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

I Background Information:

A 510(k) Number

K223690

B Applicant

Shenzhen YHLO Biotech Co., LTD.

C Proprietary and Established Names

iFlash-HCG; Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C)

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 (intact hCG) and β -hCG.

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:

iFlash-HCG is a paramagnetic particle chemiluminescent immunoassay (CLIA) for quantitative detection of the intact human chorionic gonadotropin (hCG) molecule and the hCG β-subunit (β-hCG) in human serum and plasma using the automated Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C). The iFlash-HCG assay is to be used by laboratory professionals as an aid in early detection of pregnancy together with other clinical methods.

Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) is a fully-automated, chemiluminescence immunoassay analyzer intended for quantitative or qualitative determination of analytes in human body fluids taken from clinical settings. It is used together with its supporting chemiluminescence immunoassay reagents. The Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) is intended for use in clinical laboratories.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C)

IV Device/System Characteristics:

A Device Description:

The iFlash hCG is provided in kits comprised of 100 tests, 2 packs, 50 tests/pack, Catalog # C86012 contain the following:

R1 (2 x 3.5 mL)	Anti-HCG antibody-coated paramagnetic micro-particles (mouse, 0.1 mg/mL), 0.05% ProClin 300 as preservative.
R2 (2 x 4.0 mL)	Acridinium-ester-labeled anti-HCG antibody conjugate (mouse, 0.4 μg/mL), 0.1% ProClin 300 as preservative.
CAL1 (1 x 1.0 mL)	Calibrator 1, TRIS buffer with protein stabilizer (BSA), 0.05% ProClin 300 as preservative, lyophilized.
CAL2 (1 x 1.0 mL)	Calibrator 2, HCG protein (human) in TRIS buffer with protein stabilizer (BSA), 0.05% ProClin 300 as preservative, lyophilized.
CAL3 (1 x 1.0 mL)	Calibrator 3, HCG protein (human) in TRIS buffer with protein stabilizer (BSA), 0.05% ProClin 300 as preservative, lyophilized.

Chemiluminescence immunoassay analyzer (Model: iFlash 3000-C) is a fully- automated, analyzer intended for quantitative or qualitative determination of analytes in human body fluids. It is intended for use in clinical laboratories. The Chemiluminescence immunoassay analyzer (Model: iFlash 3000-C) measures analytes in samples using the reagents, calibrators, quality

control materials and other consumables that can be in the iFlash-HCG instruction for use manual. Components of the Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) include the components in the sample/reagent area, components in the consumables area, and components in the measurement area:

Components in the sample/reagent area include:

- Sample loading unit
- Sampling unit
- Reagent processing system

Components in the consumables area include:

- Reaction vessel system
- System reagent bin (Pre-trigger Solution and Trigger Solution)
- Wash buffer tank
- Waste tank
- Solid waste container

Components in the measurement area include:

- Incubation assembly
- Magnetic separation washing assembly
- Photon detection system

B Principle of Operation:

The iFlash-HCG assay is a sandwich immunoassay using a direct chemiluminometric technique. The hCG in the sample reacts with anti-HCG antibody-coated paramagnetic microparticles and acridinium-labeled anti-HCG antibody conjugate to form a sandwich complex. After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then the pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by the iFlash optical system. The RLUs detected production is directly proportional to the analyte in the sample. Analyte concentration is determined from a calibration curve, which is generated by 3-point calibration and a master curve provided via the reagent QR code.

C Instrument Description Information:

1. Instrument Name:

   Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C)

2. Specimen Identification:

   Human serum and lithium heparin, sodium heparin and K_2-EDTA specimens are identified using the built-in barcode reader.

3. Specimen Sampling and Handling:

   Specimen sampling and handling procedures are described in the Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) Analyzer's instruction for use manual.

Assay specific specimen collection and preparation procedure can be found in the iFlash-HCG instruction for use manual.

4. Calibration:

Calibration methods and procedures are described in the Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) Analyzer's instruction for use manual. Assay specific specimen collection and preparation procedure can be found in the iFlash-HCG instruction for use manual.

5. Quality Control:

YHLO Immunoassay Multi Control, EVER-BLUE Immunoassay Multi Control are recommended.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Elecsys Hcg And Beta Test System, Elecsys TSH assay, cobas e 801 Immunoassay analyzer

B Predicate 510(k) Number(s):
K003178, K162606

C Comparison with Predicate(s):

Assay

Device & Predicate Device(s):	K223690	K003178
Device Trade Name	iFlash-HCG	Roche Elecsys HCG and Beta Test System
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of intact human chorionic gonadotropin (hCG) and the hCG β-subunit (β-hCG) in human serum and plasma to aid in the early detection of pregnancy	Same
Type of Test	Quantitative	Same
Technology	Sandwich Immunoassay	Same
Method	Automated	Same
Specimen Type	Human Serum and Plasma	Same

General Device Characteristic Differences		
Assay Range	0.5-10000 mIU/mL	0.2 – 10000 mIU/mL
Calibrator	Three-level Calibrators	Five-level Calibrators
Traceability	WHO International Standard 5th WHO IS Chorionic Gonadotrophin 07/364	4th International Standard (NIBSC) code 75/589

Instrument

Device & Predicate Device(s):	K223690	K162606
Device Trade Name	Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C)	Cobas e 801 analyzer
General Device Characteristic Similarities		
Intended Use/Indications For Use	For quantitative or qualitative determination of analytes in human body fluids.	Same
Calibration	Utilizes a stored calibration curve	Same
General Device Characteristic Differences		
Detection System	Chemiluminescent	Electrochemiluminescent

VI Standards/Guidance Documents Referenced:

- CLSI EP05- A3 Evaluation of Precision of Quantitative Measurement Methods-Approved Guideline-Third Edition
- CLSI EP06 2nd Edition – Evaluation of the Linearity of Quantitative Measurement Procedures
- CLSI EP07 3rd Edition – Interference Testing in Clinical Chemistry
- CLSI EP37 1st Edition – Supplemental Tables for Interference Testing in Clinical Chemistry
- CLSI EP17-A2 – Evaluation of Detection capability for Clinical Laboratory Measurement Procedures; Approved Guideline 2nd Edition
- CLSI EP09c 3rd Edition – Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP34 1st Edition – Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking
- CLSI 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:

Precision of the iFlash-HCG Assay was evaluated according to CLSI EP05-A3 using nine serum samples and two levels of controls. Each sample was measured in two replicates per run with two runs per day over 20 days by three different operators at three different sites using three lots of the reagents on three different Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) resulting in a total of 240 test results per level. Results from one analyzer are provided in the table below.

Sample	Mean (mIU/mL)	Repeatability		Between-Run		Between-Day		Total imprecision	
	N=240	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	0.57	0.028	4.9	0.015	2.6	0.013	2.3	0.041	7.2
Sample 2	4.68	0.199	4.3	0.072	1.5	0.045	1.0	0.216	4.6
Sample 3	14.9	0.589	3.9	0.078	0.5	0.172	1.2	0.978	6.6
Sample 4	23.5	0.838	3.6	0.269	1.1	0.116	0.5	0.914	3.9
Sample 5	91.5	1.98	2.2	0.213	0.2	0.382	0.4	4.18	4.6
Sample 6	957	13.9	1.5	6.61	0.7	6.31	0.7	22.5	2.4
Sample 7	2823	34.8	1.2	0.000	0.0	14.3	0.5	46.6	1.6
Sample 8	5627	63.1	1.1	26.5	0.5	11.9	0.2	86.0	1.5
Sample 9	9023	100	1.1	33.8	0.4	14.1	0.2	138	1.5
Control 1	2.71	0.127	4.7	0.045	1.7	0.029	1.1	0.200	7.4
Control 2	101	1.96	1.9	0.486	0.5	0.620	0.6	5.44	5.4

2. Linearity:

Linearity of the iFlash-HCG Assay was evaluated following recommendations in CLSI EP06-Ed2. Three linearity studies were performed using eleven serum samples each. Varying concentrations of hCG were obtained by mixing low level serum samples and high level serum samples. All three studies were performed using three lots of reagents on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C).

In the first study, linearity was established by testing samples with concentrations ranging from 0.43 to 11786.53 mIU/mL. Each sample was tested in four replicates.
A regression analysis was conducted resulting in the following linear regression equation:
$y = 0.9824x + 38.719$, $R2 = 0.9994$

In the second study, mid-range linearity was established by testing samples with hCG concentrations ranging from 0.45 to 1,050.30 mIU/mL. Each sample was tested in four

replicates. A regression analysis was conducted resulting in the following linear regression equation: y = 0.9994x - 0.545, R2 = 0.9996

In the third study, low-range linearity was established by testing samples with concentrations ranging from 0.46 to 128.02 mIU/mL. Each sample level was tested in four replicates. A regression analysis was conducted resulting in the following linear regression equation: y = 1.014x -2.857, R2 = 0.9995

The results from the linearity studies and limit of quantitation studies (LOQ = 0.50 ng/mL, see below), supports the sponsor's claimed analytical measuring interval of 0.50 to 10,000 mIU/mL.

Dilution Studies

Dilution studies were performed to determine the sample recovery after a 1:100 dilution either manually or automatically by the Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C). The dilution study results support labeling claims that samples with hCG concentrations above 10,000 mIU/mL may be diluted 1:50 or 1:100 with sample diluent either manually or automatically on-onboard the analyzer.

3. Analytical Specificity/Interference:

Interference

The potential interference of certain exogenous and endogenous substances was assessed using female human serum pool samples containing approximately 6 mIU/mL and 5000 mIU/mL ("low" and "high" respectively) hCG spiked with various concentrations of substances based on 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).

The following table lists the concentration of each endogenous substance at which no significant interference was found in the study.

Substance	**Concentration**
Bilirubin (Conjugated)	40mg/dL
Bilirubin (Unconjugated)	40mg/dL
Hemoglobin	1000mg/dL
Triglycerides	3000 mg/dL
Total protein	≤10000 mg/dL
Rheumatoid factors	2000 IU/mL
Human Anti-Mouse Antibodies (HAMA)	600 ng/mL
Antinuclear antibody (ANA)	500 AU/mL

The following table lists the concentration of each exogenous substance at which no significant interference was found in the study.

Substance	**Concentration**
Phenylbutazone	400 μg/mL

Aspirin (Acetylsalicylic Acid)	1000 µg/mL
Acetaminophen	200 µg/mL
Ibuprofen	500 µg/mL
N-acetylcysteine	150 µg/mL
Methyldopa	25 µg/mL
Theophylline	60 µg/mL
Metformin	12 µg/mL
Isosorbide dinitrate	6 µg/mL
Rifampicin	48 µg/mL
Tetracycline hydrochloride	24 µg/mL
Cefoxitin	6600 µg/mL
Cyclosporine	2 µg/mL
Metronidazole	125 µg/mL
Ascorbic acid	60 µg/mL
Ampicillin-Na	100 µg/mL
Levodopa	20 µg/mL

Cross-Reactivity

Cross-reactivity of the iFlash-HCG assay was determined with three lots of reagent on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) using female human serum samples with approximately 0.1, 5, and 4000 mIU/mL ("low", "medium", and "high" respectively) 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 three lots of reagents on one iFlash 3000-C analyzer. The results demonstrated no cross-reactivity from potential cross-reactants up to 500 mIU/mL LH, 200 mIU/mL FSH, and 10000 mIU/mL TSH

Hook Effect

No high dose hook effect was observed for hCG concentrations in female human serum up to 1,250,000 mIU/mL hCG.

4. Assay Reportable Range:

The reportable range is 0.50 – 10,000 mIU/mL.

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

Traceability: This assay is traceable to the World Health Organization (WHO) 5th International Standard Chorionic Gonadotropin, Human, National Institute for Biological Standards and Control (NIBSC), code 07/364.

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the iFlash-HCG Assay were evaluated based upon recommendations in CLSI EP17-A2.

Limit of Blank
For determination of limit of blank (LoB), five analyte free samples were tested with three lots of reagents over at least three days on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) in four runs with five replicates per run over three days. In total 60 measured values of analyte free samples were obtained per lot. The LOB was determined to be 0.10 mIU/mL.

Limit of Detection
For determination of limit of detection (LoD), five serum samples containing low – analyte concentration (approximately 1-5 times LoB) were tested with three lots of reagents on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) in four runs with five replicates per run over three days. In total 60 measured values of samples with low analyte concentrations were obtained per lot. The LoD was determined to be 0.20 mIU/mL.

Limit of Quantitation
For determination of limit of quantitation (LoQ), five human serum samples covering the range between 0.30 and 70 mIU/mL were tested with three lots of reagents on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) in four runs with five replicates per run over three days. Limit of quantitation (LoQ) is the lowest concentration which is the design requirements of TEa%(Total Error Allowance) of ≤30%. The LoQ was determined to be 0.50 mIU/mL.

7. Assay Cut-Off:

Not applicable; this is a quantitative assay.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

Samples with high hCG (1,250,000 mIU/mL) and low hCG (3.03 mIU/mL) hCG were tested alternating five times (i.e. HH,LL,HH,LL, etc.) in replicates of three, using one reagent lot on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C). No significant sample carryover effect was observed.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were conducted following recommendations in CLSI EP09-A3 Guideline. A total of 110 female human serum samples were tested in singlicate using iFlash-HCG Assay on Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C)

(candidate device) and Elecsys HCG+β (comparator device). The regression results are shown in the following table:

Parameter	Passing Bablok
n	110
Slope	0.9860
95% CI	0.970-1.008
Intercept	-0.0470
95% CI	3.161 - 0.312
Correlation Coefficient (R2)	0.9980
Sample Range	0.531-9717

2. Matrix Comparison:

A matrix comparison study was performed to evaluate matrices suitable for use with the iFlash-HCG assay. Serum samples were compared to matched Lithium Heparin, Sodium Heparin, and K_2-EDTA plasma samples using three lots of reagents on one Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C). The results were assessed using Passing/Bablok regression analysis.

Serum/Lithium-Heparin Plasma Summary Results

Parameter	Passing Bablok
n	97
Sample Range (mIU/mL)	0.62 - 8398
Slope	0.988
Intercept	0.189
Correlation Coefficient (R2)	0.999

Serum/Sodium Heparin Plasma Summary Results

Parameter	Passing Bablok
n	97
Sample Range (mIU/mL)	0.65 - 8952
Slope	1.011
Intercept	-0.319
Correlation Coefficient (R2)	0.999

Serum/K_2-EDTA Plasma Summary Results

Parameter	Passing Bablok
n	97
Sample Range (mIU/mL)	0.65 - 8952
Slope	1.0005
Intercept	-0.0331
Correlation Coefficient (R2)	0.990

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:

Serum samples from 130 healthy, non-pregnant premenopausal women and 125 healthy, postmenopausal women were tested using the iFlash-HCG on the Chemiluminescence Immunoassay Analyzer (Model: iFlash 3000-C) according to CLSI EP28-A3c. The reference interval was calculated using the nonparametric method according to CLSI C28-A3, and was determined to be 0.05 - 0.85 mIU/mL for women ≤ age 50, and 0.1 - 4.4 mIU/mL for women over age 50. The central 95% reference interval was established to be 0.6 mIU/mL for premenopausal women and 5.4 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.