interval for the modified VITROS Immunodiagnostic Products CEA Reagent Pack is the same as the claimed analytical measuring range of the predicate: 0.31 – 400 ng/mL. The instrument has a 1:100 auto-dilution feature for the device. The claimed extended measuring range is from 400 ng/mL to 40,000 ng/mL. Samples with concentrations above 40,000 ng/mL will be reported as > 40,000 ng/mL.

The reportable range of the VITROS Immunodiagnostic Products CEA Reagent Pack is 0.31 – 40,000 ng/mL.

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

Traceability:

The calibration of the VITROS Immunodiagnostic Products CEA Reagent Pack is traceable to NIBSC First International Reference Preparation 72/225.

Stability:

Long term stability and on-board storage performance was evaluated consistent with methods based on CLSI EP25-A.

Shelf-life (Long Term) of the modified VITROS Immunodiagnostic Products CEA Reagent Pack was evaluated by using three lots of reagents stored at 2–8°C. At each monthly interval, three serum samples with CEA concentrations of 5, 20 and 250 ng/mL were tested in four runs on each of three lots. The data of each sample at each testing time point were compared to the data of initial value tested at T0. The data support a shelf-life of the modified VITROS Immunodiagnostic Products CEA Reagent Pack up to 52 weeks when stored at 2–8°C.

On-board stability of the modified VITROS Immunodiagnostic Products CEA Reagent Pack was evaluated using three lots of Reagent Packs and Calibrators that were stored opened on the instrument for up to 12 weeks. Four runs were performed by testing Calibrators and QC In-house Controls on each lot at each time point for fresh and open. The results support the claim of 8 weeks on-board stability.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the VITROS Immunodiagnostic Products CEA Reagent Pack were verified based on the CLSI EP17-A2 guideline.

The LoB was determined by testing four analyte stripped serum sample pools with three reagent lots. For each lot, samples were tested two replicates per run, two runs per day over five test days, resulting a total of 80 datapoints per lot. LoB was defined as the value

corresponding to the 95th percentile of the rank position of the distribution of values. The highest LoB value observed across three reagent lots was 0.08 ng/mL.

The LoD was determined by testing five samples containing CEA at one to five times the LoB concentration with three reagent lots. For each lot, the samples were run in six replicates per run, two runs per day, for five days, yielding a total of 300 datapoints per lot. The LoD was calculated using a parametric approach. The highest LoD value observed across lots was 0.15 ng/mL. The claimed LoD is 0.31 ng/mL.

The LoQ was determined based on the data from the LoD study described above, using precision profile approach, with goal of precision of 20% CV. The highest LoQ value observed across lots was 0.15 ng/mL. The result supports the claimed LoQ as 0.31 ng/mL.

7. Assay Cut-Off:

Refer to K041322

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were conducted by testing 110 serum samples in singlicate using one reagent lot of the modified VITROS Immunodiagnostic Products CEA Reagent Pack (candidate device) and one reagent lot of the unmodified VITROS Immunodiagnostic Products CEA Reagent Pack (predicate device) on one VITROS 5600 System. The samples covered the full expected measuring interval of the assay. Weighted Deming regression was performed by comparing the data collected from the modified (y) and unmodified (x) devices. The results are summarized in the following table:

N	Range (ng/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2
110	0.56 – 396	1.01 (0.997; 1.012)	0.106 (-0.016; 0.373)	0.999

2. Matrix Comparison:

A study was performed to demonstrate that lithium heparin plasma and EDTA plasma matrices yield comparable values as serum on the modified VITROS Immunodiagnostic Products CEA Reagent Pack. The study included 40 matched samples with CEA concentration covering the measuring range of the assay were tested. Deming regression analysis was performed, and the corresponding slopes of regression and coefficient determination are summarized in the table below:

	N	Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	R
Li-heparin plasma vs serum	40	0.950 - 351	0.998 (0.977; 1.019)	-0.118 (-0.835; -0.600)	0.999
K2-EDTA plasma vs serum	40	0.910 - 372	0.995 (0.946; 1.044)	-0.877 (-3.056; 1.302)	0.998

C Clinical Studies:

1. Clinical Sensitivity:

   Refer to K041322

2. Clinical Specificity:

   Refer to K041322

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

   Refer to K041322

D Clinical Cut-Off:

Refer to K041322

E Expected Values/Reference Range:

The Expected Values for the unmodified VITROS Immunodiagnostic Products CEA Reagent Pack (predicate) was established based on the results of a study of 768 specimens obtained from healthy subjects and diseased patients.

To verify the expected results, serum samples from a total 68 healthy nonsmokers and 72 healthy smokers were tested in singlicate, on the VITROS 5600 System, using one lot of the modified VITROS Immunodiagnostic Products CEA Reagent Pack (candidate device), according to CLSI EP28-A3c. The results are summarized in the following table:

Category	N	Percent (%)			
		0–3.0 ng/mL	>3.0–5.0 ng/mL	>5.0–10.0 ng/mL	>10.0 ng/mL
Healthy Subjects					
Nonsmokers	68	89.7	7.4	2.9	0
Smokers	72	72.2	22.2	5.6	0

The results verified the reference range established previously.

The labeling of the VITROS Immunodiagnostic Products CEA Reagent Pack includes the following expected values for CEA among healthy subjects and diseased patients.

	N	Percent (%) of Population by CEA Concentration			
		0 – 3.0 ng/mL	>3.0 – 5.0 ng/mL	>5.0 – 10.0 ng/mL	>10.0 ng/mL
Healthy Subjects					
Nonsmokers	149	91.9	6.0	0.7	1.3
Smokers	101	67.3	22.8	8.9	1.0
Total	250	82.0	12.8	4.0	1.2
Malignant Diseases					
Colorectal	114	7.0	10.5	9.7	72.8
Breast	69	30.4	13.0	11.6	44.9
Pulmonary	56	28.6	19.6	16.1	35.7
Ovarian	51	72.6	15.7	5.9	5.9
Gastrointestinal	40	22.5	17.5	12.5	47.5
Nonmalignant Disease					
Gastrointestinal	42	73.8	19.0	4.8	2.4
Cirrhosis	65	50.8	16.9	20.0	12.3
Pulmonary	50	76.0	16.0	4.0	4.0
Hepatitis	31	77.4	16.1	6.5	0.0

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.