510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K212418

B Applicant

Healstone Biotech Inc.

C Proprietary and Established Names

hCG One Step Pregnancy Test

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Human chorionic gonadotropin (hCG)

C Type of Test:

Qualitative chromatographic immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The hCG One Step Pregnancy Test is an in vitro diagnostic test for the qualitative determination of human chorionic gonadotropin hormone in human urine samples. It is used as an aid in early detection of pregnancy, in some cases as early as five (5) days before the expected period, i.e., as early as six (6) days before the day of the missed period.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

None.

IV Device/System Characteristics:

A Device Description:

The hCG One Step Pregnancy Test is a qualitative immunochromatographic assay containing a lateral flow test strip housed in a cassette. The test strip can be used in midstream and dip mode.

B Principle of Operation:

The hCG One Step Pregnancy Test is a lateral flow sandwich immunochromatographic assay that utilizes a combination of monoclonal antibodies for qualitative detection of hCG in human urine samples. The test uses two lines to show the results. When the urine sample is added to the absorbent tip, the sample migrates via capillary action along the membrane. If present in the sample, hCG will react with the specific monoclonal hCG antibody and form a colored line at the test line region of the membrane, indicating a positive result (pregnant). Absence of a colored line at the test line region suggests a negative result (not pregnant). To serve as a procedural control, a colored line will always appear in the control line region. If the control line does not appear (no color appears at the control line region 3 minutes after application of the sample), the test is invalid and the specimen should be retested with a new device.

V Substantial Equivalence Information:

A Predicate Device Name(s):

First Response Early Result Pregnancy Test

B Predicate 510(k) Number(s):

K123436

C Comparison with Predicate(s):

Device & Predicate Device(s):	K212418	K123436
Device Trade Name	hCG One Step Pregnancy Test	First Response Early Result Pregnancy Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	For qualitative detection of hCG as an aid in early detection of pregnancy	Same
Product Usage	Over-the-counter use	Same
Methodology	Chromatographic immunoassay	Same
Specimen Collection Method	Midstream or dip	Same
Specimen type	Urine	Same
Sensitivity	10 mIU/mL	Same
Time to Result	3 minutes	Same
General Device Characteristic Differences		
hCG Isoforms Detected	Intact hCG	Intact hCG, hyperglycosylated hCG, hCG β-subunit, hCG β-core fragment

VI Standards/Guidance Documents Referenced:

- CLSI guideline EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition – 2012.
- CLSI Guidelines EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- Third Edition (2014)
- CLSI EP09c-A3: Measurement Procedure Comparison And Bias Estimation Using Patient Samples; Approved Guideline - Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   Precision studies were performed using both formats of the device (midstream and dip) and negative urine samples spiked with hCG to obtain samples with hCG concentrations of 0, 5, 7.5, 8.75, 10, 12.5 and 25 mIU/mL. The study was conducted over 5 non-consecutive days by 6 operators using 3 lots per test format. A total of 150 samples were tested per lot, for a total

of 450 replicates per device format per hCG concentration. The device cutoff is 10 mIU/mL. The results are summarized in the tables below:

Overall precision results (Simulated midstream method)

hCG Concentration (mIU/mL)	Total (n)	Positive (n)	Negative (n)	% Positive Results
0	450	0	450	0%
5	450	0	450	0%
7.5	450	214	236	47.6%
8.75	450	412	38	91.6%
10	450	450	0	100%
12.5	450	450	0	100%
25	450	450	0	100%

Precision results per device lot (Simulated midstream method)

hCG Concentration (mIU/mL)	Lot 1		Lot 2		Lot 3	
	# positive/ # negative	% Positive	# positive/ # negative	% Positive	# positive/ # negative	% Positive
0	0/150	0%	0/150	0%	0/150	0%
5	0/150	0%	0/150	0%	0/150	0%
7.5	74/150	49.3%	72/150	48%	68/150	45.3%
8.75	135/150	90%	137/150	91.3%	140/150	93.3%
10	150/150	100%	150/150	100%	150/150	100%
12.5	150/150	100%	150/150	100%	150/150	100%
25	150/150	100%	150/150	100%	150/150	100%

Overall precision results (dip method)

hCG Concentration (mIU/mL)	Total (n)	Positive (n)	Negative (n)	% Positive Results
0	450	0	450	0%
5	450	0	450	0%
7.5	450	209	241	46.4%
8.75	450	406	44	90.2%
10	450	450	0	100%
12.5	450	450	0	100%
25	450	450	0	100%

Precision results per device lot (dip method)

hCG Concentration (mIU/mL)	Lot 1		Lot 2		Lot 3	
	# positive/ # negative	% Positive	# positive/ # negative	% Positive	# positive/ # negative	% Positive
0	0/150	0%	0/150	0%	0/150	0%
5	0/150	0%	0/150	0%	0/150	0%
7.5	70/150	46.7%	67/150	44.7%	72/150	48%
8.75	135/150	90%	133/150	88.7%	138/150	92%
10	150/150	100%	150/150	100%	150/150	100%
12.5	150/150	100%	150/150	100%	150/150	100%
25	150/150	100%	150/150	100%	150/150	100%

2. Linearity:

Not applicable. This is a qualitative device.

3. Analytical Specificity/Interference:

Interference from exogenous and endogenous substances

To evaluate the potential for interference from certain exogenous and endogenous compounds, female urine containing 0 (negative) and 10 mIU/mL (positive) hCG were spiked with potentially interfering exogenous and endogenous substances and tested with both formats of the device (midstream and dip). Control samples containing no test substance were also tested and compared to test samples. Samples were tested in five replicates results per device format. The results demonstrated no interference from substances at the concentrations shown in the table below.

Potentially Interfering Substances

Analyte	**Concentration**
Caffeine	0.2 mg/mL
Ascorbic acid	0.2 mg/mL
Ethanol	1% v/v
Atropine	0.2 mg/mL
Ampicillin	0.2 mg/mL
Acetaminophen	0.2 mg/mL
Gentisic acid	0.2 mg/mL
Acetylsalicyclic acid	0.2 mg/mL
Phenothiazine	0.01 mg/mL
Tetracycline	0.2 mg/mL
Estriol	0.01 mg/mL
Glucose	20 mg/mL
Hemoglobin	2 mg/mL
Albumin	60 mg/mL
Bilirubin	0.1 mg/mL

Cross Reactivity Study:

The hCG One Step Pregnancy Test was evaluated for potential cross-reactivity from luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). Urine specimens from non-pregnant females were used to prepare samples with hCG levels at 0 and 10 mIU/mL hCG and were spiked with the potential cross-reactants to obtain the desired test concentration. The results demonstrated no cross-reactivity from potential cross-reactants up to 800 mIU/mL LH, 1000 mIU/mL FSH, and 1 mIU/mL TSH in either negative or positive urine samples.

Effect of urine pH and Specific Gravity:

Negative urine samples from non-pregnant females were spiked with hCG standard substance at two different hCG concentrations (0 mIU/mL and 10 mIU/mL hCG) and were adjusted to pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 before tested using the candidate device. These samples were tested in 15 replicates using 3 lots of the proposed device by 3 different lab technicians (5 replicates per lot for each pH value). Two lots of test device were performed using dip method, one lot of test device was performed using simulated stream method. The results demonstrated that samples with pH range of 4 and 9 do not interfere with either positive or negative results from the device.

Negative urine samples from nonpregnant females with different urinary specific gravities between the range of 1.000 and 1.035 were spiked with hCG standard substance at two different hCG concentrations (low concentration of 0 mIU/mL and 10 mIU/mL). The specific gravity values for the samples collected and tested with the candidate device were 1.001, 1.003, 1.013, 1.021, 1.024, 1.026, 1.017, 1.019, 1.022, 1.030, and 1.031. The positive and negative hCG results were not affected by urine specific gravity ranges between 1.001 and 1.031.

Effect of hCG β-core fragment:

Urine samples (0 and 10 mIU/mL hCG) were spiked with hCG β-core fragment at concentrations of 102,000 pmol/L, 204,000 pmol/L, and 408,000 pmol/L and tested using 3 lots of the candidate device. All samples yielded correct results with hCG β-core fragment concentrations up to 408,000 pmol/L.

Hook Effect:

Negative pooled human urine was spiked with hCG to concentrations up to 500,000 mIU/mL hCG. Samples were tested in 15 replicates using 3 lots of the device by 3 operators. Samples were tested using both device formats (two lots of the test device were tested using the dip method while one lot of the test device was performed using the simulated stream method). No hook effect was observed at concentrations of up to 500,000 mIU/mL hCG.

4. Assay Reportable Range:

Not applicable.

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

Traceability: The hCG One Step Pregnancy Test is traceable to the 5th WHO International Standard (IS) from NIBSC with code 07/364.

6. Detection Limit:

The limit of detection was determined by using pooled negative human urine samples spiked with 0, 3, 5, 6.25, 7.5, 8.75, 10, and 15 mIU/mL hCG. Three lots of each format of the device were tested by 3 operators. The sensitivity of the hCG One Step Pregnancy Test was determined to be 10 mIU/mL for both test formats.

7. Assay Cut-Off:

The device cut-off is 10 mIU/mL. See Precision/Reproducibility (Section VII.A.1.) and Detection Limit (Section VII.A.6.) sections above.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The performance of the hCG One Step Pregnancy Test was evaluated by professionals at 2 sites with 2 lots of the candidate device and the predicate device. Urine samples from 158 women who were suspected of being pregnant between the ages of 18 to 45 were masked and randomized prior to testing. The results are summarized below.

Candidate device (dip method)	Predicate device (dip method)		
	Positive	Negative	Total
Positive	56	0	56
Negative	0	102	102
Total	56	102	158

Candidate device (simulated midstream method)	Predicate device (simulated midstream method)		
	Positive	Negative	Total
Positive	56	0	56
Negative	0	102	102
Total	56	102	158

The test performance of the hCG One Step Pregnancy Test is 100% concordant when compared to the predicate.

Lay-User Study:

Two lay user studies ((1) Lay users tested their own urine specimen and (2) Lay users testing spiked samples (Lay user spiked sample study)) were conducted at an internal site by lay users from the intended use population.

For study 1, a total of 202 female subjects with ages ranging from 18 and 45 years tested their own urine specimen. Each subject performed the hCG One Step Pregnancy Test by dip method or midstream method following the package insert instructions without any assistance. There were 98 subjects who used the midstream method and 104 who used the dip method. The data demonstrated 100% agreement between lay user results and professional results.

Lay User (Midstream)	Professional User (Midstream)		
	Positive	Negative	Total
Positive	48	0	48
Negative	0	50	50
Total	48	50	98

Lay User (Dip)	Professional User (Dip)		
	Positive	Negative	Total
Positive	72	0	72
Negative	0	32	32
Total	72	32	104

For study 2, the same 202 female subjects subsequently tested two randomly distributed tubes of urine samples spiked with hCG with concentrations of 3.0mIU/ml, 7.5 mIU/ml, 8.75 mIU/ml or 10 mIU/ml. All samples were masked and randomized prior to testing. The data demonstrated 100% agreement between lay user results and professional results at the assay cutoff of 10 mIU/mL.

	Simulated Midstream		Dip	
hCG level (mIU/mL	Lay User % Positive	Professional % Positive	Lay User % Positive	Professional % Positive
3	0%	0%	0%	0%
7.5	8.7%	96%	13.2%	96%
8.75	69.1%	100%	64.7%	100%
10	100%	100%	100%	100%

2. Matrix Comparison:

Not applicable. The device is intended for urine samples only.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   The clinical specificity of the hCG One Step Pregnancy Test was evaluated with a total 612 women with ages ranging from 18 to 66 years old who did not expect to be pregnant at the time of testing. A total of 612 urine samples were tested with three lots of the device. Of the 612 urine samples, 606 samples were consistently negative and six (6) of the 612 samples were consistently positive on the hCG One Step Pregnancy Test. These corresponding subjects were confirmed pregnant via follow up ultrasound exams. These six subjects were excluded from the calculation of the clinical specificity. The clinical specificity was calculated as 606/606, or 100%. There was no difference between the two test methods (dip and midstream).

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

   Early Pregnancy Detection Study:

   A real-world study was conducted to demonstrate the detection of hCG in Conception Cycles relative to the day of Expected Menstrual Period (EMP) by the hCG One Step Pregnancy Test. A total of 649 urine samples were collected from 59 different women (25-45 years old) who planned to become pregnant. These women were followed throughout their conception cycles with urine collected from day -9 to day +1 of their expected period**.** A total of 59 subjects were confirmed pregnant with an ultrasound exam. The hCG One Step Pregnancy Test (both dip and stream methods used for each sample) detected hCG in 68% of samples from five days before the expected menstrual period and 100% of samples from two days before the expected menstrual period. All positive hCG One Step Pregnancy Test results were confirmed with ultrasound exams. There was no difference in results between the two test methods, dip or stream.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

VIII Proposed Labeling:

The proposed labeling supports substantial equivalence for this device.

IX Conclusion:

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