510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K243573

B Applicant

Assure Tech LLC

C Proprietary and Established Names

FaStep Early Pregnancy Rapid Test Strip; FaStep Early Pregnancy Rapid Test Cassette; FaStep Early Pregnancy Rapid Test Midstream

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 lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

FaStep Early Pregnancy Rapid Test Strip is used for qualitative detection of Human Chorionic Gonadotropin (HCG) in human urine. This test is intended for use as an aid in early detection of pregnancy, in some cases as early as six (6) days before the day of the missed period, (i.e. as early as five (5) days before the day of the expected period). For over-the-counter use.

FaStep Early Pregnancy Rapid Test Cassette is used for qualitative detection of Human Chorionic Gonadotropin (HCG) in human urine. This test is intended for use as an aid in early detection of pregnancy, in some cases as early as six (6) days before the day of the missed period, (i.e. as early as five (5) days before the day of the expected period). For over-the-counter use.

FaStep Early Pregnancy Rapid Test Midstream is used for qualitative detection of Human Chorionic Gonadotropin (HCG) in human urine. This test is intended for use as an aid in early detection of pregnancy, in some cases as early as six (6) days before the day of the missed period, (i.e. as early as five (5) days before the day of the expected period). For over-the-counter use.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

None.

IV Device/System Characteristics:

A Device Description:

The test comes in three formats (strip, cassette, and midstream) named FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream, respectively. The strip format is a single test strip designed to be used in dip sampling mode only. The cassette format consists of a single test strip assembled in a plastic housing and is designed to be used in drop sampling mode only. A pipette dropper is included in the packaging. The midstream format consists of a single test strip assembled in a plastic housing with an absorbent tip and is designed to be tested in dip or in-stream sampling mode. The result is displayed in the test window of all formats as two lines for a ‘Pregnant’ positive result and one line for a ‘Not Pregnant’ negative result.

B Principle of Operation:

FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream use lateral flow immunoassay technology for in

vitro qualitative detection of human chorionic gonadotropin (hCG) in human urine. The devices utilize a combination of antibodies to detect hCG in urine as well as to serve as a control. Each device contains mouse monoclonal anti-β-hCG antibody colloidal gold conjugate pre-dried on the sample pad. Mouse monoclonal anti-α-hCG antibody (on the test line) and goat anti mouse IgG polyclonal antibody (on the control line) are coated and immobilized on a nitrocellulose membrane. When the test is performed properly, a colored line will always appear in the control zone (C). The test result is shown in the result window and read visually within 3-10 minutes of urine addition. Two distinct colored lines, one in the test zone (T) and another in the control zone (C) indicate a positive test result (pregnant). Absence of a colored line in the test zone (T) and only a colored line in the control zone (C) indicates a negative test result (not pregnant). Absence of a colored line in the control zone (C) even in the presence of a colored line in the test zone (T) indicates an invalid test result.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Wondfo One Step HCG Urine Pregnancy Test Strip, Wondfo One Step HCG Urine Pregnancy Test Cassette, Wondfo One Step HCG Urine Pregnancy Test Midstream

B Predicate 510(k) Number(s):

K150022

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243573	K150022
Device Trade Name	FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, FaStep Early Pregnancy Rapid Test Midstream	Wondfo One Step HCG Urine Pregnancy Test Strip, Wondfo One Step HCG Urine Pregnancy Test Cassette, Wondfo One Step HCG Urine Pregnancy Test Midstream
General Device Characteristic Similarities		
Intended Use/Indications For Use	Qualitative detection of human chorionic gonadotropin (hCG) in human urine as an aid in early detection of pregnancy	Same
Early Detection Claim	Pregnancy can be detected in some cases as early as six (6) days before the day of the missed period, i.e., as early as five (5) days before the day of the expected period	Same

Specimen type	Urine	Same
Methodology	Immunochromatographic assay	Same
Claimed analytical sensitivity	10 mIU/mL	Same
Device format	Strip, cassette, midstream	Same
General Device Characteristic Differences		
Target User	Over-the-counter	Prescription and over-the-counter use
Read Time	3-10 minutes	5 minutes
Traceability	World Health Organization (WHO) 5th International Standard (IS)	World Health Organization (WHO) 4th International Standard (IS)

VI Standards/Guidance Documents Referenced:

None referenced.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A precision study was performed using pooled negative female urine spiked with hCG (traceable to the 5th World Health Organization International Standard (WHO IS)) to obtain samples with hCG contractions of 0, 5, 7.5, 8, 10, 15, 20, 25 and 50 mIU/mL using 3 lots each of the FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream (both in-stream and dip sampling modes). Samples were tested in replicates of ten per day for five days for each device lot by three operators. The results are summarized in the table below.

Summary results of FaStep Early Pregnancy Rapid Test Strip (dip sampling mode)

hCG Concentration (mIU/mL)	Operator 1		Operator 2		Operator 3		Total result		% Negative	% Positive
	Lot 1		Lot 2		Lot 3					
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0%
5	50	0	50	0	50	0	150	0	100%	0%
7.5	31	19	29	21	30	20	90	60	60.0%	40.0%
8	24	26	25	25	24	26	73	77	48.7%	51.3%
10	0	50	0	50	0	50	0	150	0%	100%
15	0	50	0	50	0	50	0	150	0%	100%
20	0	50	0	50	0	50	0	150	0%	100%
25	0	50	0	50	0	50	0	150	0%	100%
50	0	50	0	50	0	50	0	150	0%	100%

Summary results of FaStep Early Pregnancy Rapid Test Cassette (drop sampling mode)

hCG Concentration (mIU/mL)	Operator 1		Operator 2		Operator 3		Total result		% Negative	% Positive
	Lot 1		Lot 2		Lot 3					
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0%
5	50	0	50	0	50	0	150	0	100%	0%
7.5	29	21	30	20	30	20	89	61	59.3%	40.7%
8	25	25	23	27	24	26	72	78	48.0%	52.0%
10	0	50	0	50	0	50	0	150	0%	100%
15	0	50	0	50	0	50	0	150	0%	100%
20	0	50	0	50	0	50	0	150	0%	100%
25	0	50	0	50	0	50	0	150	0%	100%
50	0	50	0	50	0	50	0	150	0%	100%

Summary results of FaStep Early Pregnancy Rapid Test Midstream (in-stream sampling mode)

hCG Concentration (mIU/mL)	Operator 1		Operator 2		Operator 3		Total result		% Negative	% Positive
	Lot 1		Lot 2		Lot 3					
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0%
5	50	0	50	0	50	0	150	0	100%	0%
7.5	29	21	30	20	29	21	88	62	58.7%	41.3%
8	25	25	25	25	24	26	74	76	49.3%	50.7%
10	0	50	0	50	0	50	0	150	0%	100%
15	0	50	0	50	0	50	0	150	0%	100%
20	0	50	0	50	0	50	0	150	0%	100%
25	0	50	0	50	0	50	0	150	0%	100%
50	0	50	0	50	0	50	0	150	0%	100%

Summary results of FaStep Early Pregnancy Rapid Test Midstream (dip sampling mode)

hCG Concentration (mIU/mL)	Operator 1		Operator 2		Operator 3		Total result		% Negative	% Positive
	Lot 1		Lot 2		Lot 3					
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0%
5	50	0	50	0	50	0	150	0	100%	0%
7.5	30	20	28	22	31	19	89	61	59.3%	40.7%
8	26	24	24	26	25	25	75	75	50.0%	50.0%
10	0	50	0	50	0	50	0	150	0%	100%
15	0	50	0	50	0	50	0	150	0%	100%
20	0	50	0	50	0	50	0	150	0%	100%
25	0	50	0	50	0	50	0	150	0%	100%
50	0	50	0	50	0	50	0	150	0%	100%

The claimed analytical sensitivity is 10 mIU/mL hCG.

2. Linearity:

Linearity is not applicable since this is a qualitative test.

3. Analytical Specificity/Interference:

Interference from exogenous and endogenous substances:
To evaluate potential interference from certain exogenous and endogenous substances, negative urine samples from normal healthy females were spiked to hCG concentrations of 0 mIU/mL, 5 mIU/mL, and 10 mIU/mL hCG and were then spiked with potentially interfering substances at the concentrations listed below. Samples were tested by three operators, in triplicate, using three device lots. No interference was observed at the concentrations shown in the table below:

Substance	***Concentration***
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	20 mg/dL
Albumin	20 mg/dL
Amoxicillin	20 mg/dL
Ampicillin	20 mg/dL
Ascorbic acid	20 mg/dL
Aspirin	80 mg/dL
Atropine	20 mg/dL
Benzoylecgonine	10 mg/dL
Bilirubin	2 mg/dL
Caffeine	20 mg/dL
Cannabinol	10 mg/dL
Codeine	0.006 mg/dL
EDTA	80 mg/dL
Ephedrine	20 mg/dL
Ethanol	0.1%
Folic acid	0.03 mg/dL
Gentisic acid	20 mg/dL
Glucose	2000 mg/dL
Hemoglobin	20 mg/dL
Ibuprofen	40 mg/dL
Ketone	20 mg/dL
Methanol	1%
Phenothiazine	20 mg/dL
Phenylpropanolamine	20 mg/dL
Pregnanediol	1.5 mg/dL
Salicylic Acid	20 mg/dL
Tetracycline	20 mg/dL
Thiophene	20 mg/dL

Uric acid	23.5 mg/dL
Vitamin B1	80 mg/dL
β-hydroxybutyrate	2000 mg/dL

Cross-reactivity of Similar Compounds:
To evaluate cross-reactivity, negative urine samples were spiked to contain 0, 5 and 10 mIU/mL hCG and then spiked with potential cross reactants. Samples were then spiked with 500 mIU/mL luteinizing hormone (hLH), 1000 mIU/mL follicle-stimulating hormone (hFSH), and 1 mIU/mL thyroid-stimulating hormone (hTSH). The samples were tested in ten replicates using three device lots by three different operators. No cross-reactivity was observed at the tested concentrations.

Effects of Urine pH:
To evaluate the effect of urine pH on test results, urine samples containing 0, 5, and 10 mIU/mL hCG were tested at pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The samples were tested in triplicate using three lots of the device by three operators. The results demonstrated that changes in pH ranging from 4.0 to 9.0 do not interfere with either positive or negative results from the device.

Effects of Urine Specific Gravity:
To evaluate the effect of urine specific gravity on test results, urine samples containing 0, 5 and, 10 mIU/mL hCG were tested at specific gravities of 1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030 and 1.035. The samples were tested in triplicate using three device lots by three operators. The results demonstrated that changes in specific gravity ranging from 1.000 to 1.035 do not interfere with either positive or negative results from the device.

Hook Effect:
Negative urine samples were spiked with varying hCG concentrations up to 200,000 mIU/mL. The samples were tested in replicates of five using three device lots by three operators. All tested concentrations gave a positive result. The results demonstrated that no hook effect was observed at hCG concentration up to 200,000 mIU/mL.

Effects of hCG β-core fragment:
Urine samples with hCG concentrations of 0, 5, 10, and 20,000 mIU/mL were spiked with hCG β-core fragment (hCGβcf) at concentrations of 50,000 pmol/L, 125,000 pmol/L, 250,000pmol/L, and 500,000 pmol/L. The samples were tested in replicates of ten using three lots of the device by three operators. All samples yielded correct results with hCG β-core fragment concentrations up to 500,000 pmol/L.

4. Assay Reportable Range:

Not applicable.

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

FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream are calibrated against reference material traceable to WHO International Standard 5th edition, NIBSC code 07/364.

6. Detection Limit:

A detection limit study was performed using negative female urine samples spiked with 0, 5, 6, 7.5, 8, 9, 10, 12.5, 15, 20, 25 and 50 mIU/mL hCG (hCG traceable to the 5th WHO IS). The samples were tested using all three device formats (FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream (both in-stream and dip modes)). The samples were tested in replicates of 20, using three device lots of each test format, by three different operators. The claimed analytical sensitivity or cut-off of the candidate device is 10 mIU/mL hCG.

7. Assay Cut-Off:

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

B Comparison Studies:

1. Method Comparison with Predicate Device:

Urine samples were collected from 321 women who presented at three sites for pregnancy testing. Approximately half of the subjects were suspected to be pregnant in the early stage of less than 5 weeks. Ages of these women ranged from 18 to 50 years. Samples were masked and randomized by people who labeled the samples but did not participate in the testing. A total of 110 samples were tested for strip format, and 105 samples were tested for cassette format; 106 samples were tested for the midstream format in both dip sampling mode and in-stream sampling mode. Samples were tested by medical professionals using both the candidate and predicate devices. The results are summarized in the tables below.

Summary results of FaStep Early Pregnancy Rapid Test Strip (dip sampling mode)

Candidate device		Predicate device		
		Positive	Negative	Total
Strip format	Positive	61	0	61
	Negative	0	49	49
	Total	61	49	110

Summary results of FaStep Early Pregnancy Rapid Test Cassette (drop sampling mode)

Candidate device		Predicate device		
		Positive	Negative	Total
Cassette format	Positive	54	0	54
	Negative	0	51	51
	Total	54	51	105

Summary results of FaStep Early Pregnancy Rapid Test Midstream (dip sampling mode)

Candidate device		Predicate device		
		Positive	Negative	Total
Midstream format (dip method)	Positive	57	0	57
	Negative	0	49	49
	Total	57	49	106

Summary results of FaStep Early Pregnancy Rapid Test Midstream (in-stream sampling mode)

Candidate device		Predicate device		
		Positive	Negative	Total
Midstream format (in-stream method)	Positive	57	0	57
	Negative	0	49	49
	Total	57	49	106

The test performance of FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream were 100% concordant when compared to the predicate.

2. Matrix Comparison:

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

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Specificity Study to Determine False Positive Result Rate:
Urine sampled from 900 non-pregnant females were tested with FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream (both in-stream and dip modes). Urine samples from non-pregnant women in pre-menopausal (18-40 years, n=300), peri-menopausal (41-55 years, n=300), and post-menopausal (>55 years, n=300) age groups were evaluated. In each age group, 100 participants were tested with the strip format, 100 participants were tested with the cassette format, 50 participants were tested with the midstream format using in-stream sampling method, and 50 participants tested with the midstream format using dip sampling method. Three lots of each test format were used in the study. The results are summarized in the tables below.

Summary results of FaStep Early Pregnancy Rapid Test Strip

Group	Lot 1	Lot 2	Lot 3	Total result
Pre-menopausal	0+/34-	0+/33-	0+/33-	0+/100-
Peri-menopausal	0+/33-	0+/34-	0+/33-	0+/100-
Post-menopausal	0+/33-	0+/33-	0+/34-	0+/100-

Summary results of FaStep Early Pregnancy Rapid Test Cassette

Group	Lot 1	Lot 2	Lot 3	Total result
Pre-menopausal	0+/34-	0+/33-	0+/33-	0+/100-
Peri-menopausal	0+/33-	0+/34-	0+/33-	0+/100-
Post-menopausal	0+/33-	0+/33-	0+/34-	0+/100-

Summary results of FaStep Early Pregnancy Rapid Test Midstream (dip and in-stream sampling modes combined)

Group	Lot 1	Lot 2	Lot 3	Total result
Pre-menopausal	0+/34-	0+/33-	0+/33-	0+/100-
Peri-menopausal	0+/33-	0+/34-	0+/33-	0+/100-
Post-menopausal	0+/33-	0+/33-	0+/34-	0+/100-

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

Detection of hCG in Early Pregnancy Clinical Samples:
A total of 680 urine samples were collected from 68 pregnant women from the intended use population. The women ranged from 18 to 45 years old, and testing was conducted starting from 9 days before expected menstrual period to the day of the expected menstrual period (EMP). Each sample was tested using FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream (both in-stream and dip sampling modes). Pregnant women were later confirmed to be pregnant via ultrasound. Representative results are summarized in the table below.

Days Before Expected Menstrual Period (EMP)	Total (n)	Positive (n)	Percentage of Pregnancy
-9	68	0	0%
-8	68	5	7.4%
-7	68	10	14.7%
-6	68	27	39.7%
-5	68	47	69.1%
-4	68	61	89.7%
-3	68	66	97.1%
-2	68	67	98.5%
-1	68	68	100%
0	68	68	100%

Lay User Study:
A total of 321women with varying educational and occupational backgrounds with an age range of 18 to 50 years old from three clinical sites tested their own urine specimen.
A total of 110 lay users tested using the strip format, 105 lay users tested using the cassette format, 53 lay users tested using the midstream format in dip sampling mode, and 53 lay users tested using the midstream format in in-stream sampling mode. Each lay user also provided a sample for professional testing. The data demonstrated 100% agreement between lay user results and professional results.

Summary results of FaStep Early Pregnancy Rapid Test Strip (dip sampling mode)

Strip format		Professional		
		Positive	Negative	Total
Layperson	Positive	61	0	61
	Negative	0	49	49
	Total	61	49	110

Summary results of FaStep Early Pregnancy Rapid Test Cassette (drop sampling mode)

Cassette format		Professional		
		Positive	Negative	Total
Layperson	Positive	54	0	54
	Negative	0	51	51
	Total	54	51	105

Summary results of FaStep Early Pregnancy Rapid Test Midstream (dip sampling mode)

Midstream format (dip method)		Professional		
		Positive	Negative	Total
Layperson	Positive	32	0	32
	Negative	0	21	21
	Total	32	21	53

Summary results of FaStep Early Pregnancy Rapid Test Midstream (in-stream sampling mode)

Midstream format (in-stream method)		Professional		
		Positive	Negative	Total
Layperson	Positive	25	0	25
	Negative	0	28	28
	Total	25	28	53

Lay User Spiked Sample Study:
Urine samples were prepared at 5 mIU/mL, 7.5 mIU/mL, 8 mIU/mL, and 10 mIU/mL hCG concentrations by spiking hCG into negative pooled urine specimens. Each sample was aliquoted into individual containers and blind-labeled. A total of 300 lay persons performed the testing. FaStep Early Pregnancy Rapid Test Strip, FaStep Early Pregnancy Rapid Test Cassette, and FaStep Early Pregnancy Rapid Test Midstream were each tested by 100 lay

users. Each layperson tested four blind-labeled samples (5 mIU/mL, 7.5 mIU/mL, 8 mIU/mL, and 10 mIU/mL), and an aliquot of each of the spiked urine samples was also tested by the professional. The results are summarized in the tables below:

Summary results of FaStep Early Pregnancy Rapid Test Strip (dip sampling mode)

hCG Concentration (mIU/mL)	Layperson result			Professional result			Percent Agreement
	No. of Positive	No. of Negative	% positive	No. of Positive	No. of Negative	% positive	
5	0	100	0%	0	100	0%	100%
7.5	39	61	39%	41	59	41%	98%
8.0	51	49	51%	53	47	53%	98%
10	100	0	100%	100	0	100%	100%

Summary results of FaStep Early Pregnancy Rapid Test Cassette (drop sampling mode)

hCG Concentration (mIU/mL)	Layperson result			Professional result			Percent Agreement
	No. of Positive	No. of Negative	% positive	No. of Positive	No. of Negative	% positive	
5	0	100	0%	0	100	0%	100%
7.5	40	60	40%	42	58	42%	98%
8.0	51	49	51%	52	48	52%	99%
10	100	0	100%	100	0	100%	100%

Summary results of FaStep Early Pregnancy Rapid Test Midstream (dip sampling mode)

hCG Concentration (mIU/mL)	Layperson result			Professional result			Percent Agreement
	No. of Positive	No. of Negative	% positive	No. of Positive	No. of Negative	% positive	
5	0	100	0%	0	100	0%	100%
7.5	39	61	39%	42	58	42%	97%
8.0	49	51	49%	51	49	51%	98%
10	100	0	100%	100	0	100%	100%

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

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.