

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k063720

B. Purpose for Submission:

New device

C. Measurand:

Human chorionic gonadotropin

D. Type of Test:

Quantitative Immunoassay

E. Applicant:

Ortho-Clinical Diagnostics

F. Proprietary and Established Names:

VITROS Immunodiagnostic Products Total β -hCG II Reagent Pack

VITROS Immunodiagnostic Products Total β -hCG II Calibrators

VITROS Immunodiagnostic Products Total β -hCG II Range verifiers

G. Regulatory Information:

1. Regulation section:

21 CFR §862.1155 Human chorionic gonadotropin test system

21 CFR §862.1150 Calibrators

21 CFR §862.1660 Quality control material (assayed and unassayed)

2. Classification:

Class II – assay, calibrator

Class I, reserved – control material

3. Product code:

DHA Human chorionic gonadotropin (HCG) test system

JIT Calibrator, secondary

JJX Single (specified) analyte controls (assayed and unassayed)

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications(s) for use below.

2. Indication(s) for use:

For quantitative measurement of human chorionic gonadotropin (hCG) and its β -subunit in human serum and plasma (EDTA or heparin) to aid in the early detection of pregnancy.

Calibrator: For use in the calibration of the VITROS Immunodiagnostic System for the quantitative measurement of human chorionic gonadotropin (hCG) and its β -subunit in human serum and plasma (EDTA or heparin).

Range verifier: For *in vitro* use in verifying the calibration range of the VITROS Immunodiagnostic System when used for the measurement of human chorionic

gonadotropin (hCG) and its β -subunit.

3. Special conditions for use statement(s):

For Prescription Use only.

4. Special instrument requirements:

The VITROS Immunodiagnostic System

I. Device Description:

Reagent pack: VITROS Immunodiagnostic Products Total β -hCG II Reagent Pack consists of 100 streptavidin coated wells, and two vials of antibody reagent: one vial with biotin-mouse monoclonal anti- β -hCG in buffer with mouse serum, bovine serum albumin, bovine gamma globulin and antimicrobial agent, second vial with HRP-mouse monoclonal anti- β -hCG in buffer with bovine serum albumin and antimicrobial agent.

Calibrator: Three vials of freeze-dried, recombinant hCG in human plasma with antimicrobial agent; concentrations approximately 0, 3000, and 14,000 mIU/mL.

Range Verifiers: Two vials of freeze-dried, human serum (low range) or recombinant hCG in human serum (high range) with antimicrobial agent; concentrations at 0 and 14,250 mIU/mL (4th IS 75/589).

All human source material was tested by FDA-approved methods for HIV-1/2, HBsAg, and HCV and found to be negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Abbott Laboratories ARCHITECT SYSTEM Total β -hCG assay, VITROS Immunodiagnostic Products Total β -hCG II Range verifiers

2. Predicate 510(k) number(s):

k983424, k973517

3. Comparison with predicate:

VITROS Total β -hCG II assay compared to the predicate, Abbott Laboratories ARCHITECT SYSTEM Total β -HCG assay:

Similarities		
	Predicate device	Proposed device
Test principle	Chemiluminescent immunoassay	Same
Sample type	Serum and plasma	Same
Top of measuring range	15,000 mIU/mL	Same
Antibody (1 st of 2 in assay; see below in differences)	Conjugated monoclonal mouse anti- β -hCG	Same

Differences		
	Predicate device	Proposed device
Sample volume	75 µl	40 µl
Luminescent label	Acridinium	Horseradish peroxidase
Antibody (2 nd of 2 in assay; see above)	Mouse monoclonal anti-β-hCG-coated microparticles	Biotin-conjugated mouse monoclonal anti-β-hCG

Calibrator

Similarities		
	Predicate device	Proposed device
Range of calibration	0 – 15,000 mIU/mL	0 - 14,000 mIU/mL

Differences		
	Predicate device	Proposed device
Number of levels	Six	Three

Verifiers

Similarities		
	Predicate device	Proposed device
Levels	Low and high	Same
Form	Freeze-dried	Same

Differences		
	Predicate device	Proposed device
Matrix	Bovine serum	Human plasma
Antigen	Purified from human urine hCG	Bacterial recombinant hCG

K. Standard/Guidance Documents Referenced (if applicable):

Standard:

WHO Standard for HCG, 4th IS, 75/589

Guidance:

CLSI Documents:

- *User Evaluation of Precision Performance of Clinical Chemistry Devices: Approved Guideline (EP5-A2)*
- *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline (EP6-A2)*
- *Interference Testing in Clinical Chemistry: Approved Guideline (EP7-A2)*
- *Method Comparison and Bias Estimation using Patient Samples: Approved Guideline (EP9-A2)*
- *Protocols for Determination of Limits of Detection and Limits of Quantitation (EP17-A3)*

L. Test Principle:

An immunometric immunoassay technique is used, which involves the reaction of

hCG present in the sample with a biotinylated antibody (mouse monoclonal anti- β -hCG) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti- β -hCG). The antigen-antibody complex is captured by streptavidin on the wells. 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 VITROS Immunodiagnostic System. The amount of HRP conjugate bound is directly proportional to the amount of hCG present.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was performed using CLSI Document EP5-A as a guideline. Two replicates each of three re-hydrated freeze-dried control sera and two frozen patient sample pools were assayed on 2 separate runs per day over more than 20 days. Two reagent lots and two analyzers were used.

		Mean hCG conc.	Within day		Within lab		Total precision	
			SD	%CV	SD	%CV	SD	%CV
Controls	Low	16.45	0.330	2.1	0.587	3.6	0.611	3.8
	Mid	49.76	0.53	1.1	1.49	3.0	1.52	3.1
	High	369.20	5.33	1.4	10.79	2.9	9.66	2.6
Pools	Plasma	4819.2	104.50	2.2	162.44	3.3	131.45	2.8
	Serum	9440.8	230.01	2.5	413.62	4.2	363.28	3.8

Units = mIU/mL

b. *Linearity/assay reportable range:*

The measuring range for this assay is 2.39 – 15,000 mIU/mL.

Linearity was evaluated using CLSI Document EP6-A2 as a guideline.

Three studies were performed to evaluate the measuring range. First, two plasma sample pools were mixed: a low pool which had an estimated hCG titer of 52.9 mIU/mL and a high pool estimated to be 19,520 mIU/mL. In order to evaluate the linearity at the low part of the measuring range, a low pool at 2.18 mIU/mL and a high pool at 57.2 mIU/mL were also serially mixed and evaluated.

For the first recovery study (52.9 – 19520 mIU/mL) that evaluated the entire assay range using nine samples (six replicates of each), the recoveries ranged from 94.8 - 111.6%, with a mean result of 103.6%. The second recovery study (2.18 – 57.2 mIU/mL) that evaluated the low end of the assay range using eleven samples (six replicates) had recoveries ranging from 92.9 - 118.8%, with a mean result of 101.2%.

Recovery of the upper end of the measuring range was evaluated by measuring negative male serum spiked with known amounts of the WHO 4th IS (75/589). High and low pools were made and admixtures of the two pools

were prepared generating samples ranging from 13,065 – 15,489 mIU/mL. All samples were then measured on the VITROS Total β -hCG II assay in triplicate using two reagent lots. The mean measured values of the samples were within 10% of the expected concentrations of the samples. Results are summarized below:

Expected Concentration	Mean VITROS result (mIU/mL)	% Difference
14837	15117	1.887
14649	15095	3.045
14365	15650	8.945
13862	14214	2.539
13065	13642	4.416

High sample dilution recommendations were evaluated by assaying 10 samples containing high amounts of β -hCG. The samples were diluted both manually and on the VITROS Immunodiagnostic System with VITROS Immunodiagnostic Products High Sample Diluent B (HSDB) to 1/2, 1/5, 1/10 and 1/20, 1/100, 1/200 and 1/400. No systematic differences between manual and on board dilution were observed and both demonstrated acceptable recoveries.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability and Value Assignment: The VITROS Total β -hCG Calibrators and Range Verifiers are traceable to the WHO β -hCG 4th International Standard.

Calibrators: Primary calibrators are prepared gravimetrically from the 4th International Standard. A set of 10 Working Calibrators are prepared and calibrated against the Absolute Calibrators using twenty runs on two instruments. A second set of Working Calibrators (in-house reference calibrators) are prepared gravimetrically and calibrated against the first set of Working Calibrators using 84 assays. For each Reagent Lot, the set of three Product Calibrators are assigned values using a mean of 20 determinations in a measurement procedure calibrated with the Working Calibrators. The full calibration curves from the 20 assays are also used to prepare a “Master Calibration Curve” specific for that Reagent Lot. This Master Calibration Curve is provided to each customer’s VITROS Analyzer via a magnetic card.
Range Verifiers: Range Verifiers are tested multiple times across VITROS Total β -hCG II reagent lots and instruments. The high and low range verifiers must pass predetermined acceptance criteria for each run. Included in the runs are the Working Calibrators that are traceable to the 4th International Standard (NIBSC 75/589).

Stability: Freeze-dried and reconstituted calibrator master lots were evaluated at the proposed storage temperatures as well as at high temperature to demonstrate accelerated stability of the calibrators and controls. Multiple lots of the reagent packs were evaluated for open on-board stability. Testing protocols and data summaries provided were found to be adequate. Freeze-

thaw and elevated temperature (to represent transportation conditions) stability testing protocols and summaries were provided for reagent packs, calibrators and controls, and found to be adequate.

d. Detection limit:

The method to determine detection limit is based upon the CLSI document EP17-A3 (“Protocols for Determination of Limits of Detection and Limits of Quantitation”).

The study ran ten replicates of each blank and five low positive sample pools with two lots on three instruments for two runs per day for three days using two different calibrations. The LoB was calculated to be 0.05 mIU/mL. The LoD was calculated to be 0.70 mIU/mL. The Limit of Quantitation (LoQ) was calculated to be 2.39 mIU/mL as determined by the lowest concentration at which test results of five low-analyte samples had a CV<15%.

e. Analytical specificity:

Interference: The VITROS Total β -hCG II assay was evaluated for interference consistent with CLSI Guideline EP7-A2. To calculate the percent interference, the mean value of four determinations of a solution of each test substance was compared with that of a corresponding control in two lots. Of the compounds tested, none of the compounds tested was found to cause a bias of more than 10%.

Compound Concentration Tested

Bilirubin	20 mg/dL
Biotin	1000 ng/dL
Dipyrrone	100 mg/dL
FSH	400 mIU/mL
Hemoglobin*	500 mg/dL
LH	400 mIU/mL
Sodium Azide	100 mg/dL
Triolein	3000 mg/dL
TSH	250 μ IU/mL

* Hemoglobin was added to a series of specimens with hCG concentrations of 0.00 to 10350 mIU/mL (IU/L).

Hook effect: Nine clinical samples and dilutions of the WHO 4th International Standard (NIBSC 75/589) were diluted in negative plasma. Triplicate determinations were made with each of two Master Lots. No evidence of high dose hook was observed in the study up to 1,300,000 mIU/mL.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison was evaluated using CLSI Document EP9-A2 as a guideline.

139 patient samples were assayed on both the predicate device (Abbott ARCHITECT Total β -hCG assay) and the proposed device (VITROS Total β -

hCG II assay). The relationship between the two methods, determined by Deming Regression, was as follows:

Device = 1.016(Predicate) – 95.0 mIU/mL

Correlation coefficient = 0.989

Standard error of slope = 0.0095

Standard error of intercept = 34.84

b. Matrix comparison:

Blood was drawn from 22 subjects, spiked with various concentrations of hCG that spanned the measuring range, and processed to produce a full set of matched specimen types (serum, heparin plasma, EDTA plasma, and citrate plasma). The percent difference seen when using plasma samples compared to serum samples was: $\leq -4.0\%$ for heparin plasma, $\leq 0.2\%$ for EDTA plasma, and $\leq 0.8\%$ for CAT plasma samples.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The sponsor measured the hCG levels of 540 samples on the VITROS Total β -hCG II assay to determine the reference range for normal and pregnant subjects.

The results are as follows:

Sample type	No. of samples	Mean	Min	Max	2.5 th percentile	97.5 th percentile
Normal male	98	0.02	0.00	1.06	0.00	0.08
Normal female	123	0.46	0.00	5.42	0.00	4.32
Post-menopausal	69	1.52	0.00	6.66	0.00	6.46
Total	290	0.56	0.00	6.66	0.00	4.83
Pregnant females						
Gestational age 1-10 wks	112	31,142	44.71	256,740	63.7	150,854
Gestational age 11-15 wks	43	55,425	11,556	265,380	11,795	151,996
Gestational age 16-22 wks	50	27,023	7,480.8	111,954	9,383.8	61,410
Gestational age 23-40 wks	45	24,031	1,531.1	101,566	1,737.2	98,576

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.