510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231525

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS CA 19-9

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Carbohydrate Antigen (CA 19-9)

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 In Vitro Diagnostic Use Only

For the quantitative measurement of 1116-NS-19-9 defined antigen in human serum and plasma (EDTA or heparin), using the VITROS 5600 Integrated System. The VITROS CA 19-9 test is to be used to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The VITROS CA 19-9 test can be used to monitor the disease status in patients with confirmed pancreatic cancer who show measurable CA 19-9 values over the course of their disease. Serial CA 19-9 test results should be used in conjunction with all other available clinical and laboratory data before a medical decision is determined.

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 test methods cannot be used interchangeably. 1116-NS-19-9 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 values. The results reported by the laboratory to the physician must include the identity of the CA 19-9 test used."

D Special Instrument Requirements:

VITROS 5600 Integrated System (K081543)

IV Device/System Characteristics:

A Device Description:

VITROS Immunodiagnostic Products CA 19-9 Reagent Pack consists of the following materials:

<u>Materials Provided</u>

1) Reagent Pack:
   - 100 coated wells (antibody, mouse monoclonal anti-1116-NS-19-9 defined antigen, binds >49 U 1116-NS- 19-9 defined antigen/well)
   - 13.4 mL assay reagent (buffer containing bovine serum, bovine gamma globulin and antimicrobial agent)
   - 20.0 mL conjugate reagent (HRP-mouse monoclonal anti-1116-NS-19-9 defined antigen, binds ≥326 U 1116-NS-19-9 defined antigen/mL) in buffer with bovine serum albumin, bovine gamma globulin and antimicrobial agent.

2) Calibrators:
   - 1 set of VITROS CA19-9 Calibrators 1, 2, and 3 with nominal values of 15 U, 60 U and 700 U 1116-NS-19-9 defined antigen/mL; Ready to use
   - Lot calibration card
   - Protocol card
   - 24 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 CA 19-9 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:

When the analyte (1116-NS-19-9 defined antigen) is present in the patient sample, it binds to streptavidin/biotinylated Antibody (mouse monoclonal anti-1116-NS-19-9 defined antigen) that is coated to the microwell. In the next step, a horseradish peroxidase (HRP)-labelled antibody conjugate (Mouse monoclonal anti-1116-NS-19-9 defined antigen) binds to the immobilized 1116-NS-19-9 defined 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 conjugate bound is directly proportional to the concentration of 1116-NS-19-9 defined antigen present in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Vitros CA 19-9

B Predicate 510(k) Number(s):

K052889

C Comparison with Predicate(s):

Device & Predicate Device(s):	New Device K231525	Predicate K052889
Device Trade Name	VITROS Immunodiagnostic Products CA 19-9 Reagent Pack	VITROS CA 19-9
General Device Characteristic Similarities		
Intended Use/ Indications For Use	For the quantitative measurement of 1116-NS-19-9 defined antigen in human serum and plasma (EDTA or heparin), using the VITROS 5600 Integrated System. The VITROS CA 19-9 test is to be used to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The VITROS CA 19-9 test can be used to monitor the disease status in patients with confirmed pancreatic cancer who show measurable CA 19-9 values over the course of their disease. Serial CA 19-9 test results should be used in conjunction with all other available clinical and laboratory data before a medical decision is determined.	For the quantitative measurement of 1116-NS-19-9 defined antigen in human serum and plasma (EDTA or heparin). The VITROS CA 19-9 assay is to be used to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The VITROS CA 19-9 assay can be used to monitor disease status in patients with confirmed pancreatic cancer who show measurable CA 19-9 values over the course of their disease. Serial CA 19-9 test results should be used in conjunction with all other available clinical and laboratory data before a medical decision is determined.
Type of test	Quantitative	Same
Methodology	Chemiluminescence Immunoassay	Same
Sample Matrix	Human serum, plasma (EDTA, heparin)	Same
Sample Dilution	None, auto dilution up to 20-fold	Same
Sample Volume	35 µL	Same
Analyte	CA 19-9	Same
Capture antibody	Mouse monoclonal anti-1116-NS-19-9 defined antigen	Same
Detection antibody	HRP-labelled antibody conjugate (Mouse monoclonal anti-1116-NS-19-9 defined antigen)	Same
Detection	Luminescence	Same
Calibrators	3 levels: 15, 60 and 700 U/mL	Same
Detection Limit	LoB: 1.05 U/mL LoD: 1.4 U/mL LoQ: 1.4 U/mL	Same
Analytical measuring range	1.4–1000 U/mL	Same

General Device Characteristic Differences		
Wells	biotinylated antibody pre-coated on the well	No biotinylated antibody pre-coated on the wells
Instrument platform	VITROS 5600	VITROS 3600 VITROS 5600 VITROS XT 7600 VITROS ECi/ECiQ

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, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07, Interference Testing in Clinical Chemistry – Third Edition
- 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 (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack was evaluated by testing a panel of six serum samples on one VITROS 5600 Integrated System using three reagent lots. 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 datapoints per 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	6.5	0.1	2.1%	0.2	2.4%	0.2	3.2%	0.2	2.8%	0.2	2.6%	0.3	5.0%
2	28.4	0.5	1.9%	1.7	5.9%	1.8	6.2%	0.0	0.0%	0.8	2.8%	1.9	6.8%
3	105.0	1.8	1.7%	5.1	4.9%	5.5	5.2%	2.7	2.6%	2.0	1.9%	6.4	6.1%
4	221.0	3.5	1.6%	6.7	3.0%	7.7	3.4%	4.4	2.0%	1.6	0.7%	8.9	4.0%
5	297.0	5.3	1.8%	8.3	2.8%	9.8	3.3%	7.5	2.5%	3.9	1.3%	12.9	4.4%
6	703.0	11.2	1.6%	16.4	2.3%	19.8	2.8%	14.1	2.0%	1.2	0.2%	24.4	3.5%

2. Linearity:

A linearity study was performed according to CLSI EP06-A2. One pool of human sera with CA 19-9 concentration above 1000 U/mL was mixed with a low serum pool at around 0.9 U/mL to produce a series of 16 dilution samples. Each dilution sample was tested in five replicates on one VITROS 5600 Integrated System using one reagent lot of the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack. The data was analyzed using weighted least squares linear regression analysis comparing measured results to the predicate results. The results are summarized in the tables below:

Range (U/mL)	Slope (95% CI)	Y-Intercept (U/mL) (95% CI)	R^2	% Deviation from Linearity
0.9 – 1152	0.973 (0.957; 0.989)	0.145 (0.103; 0.186)	0.999	-12.3% – 14.1%*

* Sample at 0.86 U/mL showed %Deviation of -12.3%, Sample at 1.52 U/mL showed %deviation of 14.1%, sample at 2.35 U/mL showed % deviation of 13.0%. %Deviation is less than 10% for the rest of samples.

The results support the linearity of the following claimed analytical measuring interval (AMI): 1.4 – 1000 U/mL.

Dilution study:

For automatic dilution, six native samples were diluted on the VITROS 5600 Integrated System using VITROS High Sample Diluent B Reagent Pack. Six samples were at concentrations 1310 U/mL, 1810 U/mL, 2050 U/mL, 2780 U/mL, 5000 U/mL, and 9530 U/mL. Each sample was diluted 20-fold (1-part sample to 19-parts diluent) and run in five replicates, on one reagent lot of the updated VITROS Immunodiagnostic Products CA 19-9 Reagent Pack and one lot of the predicate using one VITROS 5600 Integrated 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 20-fold for the updated VITROS Immunodiagnostic Products CA 19-9 Reagent Pack.

3. Analytical Specificity/Interference:

Potential interfering substances were tested for their ability to interfere with the performance of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack using procedures recommended in the guidelines CLSI EP07-A3 and CLSI EP37.

Endogenous Interference:

Each potential endogenous interfering substance was tested at two analyte concentrations: ~5 U/mL and ~50 U/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 CA 19-9 in the test samples and control samples were measured in five replicates using each of three reagent lots on the 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 VITROS Immunodiagnostic Products CA 19-9 Reagent Pack up to the concentrations of the potential interfering substances tested as shown in the table below:

Substance	Concentration
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Biotin	0.351 mg/dL
Cholesterol, total	400 mg/dL
HAMA (Human Anti-Mouse Antibodies)	800 µg/L
Total Protein	15 g/dL
Triglycerides, total	3000 mg/dL

The device showed interference to hemoglobin and rheumatoid factor as follows:

Substance	Interferent Concentration	Measurand (U/mL)	% Bias
Hemoglobin	1000 mg/dL	7.1	190.1%
		45.2	30.5%
	125 mg/dL	47.5	0.8%
	100 mg/dL	6.1	2.0%
Rheumatoid Factor	1035 U/mL	5.0	27.4%
	981 U/mL	43.4	2.8%
	900 U/mL	32.5	5.2%
	199 U/mL	5.0	5.2%

Exogenous Interference:

The same protocol was used to test potential interference of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack to the exogenous substances. No interference (≤ ±10% difference of test from control) for the modified device up to the concentrations of the substances tested as shown in the table below:

Substance	Concentration	Substance	Concentration
Acetaminophen	50 mg/dL	Hydralazine	1.44 mg/dL
N-Acetylcysteine	15.0 mg/dL	Hydrocodone	0.0072 mg/dL
Acetylsalicylic acid	50 mg/dL	Ibuprofen	40 mg/dL
Alpha-tocopherol	6.45 mg/dL	Intralipid	2000 mg/dL
Amoxicillin	5.40 mg/dL	Leucovorin	11.4 mg/dL

Substance	Concentration	Substance	Concentration
Ascorbic acid	300 mg/dL	Levothyroxine	0.0429 mg/dL
Cefoxitin sodium	695 mg/dL	Loratadine	0.0087 mg/dL
Cetuximab	70.5mg/dL	Megestrol Acetate	2.26 µg/mL
Cholecalciferol (D3)	19.2 µg/dL	Methotrexate	136 mg/dL
Cisplatin	5.7 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	Omeprazole	0.840 mg/dL
Cyclophosphamide	54.9 mg/dL	Paclitaxel	6.7 mg/dL
Cytarabine	3 mg/dL	Phenytoin	6.00 mg/dL
Dextran 40	2400 mg/dL	Prednisone	0.010 mg/dL
Dextromethorphan	0.00156 mg/dL	Salicylic acid	2.86 mg/dL
Doxorubicin	4 mg/dL	Streptozocin	114 mg/dL
Enoxaparin	360 U/dL	Tamoxifen	51.9 ug/dL
Ethanol	600 mg/dL	Theophylline	6.0 mg/dL
Eribulin	1.12 µg/mL	Vancomycin	12.3 mg/dL
5-Fluorouracil	39 mg/dL	Vinblastine	0.0084 mg/dL
Furosemide	1.59 mg/dL	Vincristine	0.00162 mg/dL
Gemcitabine	38.2 mg/dL	Vinorelbine	0.19 mg/dL

4. Assay Reportable Range:

   The assay reportable range for the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack is the same as the analytical measuring range, from 1.4 to 1000 U/mL.

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

   Traceability:

   The calibration of the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack is traceable to in-house reference calibrators which have been value assigned to correlate to another commercially available test. The traceability has not changed since the original clearance of the device under K052889.

   Stability:

   Shelf-life (unopened) stability: The on-going real-time stability study was performed using three lots of kit reagents according to the recommendation of CLSI EP25-A. Each lot was stored at 2–8°C and tested with three samples (with concentration at 22.7, 75.3, and 230 U/mL) in four runs at baseline and monthly thereafter. The current data support a shelf-life of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack to 20 weeks when stored at 2–8°C.

   In-use (opened) stability: The study was conducted using three lots of kit reagents stored under on-board conditions (2–8°C). Each lot was tested with three samples, described in shelf-life stability, in four runs at baseline and six additional time points up to 12 weeks. The

data support an on-board stability of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack up to 8 weeks.

The sample stability and calibrator stability were previously established per K052889.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack were verified based on the CLSI EP17-A2 guideline.

The LoB was determined by testing four unique native serum pools containing no measurable CA 19-9 using three reagent lots. For each lot, 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 0.0 U/mL. No claim is shown in the device's labeling.

The LoD was determined by testing five samples containing low levels of CA 19-9 (three low native serum sample pools and two low level samples prepared by stripping high level sample to the target levels) 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 0.17 U/mL. The claimed LoD is 1.4 U/mL.

The LoQ was determined based on the study 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 0.53 U/mL. The results support the claimed LoQ as 1.4 U/mL.

7. Assay Cut-Off:

See clinical cut-off

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were conducted by testing 149 serum native samples in singlicate using one reagent lot of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack (candidate device) and two reagent lots of the unmodified VITROS CA 19-9 (predicate device) on one VITROS 5600 System. The results of samples tested within the analytical measuring range of both the candidate and the predicate device were performed with weighted Deming regression analysis by comparing the data from the modified (y) to the unmodified (x) devices. The results are summarized in the following table:

	N	Range (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2
Candidate vs. Predicate Lot 1	117	2.8–934	0.96 (0.94; 0.97)	0.44 (0.23; 0.66)	0.99
Candidate vs. Predicate Lot 2	118	2.8–934	0.97 (0.95; 0.99)	0.15 (-0.03; 0.32)	0.99

2. Matrix Comparison:

A study was performed per CLSI EP35 to demonstrate that lithium heparin plasma and EDTA plasma matrices yield comparable values as serum on the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack. A total of 45 matched individual donor samples with analyte concentration across the analytical measuring range were tested in singlicate using one reagent lot on one VITROS 5600 Integrated System. Four samples were excluded from the data analysis due to being outside of measuring range. Weighted Deming regression analysis was performed by comparing the data from plasma samples (y) to the matched serum samples (x). The results are summarized in the table below:

	N	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Li-heparin plasma vs serum	41	2.4–866	0.97 (0.96; 0.98)	-0.00 (-0.12; 0.12)	0.99
K2-EDTA plasma vs serum	41	2.4–877	0.98 (0.97; 0.99)	-0.07 (-0.27; 0.14)	1.00

C Clinical Studies:

1. Clinical Sensitivity:

Refer to K052889

2. Clinical Specificity:

Refer to K052889

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

Not Applicable

D Clinical Cut-Off:

Refer to K052889

E Expected Values/Reference Range:

The reference range of the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack was established in K052889. To verify this reference range for the modified VITROS

Immunodiagnostic Products CA 19-9 Reagent Pack, serum samples from a total of 60 apparently healthy individuals were tested, including 30 normal healthy females and 30 normal healthy males. The samples were tested with one lot of the modified VITROS Immunodiagnostic Products CA 19-9 Reagent Pack on one VITROS 5600 Integrated System. The results are summarized below:

# of Subjects	CA 19-9 Values (U/mL)		
	≤37	37.1-70	>70
60	57	2	1

Only one sample tested >70 U/mL by the modified device. This sample was tested with the unmodified predicate device and confirmed with the same result. The established reference range was verified.

The labeling of the VITROS Immunodiagnostic Products CA 19-9 Reagent Pack contains the following expected values (refer to K052889) for CA 19-9 in malignant and non-malignant conditions, in addition to the values for CA 19-9 in healthy subjects:

Cohort	# Subjects	CA 19-9 Values (U/mL)		
		≤37	37.1 – 70	>70
Nonmalignant Disease				
Colorectal	30	27	2	1
Cirrhosis	67	59	2	6
Non-GI	40	36	4	0
H. Pylori	50	46	4	0
Malignant Disease				
Pancreatic	50	15	2	33
Colorectal	100	49	8	43
Stomach	30	19	1	10
Lung	50	31	7	12
Healthy Subjects				
Healthy males (100) and females (100)	200	194	6	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.