510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K212221

B Applicant

Diazyme Laboratories Inc.

C Proprietary and Established Names

Diazyme DZ-Lite iFlash Total beta-hCG Assay, Diazyme DZ-Lite iFlash 1800 Chemiluminescence Immunoassay Analyzer

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
JJE	Class I	21 CFR 862.2160 - Discrete photometric chemistry analyzer for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Total beta human chorionic gonadotropin (βhCG) in human serum

C Type of Test:

Quantitative chemiluminescent immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Diazyme DZ-Lite iFlash Total βhCG Assay is a chemiluminescent immunoassay intended for use for the quantitative determination of total beta-human chorionic gonadotropin (total βhCG) in human serum on DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer. The assay is intended for use as an aid in the early detection of pregnancy.

Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer is used clinically in combination with the supporting chemiluminescence immunoassay reagents for determination of analytes in human body fluids through acridinium ester-based chemiluminescence method.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Diazyme DZ-Lite iFlash 1800 Chemiluminescence Immunoassay Analyzer

IV Device/System Characteristics:

A Device Description:

The Diazyme DZ-Lite iFlash Total βhCG Assay is sandwich immunoassay. The materials included are as follows:

Reagent kit, 100 tests, 2 packs, 50 tests/pack, Catalog # C86012

R1 (2 x 3.5 mL)	Anti-HCG coated microparticles, 0.05% ProClin 300.
R2 (2 x 4.0 mL)	Anti-HCG acridinium-ester-labeled conjugate; 0.05% ProClin 300.
CAL1 (1 x 1.0 mL)	Calibrator 1, 1 bottle, phosphate buffer with protein stabilizers, 0.05% ProClin 300, lyophilized.
CAL2 (1 x 1.0 mL)	Calibrator 2, 1 bottle, HCG in phosphate buffer with protein stabilizers, 0.05% ProClin 300, lyophilized.
CAL3 (1 x 1.0 mL)	Calibrator 3, 1 bottle, HCG in phosphate buffer with protein stabilizers, 0.05% ProClin 300, lyophilized.

The reagents in the pack are ready for use. Calibrators are lyophilized powder that are reconstituted with distilled water before use.

The Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer is a microcomputer controlled, random and continuous access analyzer that performs immunoassays utilizing a magnetic particle solid phase and chemiluminescent detection.

B Principle of Operation:

The HCG in the sample is incubated with anti-HCG coated paramagnetic microparticles and anti-HCG acridinium-ester-labeled conjugate react to form a sandwich complex. The unbound materials are washed away from the solid phase in a magnetic field. Afterwards, the Pre-Trigger and Trigger Solutions are added to the reaction mixture and the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HCG in the sample and the RLUs detected by the iFlash optical system. Results are determined via a calibration curve, which is instrument-specifically generated by 3-point calibration and a master curve provided via the reagent QR code.

The Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer operates based on chemiluminescence using magnetic particle solid phase and chemiluminescent tracer.

C Instrument Description Information:

1. Instrument Name:

   Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer

2. Specimen Identification:

   Human serum specimens are identified using the built-in barcode reader.

3. Specimen Sampling and Handling:

   The sample supply system is composed of sample loading area, sample supply unit, and sample bar code scanner. Samples are loaded in the loading area and transferred by the sample supply unit. The bar code scanner reads the information on the bar code of the sample rack and sample tube.

   The sample handling system consists of the sample/reagent probe assembly and wash well assembly. The sample/reagent probe assembly, located near the sample supply unit, aspirates and dispenses samples and reagents. Under the sample/reagent probe is a wash well for washing the probe in order to prevent carryover and reagent contamination.

4. Calibration:

   Calibration and QC are usually performed at the beginning of routine tests (before sample processing). However they can be performed anytime during routine operation.
   The calibration and QC include the following two steps:

- Test calibrators and controls
- Validate the result

Calibrators are prepared according to the reagent kit instructions.

5. Quality Control:

Quality control can be performed using Diazyme DZ-Lite hCG controls.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Elecsys HCG and Beta Test System, Roche cobas c6000 Series system with electrochemiluminescence (ECL) module e601

B Predicate 510(k) Number(s):
K003178, K060373

C Comparison with Predicate(s):

Assay Predicate

Device & Predicate Device(s):	K212221	K003178
Device Trade Name	Diazyme DZ-Lite iFlash Total βhCG Assay	Roche Elecsys HCG and Beta Test System
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of total beta-human chorionic gonadotropin (total βhCG) in human serum to aid in the early detection of pregnancy	Same
Type of Test	Quantitative	Same
Technology	Sandwich Immunoassay	Same
Method	Automated	Same
Calibration	Utilizes a stored calibration curve	Same
General Device Characteristic Differences		
Specimen Type	Human Serum	Human Serum and Plasma
Assay Range	0.6 – 10,000 mIU/mL	0.1 – 10,000 mIU/mL

Device & Predicate Device(s):	K212221	K003178
Calibrator	Three-level Calibrators	Five-level Calibrators

Instrument Predicate

Device & Predicate Device(s):	K212221	K060373
Device Trade Name	Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer	Roche cobas c6000 Series system with electrochemiluminescence (ECL) module e601
General Device Characteristic Similarities		
Intended Use/Indications For Use	Immunoassay Analyzer used clinically in combination with the supporting immunoassay reagents for determination of analytes in human body fluids	Same
Device Description	Microcomputer controlled, random and continuous access analyzer that performs immunoassays utilizing a magnetic particle solid phase and chemiluminescent detection	Same
Sample Aspiration	Directly from sample tube in the sample bay of the analyzer	Same
Sample Identification	Bar-coded sample tubes read directly by analyzer bar code reader	Same
General Device Characteristic Differences		
Detection System	Chemiluminescent	Electrochemiluminescent
Throughput	180 tests/hour	170 tests/hour

VI Standards/Guidance Documents Referenced:

- Clinical and Laboratory Standards Institute **EP05- A3** Evaluation of Precision of Quantitative Measurement Methods-Approved Guideline-Third Edition
- Clinical and Laboratory Standards Institute **EP06-A** – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- Clinical and Laboratory Standards Institute **EP07-A2** – Interference Testing in Clinical Chemistry; Approved Guideline

- Clinical and Laboratory Standards Institute **EP17-A2** – Evaluation of Detection capability for Clinical Laboratory Measurement Procedures; Approved Guideline 2nd Edition
- Clinical and Laboratory Standards Institute **EP28-A3c** – Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the Diazyme DZ-Lite iFlash Total βhCG Assay was evaluated based upon recommendations in the CLSI EP05-A2 guideline. In the study, eight serum samples and two levels of serum based controls were tested in duplicates per run, 2 runs per day for 20 days using three lots of the reagents on two different Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay analyzers. The results of the within-run, between-run, between-day, and total CV% for three lots of the reagent combined are listed in the following table:

Analyzer 1(N=240):

Sample	Mean N=240	Within-Run		Between-Run		Between-day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum1	1.14	0.073	6.4%	0.062	5.4%	0.095	8.3%	0.13	11.8%
Serum2	4.47	0.18	4.1%	0.13	3.0%	0.17	3.8%	0.28	6.3%
Serum3	17.1	0.67	3.9%	0.62	3.6%	0.61	3.6%	1.10	6.4%
Serum4	98.3	1.68	1.7%	1.83	1.9%	3.03	3.1%	3.92	4.0%
Serum5	1830.4	24.9	1.4%	21.8	1.2%	50.1	2.7%	60.0	3.3%
Serum6	4317.0	49.7	1.2%	54.1	1.3%	113.1	2.6%	134.9	3.1%
Serum7	5948.7	84.5	1.4%	50.3	0.8%	166.7	2.8%	193.5	3.3%
Serum8	9078.6	93.8	1.0%	112.6	1.2%	250.1	2.8%	289.9	3.2%
Control 1	1.22	0.066	5.4%	0.045	3.7%	0.125	10.2%	0.148	12.1%
Control 2	125.6	2.2	1.7%	1.8	1.5%	3.7	2.9%	4.6	3.7%

Analyzer 2 (N=240):

Sample	Mean N=240	Within-Run		Between-Run		Between-day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum1	1.20	0.078	6.5%	0.073	6.1%	0.021	1.8%	0.109	9.1%
Serum2	4.29	0.173	4.0%	0.137	3.2%	0.161	3.8%	0.273	6.4%
Serum3	16.9	0.75	4.4%	0.74	4.4%	0.49	2.9%	1.16	6.9%
Serum4	100.2	1.78	1.8%	2.55	2.5%	2.78	2.8%	4.17	4.2%
Serum5	1871.8	23.6	1.3%	33.3	1.8%	34.1	1.8%	53.2	2.8%
Serum6	4446.1	53.2	1.2%	152.9	3.4%	40.2	0.9%	166.8	3.8%
Serum7	6058.3	65.3	1.1%	173.1	2.9%	119.4	2.0%	220.2	3.6%
Serum8	9347.0	93.8	1.0%	114.5	1.2%	158.4	1.7%	216.8	2.3%
Control 1	1.25	0.075	6.0%	0.052	4.2%	0.062	5.0%	0.110	8.8%
Control 2	125.7	2.1	1.7%	3.2	2.5%	4.3	3.4%	5.7	4.6%

2. Linearity:

The linearity of the Diazyme DZ-Lite iFlash Total βhCG Assay was evaluated by conducting three studies based upon recommendations in the CLSI EP06-A guideline.

In the first study, a high concentration serum sample was diluted with a serum sample low pool to create eleven sample dilutions with concentrations that ranged from 0.41 to 13,614 mIU/mL. In the second study, the sponsor assessed linearity of the low concentration interval by preparing eleven samples with hCG concentrations that ranged from 0.36 to 1,594 mIU/mL. The third study focused on evaluating linearity at the very low end of the analytical measuring interval using samples with hCG concentrations that encompass important medical decision limits, including a sample with a measurable hCG concentration at, or just below, the lowest point of the claimed reportable range. In all the studies each sample level was tested in triplicates using one reagent lot. The maximum deviation from linearity was found to be acceptable and supported by the results.

The results from the linearity studies and limit of quantitation studies (LOQ = 0.60 ng/mL, see below), supports the sponsor's claimed analytical measuring interval of 0.6 to 10,000 mIU/mL.

Dilution Studies

Dilution studies were performed to determine the sample recovery after a 1:100 dilution is performed either manually or automatically by the Diazyme DZ-Lite iFlash 1800 Chemiluminiscence Immunoassay Analyzer. The dilution study results support the sponsor's labeling claims that samples with hCG concentrations above 10,000 mIU/mL may be diluted 1:100 either manually or on-onboard the analyzer.

3. Analytical Specificity/Interference:

Interference

To determine the level of interference from the substances present in serum, the Diazyme DZ-Lite iFlash Total βhCG Assay was used to test three human serum samples with approximately 6 mIU/mL, 60 mIU/mL, and 600 mIU/mL ("low", "medium", and "high" respectively) hCG concentrations spiked with various concentrations of substances based upon recommendations in CLSI EP7-A2. The following endogenous substances do not interfere with this assay at the levels tested (less than 10% bias between the test sample and the control sample).

Interferent	Concentration
Ascorbic Acid	20 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	20 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor	2000 IU/mL
Triglycerides	3000 mg/dL

The following substances showed no significant interference (< ± 10%) up to the concentrations summarized below.

Interference Substance	Concentration Showing No Significant Interference
Total Protein	60 mg/mL
Luteinizing Hormone	500 mIU/mL
Follicle-Stimulating Hormone	200 mIU/mL
Human Growth Hormone	100 ng/mL
Thyroid-Stimulating Hormone	37 mIU/mL
Acetylsalicylic Acid	0.2 mg/mL
EDTA	0.8 mg/mL
Ethanol	1%
Gentisic Acid	0.2 mg/mL
Glucose	5 mg/mL
Salicylic Acid	0.2 mg/mL

Hook Effect

No High dose hook effect was observed for hCG concentrations in serum up to 1,000,000 mIU/mL.

4. Assay Reportable Range:

   The Analytical Measuring Range (AMR) is claimed to be 0.6 – 10,000 mIU/mL.

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

   Traceability: This assay is traceable to the WHO 5th International Standard 07/364.

6. Detection Limit:

   The LoB, LoD, LoQ of the Diazyme DZ-Lite iFlash Total βhCG Assay was determined based upon recommendations in CLSI EP17-A2.

   Limit of blank (LoB) is the highest concentration that is likely to be observed for a blank sample. To calculate the LoB, six hCG free blank samples collected were run with 5 replicates on at least three different days with three lots of hCG reagent on three Diazyme DZ-Lite iFlash 1800 analyzers respectively.. The LOB was determined to be 0.06 mIU/mL.

   Limit of detection (LoD) is the lowest concentration of analyte that can be detected reliably. Five serum samples with hCG collected from medical laboratory were diluted with a normal serum to a level approximately 1-4 times LoB and tested with three lots of HCG reagent on the iFlash 1800 with 15 replicates over three days (5 replicates per day).. The LoD was determined to be 0.14 mIU/mL.

Limit of quantitation (LoQ) is the lowest concentration which is the design requirements of TEa%(Total Error Allowance) of ≤32%. Seven hCG free serum samples were spiked with 5th WHO international standard to target concentrations respectively. The diluted serum samples were tested with three lots of the Diazyme DZ-Lite iFlash Total βhCG Assay reagents on three Diazyme DZ-Lite iFlash 1800 analyzers with 5 replicates per run, one run per day for 3 days (15 replicates total per sample) on corresponding iFlash 1800 using three lots of the reagents. The LoQ was determined to be 0.60 mIU/mL.

7. Assay Cut-Off:

   Not applicable.

8. Accuracy (Instrument)

   Not applicable.

9. Carryover

   High sample containing 9285mIU/mL hCG and a low sample containing 4.22mIU/mL hCG were tested in duplicate, alternating 18 times (i.e. HH,LL,HH,LL, etc.) on the iFlash 1800 analyzer with one reagent lot with no carryover effect.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   A total of 141 female human serum samples were tested with the Diazyme DZ-Lite iFlash Total βhCG Assay and the results obtained were compared to the predicate method. The regression results are shown in the following table:

Parameter	Passing Bablok
n	141
Slope	0.9976
95% CI	0.9806-1.0165
Intercept	0.3227
95% CI	-4.3128-5.0253
Correlation Coefficient (R^2)	0.9936
Sample Range	2.7-9922

2. Matrix Comparison:

   Not applicable. The assay has been validated for use with serum samples only.

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:

Expected Values: To establish the reference interval for the iFlash-HCG Assay, serum samples from 120 apparently healthy, non-pregnant premenopausal women of Ages 21-50 and 124 postmenopausal women of Ages ≥ 50 were tested using the iFlash-HCG Assay on the iFlash 1800 analyzer based upon recommendations in the CLSI C28-A3 guideline. The central 95% reference interval was established to be ≤ 0.4 mIU/mL for premenopausal women and ≤ 3.0 mIU/mL for post-menopausal women.

It is recommended that each laboratory establish its own expected reference range for the specific population.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

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.