510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K213626

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products AFP Reagent Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LOJ	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 and update the unit conversion factor.

B Measurand:

Alpha-fetoprotein (AFP)

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 alpha-fetoprotein (AFP) concentrations in human serum using the VITROS 5600 Integrated system to aid in the management of patients with non-seminomatous testicular cancer.

C Special Conditions for Use Statement(s):

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

D Special Instrument Requirements:

VITROS 5600 Integrated System (K081543)

IV Device/System Characteristics:

A Device Description:

VITROS Immunodiagnostic Products AFP Reagent Pack:

- 100 coated wells (antibody, sheep anti-AFP, binds ≥25 IU AFP/well)
- 20.6 mL conjugate reagent (HRP-mouse monoclonal anti-AFP, binds ≥156 IU AFP/mL) in buffer with bovine serum and antimicrobial agent
- 15.8 mL assay reagent (buffer containing bovine serum albumin and antimicrobial agent)

Calibrators:

- 1 set of VITROS AFP Calibrators 1, 2 and 3 (human cord serum/plasma derived AFP in human plasma with antimicrobial agent, 2 mL); nominal values 2, 22 and 220 IU/mL (1st International Reference Preparation 72/225) (2.42, 26.6 and 266 ng/mL)
- Lot calibration card
- Protocol card
- 24 calibrator bar code labels (8 for each calibrator)

The VITROS Immunodiagnostic Products AFP 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. In addition, the factor used for converting IU/mL to ng/mL has been changed from 1.04 to 1.21 in order to align the VITROS Immunodiagnostic Products AFP Reagent Pack to other vendors using the same conversion factor.

B Principle of Operation:

An immunometric chemiluminescent immunoassay is used. AFP present in the sample reacts with a biotinylated antibody (sheep anti-AFP) 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-AFP) binds to the immobilized AFP. 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 AFP present.

V Substantial Equivalence Information:

A Predicate Device Name(s):

VITROS Immunodiagnostic Products AFP Assay

B Predicate 510(k) Number(s):

K983031

C Comparison with Predicate(s):

Device & Predicate Device(s):	K213626	K983031
Device Trade Name	VITROS Immunodiagnostic Products AFP Reagent Pack	VITROS Immunodiagnostic Products AFP Assay
General Device Characteristic Similarities		
Intended Use/ Indications For Use	For the quantitative measurement of alpha-fetoprotein (AFP) concentrations in human serum using the VITROS 5600 Integrated system to aid in the management of patients with non-seminomatous testicular cancer.	Rx ONLY For in vitro diagnostic use only. For the quantitative measurement of alpha-fetoprotein (AFP) concentrations in human serum using the VITROS ECi/ECiQ/ 3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems to aid in the management of patients with non-seminomatous testicular cancer
Antibody	Monoclonal anti-AFP and Sheep anti-AFP	Same
Sample Type	Human serum	Same
Traceability	Calibrated against First International Reference Preparation 72/225.	Same

Measuring Range	0.800–500 IU/mL	Same
Detection Limit	LOB: 0.229 IU/mL LOD: 0.476 IU/mL LOQ: 0.800 IU/mL	LOB: Same LOD: Same LoQ: Not determined
Basic Principle	Sandwich immunoassay	Same
General Device Characteristic Differences		
Assay Plate/ Protocol	Biotinylated sheep anti-AFP pre-bound to the well	The biotinylated sheep anti-AFP is not pre-bound to the well
Conversion Factor between Units	IU/mL x 1.21 = ng/mL.	IU/ml x 1.04 = ng/mL
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:

- CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
- CLSI EP09c 3rd Edition Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- 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-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- 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 AFP Reagent Pack was evaluated by testing a panel of five serum samples on one VITROS 5600 System using three reagent lots. The samples with AFP concentrations targeted at 3, 12, 75, 240 and 400 IU/mL were prepared from normal and elevated AFP serum samples. Each sample was tested in two

replicates per run, two runs per day, for 20 days, for a total of 80 measurements for each reagent lot.

The within-laboratory precision for one representative lot is shown in the table below:

Sample	Mean (IU/mL)	Within-Run (Repeatability)		Between-Run		Between-Day		Within-Lab	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP1	2.9	0.06	1.9%	0.02	0.6%	0.04	1.2%	0.07	2.3%
PP2	11.9	0.20	1.6%	0.12	1.0%	0.24	2.0%	0.33	2.8%
PP3	77.0	1.51	2.0%	0.43	0.6%	1.43	1.9%	2.12	2.8%
PP4	235.6	7.5	3.2%	3.60	1.5%	4.86	2.1%	9.67	4.1%
PP5	395.4	9.6	2.4%	13.41	3.4%	6.90	1.7%	17.88	4.5%

Lot-to-lot imprecision was calculated by combining the data for three reagent lots and is summarized in the table below:

Sample	Mean (IU/mL)	Within-Run (Repeatability)		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
PP1	2.9	0.05	1.6%	0.04	1.3%	0.05	1.6%	0.04	1.5%	0.09	3.0%
PP2	11.8	0.17	1.5%	0.11	1.0%	0.19	1.6%	0.07	0.6%	0.29	2.4%
PP3	77.1	1.26	1.6%	1.30	1.7%	1.02	1.3%	0.21	0.3%	2.09	2.7%
PP4	240.0	6.30	2.6%	7.10	3.0%	3.60	1.5%	3.80	1.6%	10.80	4.5%
PP5	408.5	13.60	3.3%	11.10	2.7%	9.10	2.2%	11.70	2.9%	23.00	5.6%

2. Linearity:

A linearity study was performed according to CLSI EP06-A2. One pool of human sera with AFP concentration above 500 IU/mL was mixed with a serum pool that had been stripped of AFP to produce a series of 15 dilution samples. Each dilution sample were tested in five replicates on one VITROS 5600 System using three reagent lots of the VITROS Immunodiagnostic Products AFP Reagent Pack. The results are summarized in the table below.

Lot	Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI) (IU/mL)	R^2	% Recovery
1	0.412 to 723	0.937 (0.927; 0.948)	0.064 (0.047; 0.082)	0.995	88.9% to 114.0%
2	0.362 to 629	1.030 (1.016; 1.045)	0.037 (0.023; 0.050)	0.995	97.6% to 115.0%
3	0.329 to 665	0.992 (0.982; 1.001)	-0.004 (-0.013; 0.004)	0.998	94.9% to 104.0%

The results support the linearity of the claimed measuring range: 0.800 – 500 IU/mL.

Dilution Study

Studies of dilutional recovery were performed. For automatic dilution, five samples were diluted on the VITROS 5600 System using VITROS High Sample Diluent A (HSDA). Each sample was diluted 1:20 (1-part sample to 19-parts diluent) and 1:400 (1-part sample and 399-parts diluent) and run in five replicates, on one reagent lot of the updated VITROS Immunodiagnostic Products AFP Reagent Pack and one lot of the predicate using one VITROS 5600 System. For manual dilution, five samples were each diluted by two laboratory scientists using VITROS HSDA at 1:1000 (1-part sample to 999-parts diluent) and 1:4000 (1-part sample to 3999-parts diluent). Each sample was tested using one reagent lot of the modified VITROS Immunodiagnostic Products AFP Reagent Pack and one reagent lot of the predicate on one VITROS 5600 System.

The results support an automatic sample dilution ratio up to 1:400 and a manual sample dilution ratio up to 1:4000 for the updated VITROS Immunodiagnostic Products AFP Reagent Pack.

3. 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 AFP Reagent Pack using procedures based on the guidelines CLSI EP07 and CLSI EP37.

Interference:

Each potential interfering substance was tested at two analyte concentrations: 4.8 IU/mL (5.81 ng/mL) and 19.2 IU/mL (23.2 ng/mL). Test samples were prepared by spiking with the potential endogenous and exogenous interfering substances at two different levels. Results were compared to matched control samples which were spiked with an equal volume of solvent (blank) where appropriate. The AFP 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 ($\leq \pm 10\%$ difference of test from control) for the VITROS Immunodiagnostic Products AFP Reagent Pack up to the concentrations of the potential interfering substances tested as shown in the tables 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	500 mg/dL
Intralipid	2000 mg/dL
Rheumatoid factor	900 IU/mL
Total protein	15 g/dL
Triglycerides	1500 mg/dL

Exogenous Substance	Concentration	Exogenous Substance	Concentration
5-Fluorouracil	17 mg/dL	Etoposide	25 mg/dL
Acetaminophen	20 mg/dL	Furosemide	1.59 mg/dL
N-Acetylcysteine	15 mg/dL	Hydralazine	1.44 mg/dL
Acetylsalicylic acid	50 mg/dL	Hydrocodone	0.0072 mg/dL
Actinomycin D	50 mg/dL	Ibuprofen	40 mg/dL
Alpha-tocopherol	6.45 mg/dL	Levothyroxine	0.0429 mg/dL
Amoxicillin	5.4 mg/dL	Loratadine	0.0087 mg/dL
Ascorbic acid	300 mg/dL	Methotrexate	450 mg/dL
Biotin	0.351 mg/dL	Mitomycin C	0.72 mg/dL
Bleomycin sulfate	300 mg/dL	Morphine	0.78 mg/dL
Cefoxitin sodium	695 mg/dL	Naproxen	36 mg/dL
Cisplatin	100 mg/dL	Omeprazole	0.84 mg/dL
Codeine	0.141 mg/dL	Phenytoin	6 mg/dL
Cholecalciferol	19.2 µg/dL	Prednisone	0.01 mg/dL
Cotinine	0.24 mg/dL	Sorafenib	3 mg/dL
Cyclophosphamide	25 mg/dL	Theophylline	6 mg/dL
Dextran 40	2400 mg/dL	Vinblastine	100 mg/dL
Doxorubicin hydrochloride	1 mg/dL	Vancomycin hydrochloride	12.3 mg/dL
Enoxaparin	360 U/dL	Vincristine	70 mg/dL
Ethanol	600 mg/dL		

Cross Reactivity:

Each potential cross-reactant was spiked into a buffer matrix containing no AFP at the prescribed test levels. Test substances were spiked at a volume that constituted no more than 5% of the volume of the test sample. The AFP 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 detectable AFP (the observed value below the LoQ of the assay) for the VITROS Immunodiagnostic Products AFP Reagent Pack up to the concentrations of the following potential interfering substances tested: Human α-1-acid glycoprotein (200 mg/d), Human α-1- antitrypsin (500 mg/dL), Human ceruloplasmin (250 mg/dL), Human chorionic gonadotrophin (1,000,000 mIU/mL), Human IgG (6000 mg/dL), Human placental lactogen (2000 µg/dL), Human serum albumin (6000 mg/dL), Human transferrin (2500 mg/dL), and Prolactin (50,000 mIU/L).

4. Assay Reportable Range:

The analytical measuring range for the VITROS Immunodiagnostic Products AFP Reagent Pack is 0.800 IU/mL (0.968 ng/mL) to 500 IU/mL (605 ng/mL).

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

Traceability:

The calibration traceability of the VITROS Immunodiagnostic Products AFP Reagent Pack has not changed since the original clearance K983031. This assay has been standardized against the WHO/NIBSC First International Reference Preparation 72/225.

Stability:

Shelf-life (unopened) of the VITROS Immunodiagnostic Products AFP 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 the VITROS Immunodiagnostic Products AFP Reagent Pack up to 32 weeks when stored at 2–8 °C.

On-board stability of the VITROS Immunodiagnostic Products AFP Reagent Pack was assessed using three lots of kit reagents stored opened and refrigerated (2–8 °C). Each lot was tested in four runs at baseline and six additional time points over 12 weeks. Fresh reagent packs were tested at each time point as reference. The data support an on-board stability of the VITROS Immunodiagnostic Products AFP Reagent Pack up to 8 weeks.

6. Detection Limit:

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

The LoB was determined by testing four stripped serum pools containing no measurable AFP using three reagent lots. For each lot, LoB samples were run in two replicates per run, two runs per day for five days. LoB was defined as the value corresponding to the 95th percentile of rank position of the distribution of values. The highest LoB value observed across three lots was 0.112 IU/mL. The claimed LoB is 0.229 IU/mL.

The LoD was determined by testing five samples containing low levels of AFP 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. The LoD was calculated using a parametric approach. The highest LoD value observed across three lots was 0.161 IU/mL. The claimed LoD is 0.476 IU/mL.

The LoQ was determined by testing the same samples used for the LoD study using three reagent lots on one VITROS 5600 System over eight calendar days. The LoQ was defined as the value with precision less than or equal to the precision of 20% CV. The results support the claimed LoQ as 0.800 IU/mL.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Method comparison studies were conducted by testing 150 serum samples in singlicate using three reagent lots of the modified VITROS Immunodiagnostic Products AFP Reagent Pack (candidate device) and in duplicate using two reagent lots of the unmodified VITROS Immunodiagnostic Products AFP Reagent Pack (predicate device) on one VITROS 5600 System. For the modified device, each sample was tested with each of three reagent lots. For the unmodified device, each sample was tested using each lot and the mean for each sample was used in the comparison analysis. Weighted Deming regression was performed, and the results are summarized in the following table:

	N	Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI) (IU/mL)	R^2
Lot 1 vs Predicate	150	0.87 to 458	0.99 (0.99; 1.00)	0.02 (-0.03; 0.07)	1.00
Lot 2 vs Predicate	150	0.87 to 458	1.00 (0.99; 1.00)	0.05 (0.02; 0.08)	0.99
Lot 3 vs Predicate	150	0.87 to 458	1.01 (1.01; 1.02)	-0.03 (-0.07; 0.02)	1.00

2. Matrix Comparison:

   Not applicable; serum is the only claimed sample matrix.

C Clinical Studies:

1. Clinical Sensitivity:

   Refer to K983031

2. Clinical Specificity:

   Refer to K983031

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

   Not applicable

D Clinical Cut-Off:

Refer to K983031

E Expected Values/Reference Range:

The references range for the unmodified VITROS Immunodiagnostic Products AFP Reagent Pack (predicate) was established based the upper 97.5% of results of a study of 408 samples from normal blood donors as follows:

Unit = IU/mL	Unit = ng/mL
< 7.22	< 8.74

To verify this reference range, serum samples from a total of 60 apparently healthy non-smoking individuals including 30 females and 30 males were each tested on three lots of the modified VITROS Immunodiagnostic Products AFP Reagent Pack (candidate device) according to CLSI EP28-A3c. The results indicated that one male sample (1.67%) tested as 8.19, 8.77 and 8.42 IU/mL for three lots, and another male sample tested as 6.98, 7.26 and 7.00 IU/mL for three lots. The rest of the samples are all below <7.22 IU/mL across three lots. The established reference range was verified.

The labeling of the VITROS Immunodiagnostic Products AFP Reagent Pack includes the following expected values for AFP in malignant and non-malignant conditions, in addition to the values for AFP in 408 health subjectss:

	N	AFP concentration				
		0 – 7.22 IU/mL (0–8.74 ng/mL)	**7.23–15 IU/mL (8.75–18.2 ng/mL**	**15.1–100 IU/mL (18.3–121 ng/mL)**	**101–500 IU/mL (122–605 ng/mL**	**>500 IU/mL (>605 ng/mL)**
Cancer						
Primary Liver	36	2	2	1	7	24
Testicular non-seminomatous	117	25	10	24	17	41
Non-malignant liver disease						
Hepatitis A	50	47	3	0	0	0
Other	53	49	3	1	0	0
Healthy Subjects						
Males	210	202	8	0	0	0
Females	198	195	11	0	0	0

The conversion factor from IU/mL to ng/mL has changed from 1.04 to 1.21; the reference interval and expected ranges in clinical categories are increased by 16% when the result interpretation uses ng/mL. The labeling states: "*AFP results must be interpretated by comparison with the updated intervals.*"; "*Serial testing for monitoring of patients should be reported and interpreted with the same conversion factor and cutoff values.*"; "*It is recommended that each laboratory establish its own expected values for the population it serves."*

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.