510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K233581

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products Total ß-hCG II Reagent Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DHA	Class II	21 CFR 862.1155 - Human Chorionic Gonadotropin (HCG) Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modification to a previously cleared device

B Measurand:

Human chorionic gonadotropin

C Type of Test:

Quantitative

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 human chorionic gonadotropin (hCG) and its β-subunit in human serum and plasma (heparin and EDTA) using the VITROS 5600 Integrated System to aid in the early detection of pregnancy.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

VITROS 5600 Integrated System

IV Device/System Characteristics:

A Device Description:

The VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack (test) is performed using the VITROS Immunodiagnostic Products Total β-hCG II Reagent and the VITROS Total β-hCG II Calibrators on the VITROS 5600 Integrated System.

VITROS Immunodiagnostic Products Total β-hCG II Reagent is comprised of the following:

- 100 coated wells (antibody, mouse monoclonal anti-β-hCG, binds >600 mIU hCG/well)
- 14.4 mL assay reagent (buffer containing mouse serum, bovine serum albumin, bovine gamma globulin and antimicrobial agent)
- 19.2 mL conjugate reagent (HRP-mouse monoclonal anti-β-hCG, binds ≥4005 mIU hCG/mL) in buffer with bovine serum albumin and antimicrobial agent

B Principle of Operation:

An immunometric immunoassay technique is used, which involves the reaction of human chorionic gonadotropin (hCG) present in the sample with a microwell coated with biotinylated antibody (mouse monoclonal anti-β-hCG) bound to streptavidin, and a Horseradish Peroxidase (HRP)-labelled antibody conjugate (mouse monoclonal anti-β-hCG). 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 hCG present in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack

B Predicate 510(k) Number(s):

K063720

C Comparison with Predicate(s):

Device & Predicate Device(s):	K233581	K063720
Device Trade Name	VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack	VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack
General Device Characteristic Similarities		
Intended Use/Indications for Use	For the quantitative measurement of human chorionic gonadotropin (hCG) and its β- subunit in human serum and plasma (heparin and EDTA) to aid in the early detection of pregnancy.	Same
Sample Type	Serum and Plasma	Same
Measuring Range	2.39– 15,000 mIU/mL	Same
Sample Volume	40 µL	Same
Test Principle	Sandwich immunoassay	Same
General Device Characteristic Differences		
Biotin-conjugated antibody	Coated onto microwell	In the reagent buffer

VI Standards/Guidance Documents Referenced:

Clinical and Laboratory Standards Institute (CLSI) EP17-A2, 2013; Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

CLSI EP05-A3, (Reaffirmed: September 2019) Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06: Evaluation of the Linearity of Quantitative Measurement Procedures; Approved Guideline – Second Edition

CLSI EP07: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition

CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition

CLSI EP35: Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures– First Edition

CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples – Third Edition

CLSI EP28-A3c: Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

CLSI EP34: Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking– First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision performance of the device was evaluated using procedures based on the Clinical and Laboratory Standards Institute (CLSI) EP05-A3 guideline.

A) 20-Day Precision Study

Six serum pools were prepared by pooling female serum samples to attain the desired concentrations of hCG. Two replicates of each of the six samples were assayed in two runs per day, over 20 days. Three reagent lots and one analyzer were used. Summary data of one representative reagent lot is shown here:

Sample	Mean (mIU/mL)	Repeatability		Between-Run		Between-Day		Total imprecision	
	N=80	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	5.33	0.095	1.80	0.190	3.6	0.183	3.4	0.281	5.3
Sample 2	15.46	0.181	1.20	0.647	4.2	0.215	1.4	0.706	4.6
Sample 3	45.67	0.666	1.50	1.260	2.8	0.632	1.4	1.559	3.4
Sample 4	297.34	4.970	1.70	4.479	1.5	5.463	1.8	8.635	2.9
Sample 5	4708.50	82.29	1.70	95.10	2.0	83.98	1.8	151.22	3.2
Sample 6	9651.00	286.71	3.00	230.87	2.4	283.26	2.9	464.48	4.8

B) Instrument-to-Instrument Reproducibility Study

A reproducibility study was conducted to assess instrument-to-instrument variability. Five serum samples with different hCG concentrations were run on three analyzers The samples

were tested across each instrument, one lot per instrument, with five replicates per run, one run per day, over five days. The within-run, between-day, between-instrument, and reproducibility was calculated for each reagent lot. Results from multiple lots were similar. Results from one representative lot are provided below:

Concentration (mIU/mL)			Repeatability (Within-run)		Between-day		Between - instrument		Total	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	75	4.38	0.10	2.3	0.14	3.2	0.37	8.5	0.41	9.4
Sample 2	75	15.00	0.23	1.5	0.42	2.8	0.90	6.0	1.02	6.8
Sample 3	75	49.55	0.77	1.6	0.94	1.9	1.96	4.0	2.31	4.7
Sample 4	75	306.35	5.11	1.7	4.50	1.5	7.19	2.3	9.90	3.2
Sample 5	75	4781.1	105.94	2.2	89.54	1.9	92.27	1.9	166.59	3.5
Sample 6	75	8329.9	249.62	3.0	219.68	2.6	220.88	2.7	399.20	4.8

2. Linearity:

Two linearity studies were performed using 10 serum samples each. Varying concentrations of hCG were obtained by mixing low level serum samples and high level serum samples. Both studies were performed using one lot of reagents on one analyzer.

In the first study, linearity was established by testing samples with concentrations ranging from 38.6-15695 mIU/mL. Each sample was tested in 10 replicates. A regression analysis was conducted resulting in the following linear regression equation:

$$y = 0.991x + 0.10, R^2 = 0.996$$

In the second study, linearity was established by testing samples with concentrations ranging from 1.51 to 51.18 mIU/mL. Each sample was tested in 10 replicates. A regression analysis was conducted resulting in the following linear regression equation:

$$y = 1.025x + 0.07, R^2 = 0.999$$

The results from the linearity studies support the reportable range of 2.39-15000 mIU/mL (IU/L).

Dilution studies

Dilution studies were performed to determine the sample recovery after a 1:400 dilution using VITROS High Sample Diluent B performed automatically by the analyzer. The dilution study results support labeling claims that samples with hCG concentrations above 15,000 mIU/mL may be diluted 1:400 using VITROS High Sample Diluent B on-onboard the analyzer.

3. Analytical Specificity/Interference:

The effect of potential interfering substances and cross-reacting substances on the performance of the VITROS Immunodiagnostic Products Total β-hCG II Reagent were evaluated based on recommendations in CLSI EP07 Third Edition.

A) Interference:

Three female human serum pool samples with approximately 5.00 mIU/mL (IU/L), 50.00 mIU/mL (IU/L) and 10,000 mIU/mL (IU/L) ("low", "medium", and "high" respectively) hCG were spiked with various concentrations of potentially interfering substances. Each sample was divided into two aliquots: test (with added interferent) and control (with no added interferent) and measured in replicates of 5 across each of 3 reagent lots on one instrument. No interference (>10% difference) was observed with the substances and concentrations listed below.

Substance	Highest concentration at which no interference was observed
Acetaminophen	15.6 mg/dL
N-Acetylcysteine	15.0 mg/dL
Alpha-tocopherol	6.45 mg/dL
Amoxicillin	5.40 mg/dL
Ascorbic acid	5.25 mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Biotin	3510 ng/mL
Carbamazepine	4.50 mg/dL
Cefoxitin sodium	695 mg/dL
Cholesterol	400 mg/dL
Codeine	0.141 mg/dL
Colecalciferol	19.2 µg/dL
Cotinine	0.24 mg/dL
Dextran 40	2400 mg/dL
Dextromethorphan	0.00156 mg/dL
Dipyrone	100 mg/dL
Enoxaparin	360 U/dL
Ethanol	600 mg/dL
Furosemide	1.59 mg/dL
Gamma globulin	6 g/dL
HAMA (Human Anti-Mouse Antibodies)	800 µg/L
Hemoglobin	1000 mg/dL
Hydralazine hydrochloride	1.44 mg/dL
Hydrocodone	0.0072 mg/dL
Ibuprofen	21.9 mg/dL
Intralipid	2000 mg/dL
Levothyroxine	0.0429 mg/dL
Loratadine	0.0087 mg/dL

Naproxen	36.0 mg/dL
Nifedipine	0.0588 mg/dL
Nitrofurantoin	0.213 mg/dL
Omeprazole	0.840 mg/dL
Phenytoin	6.00 mg/dL
Prednisone	0.010 mg/dL
Propranolol HCl	0.115 mg/dL
Rheumatoid factor	900 IU/mL
Salicylic acid	2.86 mg/dL
Sodium azide	100 mg/dL
Sulfamethoxazole	40 mg/dL
Theophylline	6.0 mg/dL
Total Protein	15 g/dL
Triglycerides	1500 mg/dL
Trimethoprim	4.2 mg/dL
Vancomycin hydrochloride	12.3 mg/dL

B) Cross reactivity:

Cross-reactivity of the VITROS Total β-hCG II reagent pack was determined using female human serum samples with approximately 0 and 25 mIU/mL hCG concentrations that were spiked with the following potential cross reactants: luteinizing hormone (LH), follicle stimulating hormone (FSH), or thyroid stimulating hormone (TSH). Samples were tested in replicates of five using 3 lots of reagents on one analyzer.

Cross-reactivity at the 0 mIU/mL hCG concentration was calculated using the following equation:

$$\% \text{ cross-reactivity} = \frac{\text{Mean result of cross-reactant sample}}{\text{Concentration of cross-reactant}} \times 100$$

Cross-reactivity at the 25 mIU/mL hCG concentration was calculated using the following equation:

$$\% \text{ cross-reactivity} = \frac{\text{(Mean result of cross-reactant sample - Mean result of control sample)}}{\text{Concentration of cross-reactant}} \times 100$$

No cross-reactivity at the tested concentrations was detected at 0 mIU/mL hCG and the cross-reactivity at the tested concentrations observed at 25 mIU/mL hCG is listed in the table below:

Substance	**Tested Concentration**	**VITROS 5600 - % Cross Reactivity**		
		Lot 1	Lot 2	Lot 3
FSH	400 mIU/mL	0.6%	0.3%	0.3%
LH	400 mIU/mL	-0.3%	-0.1%	-0.5%
TSH	200 mIU/mL	0.4%	0.1%	-0.3%

4. Assay Reportable Range:

   The assay reportable range is 2.39 to 15,000 mIU/mL. See section VII.A.2 - Linearity and section VII.A.6 - Detection Limit.

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

   The VITROS Total β-hCG II Reagent Pack is traceable to the World Health Organization (WHO) 4th International Standard Chorionic Gonadotropin, Human, National Institute for Biological Standards and Control (NIBSC), code 75/589.

6. Detection Limit:

   The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack were determined using procedures based on the CLSI EP17-A2 guideline.

   For determination of the LoB, four analyte-free serum samples were tested in replicates of five per run, with three lots of reagent, on one analyzer, over five days. The LoB was determined to be 0.00 mIU/mL. The results supports the claimed LoB of 0.05 mIU/mL.

   For determination of the LoD, five serum samples containing low analyte concentrations (approximately 1 – 5 times LoB) were tested in replicates of 12 per day, using three reagent lots, on one analyzer over 5 calendar days. The LoD was determined to be 0.21 mIU/mL. The results support the claimed LoD of 0.70 mIU/mL.

   For determination of the LoQ, four serum sample pools that contained low analyte concentrations were used to evaluate the LoQ using the Total Error (<20%) approach. The LoQ using the Total Error (<20%) approach was calculated to be 2.32 mIU/mL. The results support the claimed LoQ of 2.39 mIU/mL.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Method comparison studies were conducted following recommendations in the CLSI EP09c-3rd Edition guideline.

   A total of 135 serum samples were measured in singlicate using the updated VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack (candidate device) and the predicate device. The samples were tested using one reagent lot and one analyzer. The results were assessed using Weighted Deming regression analysis and the results are shown in the following table:

n	Slope (95% CI)	Correlation Coefficient	Conventional Units (mIU/mL)	
			Range of Samples	Intercept (95% CI)
135	0.99 (0.977 to 0.995)	0.998	2.78 to 14,675	-0.0215 (-0.160 to 0.117)

2. Matrix Comparison:

Performance of the VITROS Immunodiagnostic Products Total β-hCG II Reagent with different sample matrices was evaluated based on the recommendations in the CLSI EP35 guideline.

A matrix comparison study was performed to evaluate matrices suitable for use with the VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack. Serum samples with concentrations ranging from 7.55- 10580.0 mIU/mL were compared to matched Lithium Heparin and K2-EDTA plasma samples using one lot of reagents on the analyzer. The results were assessed using Weighted Deming regressions analysis.

A summary of the results, when compared to serum, are shown in table below:

Parameter	Li-Hep Plasma	K2-EDTA Plasma
Slope	0.978	0.978
Intercept	0.1708	0.0529
Correlation Coefficient r	0.999	0.999
n	41	41

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

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

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The reference range information was reviewed in K063720.

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.