510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221355

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products CA 125 II Reagent Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LTK	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:

Cancer Antigen (CA) 125

C Type of Test:

Quantitative, immunochemiluminescent assay

III Intended Use/Indications for Use:

A Intended Use(s):

For the quantitative measurement of OC 125 defined antigen concentration in human serum and plasma (EDTA or heparin) using the VITROS 5600 Integrated System. The VITROS CA 125 II assay is to be used as an aid in monitoring response to therapy for patients with epithelial ovarian cancer. Serial testing for patient CA 125 assay concentrations should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

B Indication(s) for Use:

Same as Intended Use

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Different test methods cannot be used interchangeably. OC 125 defined antigen in a given patient sample determined with different tests and from different manufacturers can vary due to differences in test methods and reagent specificity. A change to the test used during serial monitoring of a patient should be accompanied by additional sequential testing to confirm baseline concentrations. The results reported by the laboratory to the physician must include the identity of the CA 125 II test used.

D Special Instrument Requirements:

VITROS 5600 Integrated System (K081543)

IV Device/System Characteristics:

A Device Description:

VITROS Immunodiagnostic Products CA 125 II Reagent Pack is supplied ready for use and contains:

- 100 coated wells (antibody, mouse monoclonal anti-OC 125, binds ≥32.5 U per well)
- 9.4 mL conjugate reagent (Horseradish Peroxidase labeled mouse monoclonal anti-OC 125, binds ≥542 U/mL) in buffer with bovine serum albumin, bovine gamma globulin and antimicrobial agent
- 9.4 mL assay reagent (buffer containing bovine serum albumin, bovine gamma globulin and antimicrobial agent)

VITROS CA 125 II Calibrators are supplied ready to use and contains:

- 1 set of VITROS CA 125 II calibrators with 1, 2 and 3 levels (OC 125 defined antigen in buffer with bovine serum albumin and antimicrobial agent, 1.75 mL); target values 24.0; 130 and 875 U/mL
- Lot calibration card
- Protocol card

- 24 calibrator bar code labels (8 for each calibrator)

The VITROS Immunodiagnostic Products CA 125 II 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 chemiluminescent immunoassay is used. OC 125 defined antigen present in the sample reacts with a biotinylated antibody (mouse anti-OC 125) bound to streptavidin on a microwell. Unbound sample is removed by washing. In a second incubation, a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-OC 125) binds to the immobilized OC 125 antigen. 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 is directly proportional to the concentration of OC 125 present.

V Substantial Equivalence Information:

A Predicate Device Name(s):

VITROS Immunodiagnostic Products CA 125 II Calibrators, VITROS Immunodiagnostic Products CA 125 II Reagent Pack

B Predicate 510(k) Number(s):

K983875

C Comparison with Predicate(s):

Device & Predicate Device(s):	K221355 Device	K983875 Predicate
Device Trade Name	VITROS Immunodiagnostic Products CA 125 II Reagent Pack	VITROS Immunodiagnostic Products CA 125 II Reagent Pack
General Device Characteristic Similarities		
Intended Use/ Indications For Use	For the quantitative measurement of OC 125 defined antigen concentration in human serum and plasma (EDTA or heparin) using the VITROS 5600 Integrated System. The VITROS CA 125 II assay is to be used as an aid in monitoring response to therapy for	Same

	patients with epithelial ovarian cancer. Serial testing for patient CA 125 assay concentrations should be used in conjunction with other clinical methods used for monitoring ovarian cancer.	
Assay Principle	Immunometric	Same
Antibody	Mouse monoclonal anti-OC 125	Same
Sample Type	Serum and plasma (EDTA or heparin)	Same
Sample Volume	25 µl	Same
Traceability	Calibration is traceable to in-house reference calibrators which have been value assigned to correlate to another commercially available test.	Same
Analytical Measuring Range	5.5 – 1000 (U/mL)	Same
Calibrator Levels	3	Same
Instrument	VITROS 5600 Integrated System	Same
General Device Characteristic Differences		
Detection Limit	LoB: 0.2 U/mL LoD: 5.5 U/mL LoQ: 5.5 U/mL	LoB: 2.9 (U/mL) LoD: Same LoQ: Same
Assay Plate/ Protocol	Biotinylated capture antibody pre-bound to the well	No biotinylated capture antibody pre-bound to the well

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third 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 EP37, Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures
- CLSI EP28-A3c, Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack was evaluated by testing a panel of six serum samples on one VITROS 5600 System using three reagent lots. The samples with OC 125 concentrations had target concentrations of 8, 30, 100, 260, 400 and 800 U/mL. Each sample was tested in two replicates per run, two runs per day, for 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/sample). The results are summarized in the table below:

Sample	Mean (U/mL)	Within-Run		Between-Run		Within-Day		Between-Day		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV %
1	9.09	0.09	1.0	0.08	0.9	0.15	1.6	0.18	1.9	0.00	0.0	0.21	2.4
2	29.5	0.24	0.8	0.25	0.8	0.39	1.3	0.43	1.4	0.06	0.2	0.55	1.9
3	105	1.04	1.0	0.87	0.8	1.56	1.5	1.30	1.2	0.79	0.8	2.04	1.9
4	268	2.06	0.8	2.85	1.1	3.86	1.4	2.81	1.1	1.33	0.5	4.70	1.8
C1	401	3.02	0.8	4.92	1.2	6.28	1.6	4.30	1.1	1.50	0.4	7.35	1.8
C2	767	6.90	0.9	5.92	0.8	9.91	1.3	6.25	0.8	4.02	0.5	11.74	1.5

2. Linearity:

A linearity study was performed according to CLSI EP06-A2. One pool of human sera with OC 125 concentration above 1000 U/mL was mixed with a serum pool that had been stripped of OC 125 to produce a series of 15 dilution samples. Each dilution sample was tested in five replicates on one VITROS 5600 System using one reagent lot of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack. The results are summarized in the table below.

Range (U/mL)	Slope (95% CI)	Y-Intercept (U/mL) (95% CI)	R^2	% Recovery
3.6 – 1288	0.992 (0.985; 0.999)	-0.932 (-1.075; 0.788)	1.00	80.0%* to 101.0%

* The recovery of 80% was observed for sample with the concentration of 3.6 U/mL.

The results support the linearity of the following claimed analytical measuring range (AMR): 5 – 1000 U/mL.

3. Dilution study:

For automatic dilution, five samples were diluted on the VITROS 5600 System using VITROS High Sample Diluent B (HSDB). Each sample was diluted 1:20 (1-part sample to

19-parts diluent) and run in five replicates, on one reagent lot of the updated VITROS Immunodiagnostic Products CA 125 II Reagent Pack and one lot of the predicate using one VITROS 5600 System. The percent recovery results on the updated reagent lot were compared to the percent recoveries obtained on the predicate lot. The results support an automatic sample dilution ratio of 1:20 for the updated VITROS Immunodiagnostic Products CA 125 II Reagent Pack.

4. Analytical specificity/interference:

Potential interfering and cross-reacting substances were tested for their ability to cross react or interfere with the performance of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack using procedures based on the guidelines CLSI EP07 and CLSI EP37.

Each potential interfering substance was tested at two analyte concentrations: ~10 U/mL and ~50 U/mL. Test samples were prepared by spiking with the potential endogenous and exogenous interfering substances at two different levels (low level of interferant and high level of interferant). Results were compared to matched control samples which were spiked with an equal volume of solvent (blank). The CA 125 in the test samples and control samples were measured in five replicates using each of three reagent lots on the VITROS 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 VITROS Immunodiagnostic Products CA 125 II Reagent Pack up to the concentrations of the potential interfering substances tested as shown in the table below:

Endogenous Substance	Concentration
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Cholesterol	400 mg/dL
HAMA (Human Anti-Mouse Antibodies)	800 µg/L
Hemoglobin*	750 mg/dL
Rheumatoid Factor (RF)**	975 U/mL
Total protein ***	11.5 g/dL
Triglycerides	1500 mg/dL

* The bias showed ≥10% for hemoglobin when tested at level of 1000 mg/dL.
** The bias showed ≥10% for RF when tested at level of 1043 U/mL.
*** The bias showed ≥10% for total protein when tested at level of 15.8 g/dL.

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Acetaminophen	20.0 mg/dL	Etoposide	6.0 mg/dL
N-Acetylcysteine	15.0 mg/dL	5-Fluorouracil	9.76 mg/dL
N-Acetylcysteine	15.0 mg/dL	5-Fluorouracil	9.76 mg/dL
Acetylsalicylic acid	50 mg/dL	Furosemide	1.59 mg/dL
Alpha-tocopherol	6.45 mg/dL	Gemcitabine	7.0 mg/dL
Amoxicillin	5.40 mg/dL	Hydralazine	1.44 mg/dL
Ascorbic acid	300 mg/dL	Hydrocodone	0.0072 mg/dL
Bevacizumab	24.0 mg/dL	Ibuprofen	70.0 mg/dL
Biotin	0.351 mg/dL	Leucovorin	15.0 mg/dL
Carboplatin	15.0 mg/dL	Levothyroxine	0.0429 mg/dL
Cefoxitin sodium	695 mg/dL	Loratadine	0.0087 mg/dL
Chlorpromazine	1.0 mg/dL	Methotrexate	454 mg/dL
Cholecalciferol (D3)	19.2 µg/dL	Metoclopramide	1.0 mg/dL
Cisplatin	100 mg/dL	Mitomycin C	300 µg/dL
Codeine	0.141 mg/dL	Morphine	0.780 mg/dL
Cotinine	0.24 mg/dL	Naproxen	36.0 mg/dL
Coumadin	1.4 mg/dL	Olaparib	1.71 mg/dL
Cyclophosphamide	54.9 mg/dL	Omeprazole	0.840 mg/dL
Dexamethasone	135 µg/dL	Paclitaxel	1.0 mg/dL
Dextran 40	2400 mg/dL	Phenytoin	6.00 mg/dL
Dextromethorphan	0.00156 mg/dL	Salicylic acid	2.86 mg/dL
Dimenhydrinate	1.0 mg/dL	Theophylline	6.0 mg/dL
Diphenhydramine	1.0 mg/dL	Topotecan	0.0019 mg/dL
Docetaxel	1.326 mg/dL	Vinblastine	0.0084 mg/dL
Doxorubicin hydrochloride	75 µg/dL	Vancomycin hydrochloride	12.3 mg/dL
Enoxaparin	360 U/dL	Vinorelbine	0.19 mg/dL
Ethanol	600 mg/dL		

5. Assay reportable range:

   The assay reportable range for the VITROS (CA 125 II) is the same as the analytical measuring range, i.e., 5.5 to 1000 U/mL.

6. Traceability, stability, expected values (controls, calibrators, or methods):

   Traceability:

   The calibration of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack is traceable to in-house reference material which has been value assigned to correlate to another commercially available test. The traceability has not changed since the original clearance of the device under K983875.

   Stability:

Shelf-life (unopened) of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack was evaluated using three lots of kit reagents according to the recommendation of CLSI EP25-A. Each lot was stored at 2–8°C and tested in four runs at baseline and monthly thereafter for 12 months with one month post expiration. The data support a shelf-life of VITROS Immunodiagnostic Products CA 125 II Reagent Pack up to four months (16 weeks) weeks when stored at 2–8°C.

Opened in-use stability of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack was assessed using three lots of kit reagents stored in on-board conditions. Each lot was tested in four runs at baseline and six additional time points over 8 weeks. Fresh reagent packs were tested at each time point as reference. The data support an on-board stability of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack up to 7 weeks. Additionally, reagent packs, when stored open off-board the VITROS systems are required to be stored in reagent pack boxes containing dried desiccant at 2–8°C. These are hermetically sealed containers, which are not subject to varying humidity levels or environmental influences that may be experienced during open, on-system storage. Therefore, the open, on-board system storage stability provides worst case conditions and as a consequence, the open off-storage claim is restricted to that supported by the Open, On-board Stability trial (up to 7 weeks).

The stability of calibrator and sample stability were previously established per K983875.

7. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the VITROS Immunodiagnostic Products CA 125 II Reagent Pack were verified based on the CLSI EP17 guideline.

The LoB was determined by testing four analyte stripped serum pools containing no measurable CA 125 using three reagent lots. For each lot, LoB samples were run in two replicates per run, two runs per day, for five days yielding a total of 80 replicates 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 0.2 U/mL. The claimed LoB is 0.2 U/mL

The LoD was determined by testing five samples containing low levels of CA 125 at 1 to 5 times the LoB concentration using 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 replicates per lot. The LoD was calculated using a parametric approach. The highest LoD value observed across lots was 0.5 U/mL. The claimed LoD is 5.5 U/mL.

The LoQ was determined based on the study data from the LoD study described above and defined as the lowest value with precision less than or equal to the precision of 20% CV. The highest LoQ value observed across lots was 0.7 U/mL. The result support the claimed LoQ as 5.5 U/mL.

8. Assay cut-off:

See clinical cut-off

B Comparison Studies:

1. Method comparison with predicate device:

   Method comparison studies were conducted by testing 146 serum samples in singlicate using one reagent lot of the modified VITROS Immunodiagnostic Products CA 125 II Reagent Pack (candidate device) and one reagent lot of the unmodified VITROS Immunodiagnostic Products CA 125 II Reagent Pack (predicate device) on one VITROS 5600 System. 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 (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2
146	5.74 -1000	1.02 (1.01; 1.03)	-0.45 (-1.44; -0.17)	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 CA 125 II Reagent Pack. A total of 49 samples spread across the assay range were assayed in singlicate using one reagent lot and one analyzer. Passing Bablok regression analysis was performed, and the corresponding slopes of regression and coefficient determination are summarized in the table below:

	N	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation
Li-heparin plasma vs serum	49	6.2-988	0.984 (0.98; 0.99)	0.160 (-0.02; 0.34)	1.00
K2-EDTA plasma vs serum	49	6.3-997	0.990 (0.98; 0.99)	0.162 (-0.10; 0.42)	1.00

C Clinical Studies:

1. Clinical sensitivity and clinical specificity:

   Refer to K983875

2. Other clinical supportive data (when 1. and 2. are not applicable):

   Not applicable

D Clinical Cut-Off:

Refer to K983875. For the VITROS Immunodiagnostic Products CA 125 II Reagent Pack, a clinically meaningful change is defined as a reading at least 25% higher of CA 125 value than the previous reading when the VITROS CA 125 result was outside the normal range (> 35 U/mL).

E Expected Values/Reference Range:

The references range for the unmodified VITROS Immunodiagnostic Products CA 125 II Reagent Pack (predicate) was previously established based on a study of testing 200 samples from normal females. The results indicated that 98.5% (197 out of 200) of the samples were found to be ≤ 35 U/mL of CA 125.

To verify this reference range, serum samples from a total of 120 apparently healthy non-smoking individuals including 30 females less than 50 years of age, 30 females more than 50 years of age and 60 males less than 50 years of age were each tested on three lots of the modified VITROS Immunodiagnostic Products CA 125 II Reagent Pack (candidate device) according to CLSI EP28-A3c. Only one female sample was found to be > 35 U/mL. The rest of the female samples (98.3%) are all < 35 U/mL across three lots.

The labeling of the VITROS Immunodiagnostic Products CA 125 II Reagent Pack includes the following expected values (refer to K983875) for CA 125 in malignant and non-malignant conditions, in addition to the values for CA 125 in healthy subjects:

	N	CA 125 Concentration			
		≤ 35 U/mL	**35.1–65 U/mL**	**65.1–100 U/mL**	**> 100 U/mL**
Malignant Disease					
Ovarian	75	21	7	7	40
Gastrointestinal	30	21	2	1	6
Others*	40	32	2	0	6
Non-malignant liver disease					
Gynecological	50	48	1	1	0
Pregnancy	30	25	1	2	2
Gastrointestinal	30	28	1	0	1
Healthy Subjects					
Normal Females	200	197	3	0	0
Normal Males	50	50	0	0	0

*Bladder, Kidney, Breast, Pancreas, and Colon

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.