Good decisions come from good information.

The FSH Menopause Rapid Test (Midstream) is a rapid lateral flow chromatographic immunoassay for the qualitative detection of Follicle-Stimulating Hormone (FSH) levels in urine to evaluate the onset of menopause in women. The FDA classifies the FSH test (Product Code NGA) (Regulation Number: 862.1300) as Class I medical devices. The device is exempt from the premarket notification as outlined in 21CFR862.1645.

This document presents analytical and clinical studies performed to assess the accuracy and performance characteristics of the FSH test by Diagnox.

Device Description

The FSH Menopause Rapid Test (Midstream) is a rapid test that qualitatively detects the FSH level in urine specimens at a 25 mIU/mL sensitivity. The test utilizes a combination of antibodies, including a monoclonal anti-FSH antibody, to selectively detect elevated levels of FSH. At the level of claimed sensitivity, the FSH menopause test shows no cross-reactivity interference from the structurally related glycoprotein hormones hCG, hLH, and hTSH at high physiological levels.

The assay is conducted by adding a urine specimen to the specimen well of the test device and observing the formation of colored lines. The specimen migrates via capillary action along the membrane to react with the colored conjugate. Positive specimens react with the specific antibody-FSH colored conjugate to form a colored line at the Test Line Region of the membrane, which is darker than or the same shade as the line in the Control Line Region. To serve as a procedural control, a colored line will always appear in the Control Line Region, indicating that the proper specimen volume has been added and membrane wicking has occurred.

Performance Characteristics

Sample Correlation

Materials:

Products

Predicate: Wondfo® One Step FSH Menopause Test

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1:161212c

Lot 2:161216c

Lot 3:161220c

Methods:

The sample correlation was used with a sample number (n) of 687. This evaluation was run as a blind study using a strip and cassette format. Samples have been randomized and tested. The operator has run his/her samples (n = 1 per test) in the new product test. Samples were rated as either positive or negative at the prescribed call time. In the case of questionable results, an asterisk accompanied the result. The data has been reported in 2 x 2 charts, as shown below.

First operation method: Point the absorbent tip downward; place the absorbent tip in the urine stream for at least 10 seconds to be thoroughly wet.

Second operation method: Collect your urine into a clean cup and dip half of the absorbent pad into the urine for at least 10 seconds.

70 out of 151 positive samples used the first operation method. The others used the second method, and 196 out of 436 negative samples used the first operation method. The others used the second method.

Results were read at 5 minutes.

Results:

Midstream Pilot 1 Test Results (n=687):

		Predicate +	Predicate -
Diagnox FSH Test	+	151	0
	-	0	436

Specificity = 100%

Sensitivity = 100%

Midstream Pilot 2 Test Results (n=687):

		Predicate +	Predicate -
Diagnox FSH Test	+	151	0
	-	0	436

Specificity = 100%

Sensitivity = 100%

Midstream Pilot 3 Test Results (n=687):

		Predicate +	Predicate -
Diagnox FSH Test	+	151	0
	-	0	436

Specificity = 100%

Sensitivity = 100%

Conclusion: Compared with predicate tests, the Diagnox FSH Menopause Rapid test showed the same sensitivity and specificity.

Analytical Sensitivity

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Methods:

The analytical sensitivity was determined by spiking negative urine specimens with intact FSH standards at 0 mIU/mL, 25 mIU/mL, and 40 mIU/mL. The FSH standards were randomized and coded. A total of 10*6 replicates for each standard were tested according to the package insert. The specimens were tested with visual interpretations 5 minutes after specimen application. The results are presented in the tables below.

Results:

Midstream Lot 1: 161212c

Specimen	FSH Conc.	#Tested	Negative (-)	Positive (+)
Urine	0mIU/mL	10	10	0
	25mIU/mL	10	0	10
	40mIU/mL	10	0	10

Midstream Lot 2: 161216c

Specimen	FSH Conc.	#Tested	Negative (-)	Positive (+)
Urine	0mIU/mL	10	10	0
	25mIU/mL	10	0	10
	40mIU/mL	10	0	10

Midstream Lot 3: 161220c

Specimen	FSH Conc.	#Tested	Negative (-)	Positive (+)
Urine	0mIU/mL	10	10	0
	25mIU/mL	10	0	10
	40mIU/mL	10	0	10

Conclusion:

As indicated in the tables above, specimens with FSH concentration equal to or higher than 25mIU/mL show positive results, and specimens with FSH concentration lower than 25 mIU/mL showed negative results. Therefore, the sensitivity concentration of the FSH Menopause Rapid Test is determined to be 25 mIU/mL.

Interfering Substances

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

Analytes were spiked at the concentrations indicated into a male urine pool containing 0 and 25 (cut-off value) mIU/mL (calibrated by the 4th WHO standard). The samples were tested in triplicate, and visual interpretations were made at the prescribed read time after sample application.

Dip half of the absorbent pad into the urine for at least 10 seconds. Read results at 5 minutes.

Results:

Analyte	Concentration	Lot 1		Lot 2		Lot 3	
		0	25	0	25	0	25
Acetaminophen*	25 mg/dL	-	+	-	+	-	+
Acetoaceto Acid	2000 mg/dL	-	+	-	+	-	+
Ascorbic Acid*	20 mg/dL	-	+	-	+	-	+
B-hydroxybutyrate	2000 mg/dL	-	+	-	+	-	+
Caffeine*	20 mg/dL	-	+	-	+	-	+
Ephedrine	20 mg/dL	-	+	-	+	-	+
Gentisic Acid*	20 mg/dL	-	+	-	+	-	+
Phenylpropanolamine	20 mg/dL	-	+	-	+	-	+
Salicylic Acid*	20 mg/dL	-	+	-	+	-	+
Phenothiazine	20 mg/dL	-	+	-	+	-	+
EDTA	20 mg/dL	-	+	-	+	-	+
Acetosalicylic Acid*	20 mg/dL	-	+	-	+	-	+
Cannabinol	10 mg/dL	-	+	-	+	-	+

Codeine	10 mg/dL	-	+	-	+	-	+
Ethanol	1.0 %	-	+	-	+	-	+
Methanol	10 %	-	+	-	+	-	+
Albumin	2000 mg/dL	-	+	-	+	-	+
Glucose*	2000 mg/dL	-	+	-	+	-	+
Bilirubin*	1000 mg/dL	-	+	-	+	-	+
Hemoglobin*	2000 mg/dL	-	+	-	+	-	+

Conclusion:

No interference was observed for the following compounds at the concentrations added with the Diagnox FSH Rapid tests.

Cross Reactivity

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

300 mIU/mL LH and 1000 uIU/mL TSH were separately spiked into negative urine samples and samples containing 25 mIU/mL FSH (calibrated by WHO standard). The samples were tested in triplicate, and visual interpretations were made at the prescribed read time after sample application.

Dip half of the absorbent pad into the urine for at least 10 seconds. Read results at 5 minutes.

Results:

Treatment	Lot 1	Lot 2	Lot 3
hLH(300 mIU/mL) 0 mIU/mL FSH	-	-	-
hLH(300 mIU/mL) 25 mIU/mL FSH	+	+	+
hTSH (1000 uIU/mL) 0 mIU/mL FSH	-	-	-
hTSH (1000 uIU/mL) 25 mIU/mL FSH	+	+	+

Conclusion:

No cross-reactivity was observed for urine samples up to the following concentrations: LH (300 mIU/mL) and TSH (1000 uIU/mL).

Urinary pH

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

Five (5) mL of negative urine was aliquoted into 5 test tubes. The pH of the urine was adjusted within the range of 5 to 9 in 1 pH unit increments using NaOH or HCl. Each pH-adjusted urine was split into two aliquots: FSH was added to one aliquot to bring the final sample concentration to 25 mIU/mL. No FSH was added to the other aliquot. This was done for each pH-adjusted sample. All pH-adjusted urine was tested in duplicate at 0 and 25 mIU/mL and read three minutes after sample application.

Dip half of the absorbent pad into the urine for at least 10 seconds. Read results at 5 minutes.

Results:

pH	Lot 1		Lot 2		Lot 3	
	0 mIU/mL	25 mIU/mL	0 mIU/mL	25 mIU/mL	0 mIU/mL	25 mIU/mL
5	-	+	-	+	-	+
5	-	+	-	+	-	+
6	-	+	-	+	-	+
6	-	+	-	+	-	+
7	-	+	-	+	-	+
7	-	+	-	+	-	+
8	-	+	-	+	-	+
8	-	+	-	+	-	+
9	-	+	-	+	-	+
9	-	+	-	+	-	+

Conclusion:

The pH of the samples, when tested from a range pH5 to pH 9, did not interfere with the performance of strips. Correct positive and negative results have been obtained with all the samples at the pH levels tested.

Temperature

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

A negative urine pool was spiked with FSH to yield samples with 0 and 25 mIU/mL FSH concentrations. An aliquot of each concentration was stored at 2°, 15°, 25°, 30°, and 37°C and tested at its respective temperature in triplicate. The results were read visually as positive or negative at the prescribed read time.

Dip half of the absorbent pad into the urine for at least 10 seconds. Read results at 5 minutes.

Results:

Treatment temp. (°C)	Lot 1		Lot 2		Lot 3	
	0 mIU/mL	25 mIU/mL	0 mIU/mL	25 mIU/mL	0 mIU/mL	25 mIU/mL
2	-	+	-	+	-	+
15	-	+	-	+	-	+
25	-	+	-	+	-	+
30	-	+	-	+	-	+
37	-	+	-	+	-	+

Conclusion:

The product could yield correct results when tested with the samples stored from 4°C to 37°C. Therefore, it can accommodate a wide fluctuation of sample temperature.

Urinary Specific Gravity

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

Fifteen (15) urine samples with different specific gravity were tested.

Negative urine samples were collected by the manufacturing contractor's employees. The urine specific gravity was measured with a Clinical Refractometer. Five urine samples with specific gravity at each following range will be needed for the study: (1) Specific gravity >1.025. (2) Specific gravity 1.015 – 1.025. (3) Specific gravity < 1.015. Each urine sample was aliquoted to three parts: (1) Neat urine (2) Urine samples spiked with FSH to 25mIU/ml. Diagnox FSH product was tested in duplicates with the above urine samples. Read the result at 5 minutes after sample application.

Dip half of the absorbent pad into the urine for at least 10 seconds. Read results at 5 minutes.

Results:

ID	Urine Specific Gravity	N*	Lot 1		Lot 2		Lot 3	
			Neg	Pos	Neg	pos	Neg	Pos
1	1.06	3	-	+	-	+	-	+
2	1.09	3	-	+	-	+	-	+
3	1.04	3	-	+	-	+	-	+
4	1.07	3	-	+	-	+	-	+
5	1.05	3	-	+	-	+	-	+
1	1.015	3	-	+	-	+	-	+
2	1.016	3	-	+	-	+	-	+
3	1.021	3	-	+	-	+	-	+
4	1.023	3	-	+	-	+	-	+
5	1.016	3	-	+	-	+	-	+

1	1.014	3	-	+	-	+	-	+
2	1.013	3	-	+	-	+	-	+
3	1.008	3	-	+	-	+	-	+
4	1.005	3	-	+	-	+	-	+
5	1.014	3	-	+	-	+	-	+

Conclusion:

No effect of urine gravity was observed on the Diagnox FSH Menopause Rapid Test for USG between 1.1014 and 1.06.

Variability (Intro lot)

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

Negative urine was spiked with 10, 25, and 40mIU/mL and run individually in a single test for ten times. Results were read as positive or negative at the prescribed read time. The percent variability of the test was determined by comparing the 10 results to each other and is expressed as a percentage of actual to expected results.

Note: All readings followed the Diagnox color card. The density (intensity of color line) was increased according to the T-line signal. G10 was taken as the highest, while G1 was taken as the lowest. When the density of the test line was the same as or darker than the control line, it was considered a positive result. If only the control line was visible or two lines were visible, but the density of the test line was lighter than the control line, it was considered a negative result.

Results:

Midstream Lot 1

	1	2	3	4	5	6	7	8	9	10
10mIU/ml FSH										
T line	G6	G6	G6	G6	G6	G6	G6	G6	G6	G6
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	-	-	-	-	-	-	-	-	-	-
25mIU/ml FSH										
T line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+
40mIU/ml FSH										

T line	G9	G9	G9	G9	G9	G9	G9	G9	G9	G9
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+

Midstream Lot 2

	1	2	3	4	5	6	7	8	9	10
10mIU/ml FSH										
T line	G6	G6	G6	G6	G6	G6	G6	G6	G6	G6
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	-	-	-	-	-	-	-	-	-	-
25mIU/ml FSH										
T line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+
40mIU/ml FSH										
T line	G9	G9	G9	G9	G9	G9	G9	G9	G9	G9
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+

Midstream Lot 3

	1	2	3	4	5	6	7	8	9	10
10mIU/ml FSH										
T line	G6	G6	G6	G6	G6	G6	G6	G6	G6	G6
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	-	-	-	-	-	-	-	-	-	-
25mIU/ml FSH										
T line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+
40mIU/ml FSH										

T line	G9	G9	G9	G9	G9	G9	G9	G9	G9	G9
C line	G8	G8	G8	G8	G8	G8	G8	G8	G8	G8
Result	+	+	+	+	+	+	+	+	+	+

Conclusion:

This study demonstrated that the actual results of each level were consistent with the expected results at reading time.

Variability (Lot-to-Lot)

Materials:

Products

Device Under Test: Diagnox FSH Test (Midstream)

Lot 1: 161212c

Lot 2: 161216c

Lot 3: 161220c

Method:

Negative urine was spiked with 10, 25, and 40mIU/mL FSH and run individually in replicates of ten in three separate lots of product. Results were read as positive or negative 5 minutes after sample application. The percent variability of the test was determined by comparing the results of each level spiked sample to the expected result and was expressed as a percentage of actual to expected result.

Notes: All readings followed the Diagnox color card. The density (intensity of color line) was increased according to the T-line signal. G10 was taken as the highest, while G1 was taken as the lowest. When the density of the test line was the same as or darker than the control line, it was considered a positive result. If only the control line was visible or two lines were visible, but the density of the test line was lighter than the control line, it was considered a negative result.

Results:

Lots		10mIU			25mIU			40mIU		
		T	C	Result	T	C	Result	T	C	Result
Lot 1	1	G6	G8	-	G8	G8	+	G9	G8	+
	2	G6	G8	-	G8	G8	+	G9	G8	+
	3	G6	G8	-	G8	G8	+	G9	G8	+
	4	G6	G8	-	G8	G8	+	G9	G8	+
	5	G6	G8	-	G8	G8	+	G9	G8	+
	6	G6	G8	-	G8	G8	+	G9	G8	+
	7	G6	G8	-	G8	G8	+	G9	G8	+
	8	G6	G8	-	G8	G8	+	G9	G8	+
	9	G6	G8	-	G8	G8	+	G9	G8	+
	10	G6	G8	-	G8	G8	+	G9	G8	+

Lot 2	1	G6	G8	-	G8	G8	+	G9	G8	+
	2	G6	G8	-	G8	G8	+	G9	G8	+
	3	G6	G8	-	G8	G8	+	G9	G8	+
	4	G6	G8	-	G8	G8	+	G9	G8	+
	5	G6	G8	-	G8	G8	+	G9	G8	+
	6	G6	G8	-	G8	G8	+	G9	G8	+
	7	G6	G8	-	G8	G8	+	G9	G8	+
	8	G6	G8	-	G8	G8	+	G9	G8	+
	9	G6	G8	-	G8	G8	+	G9	G8	+
	10	G6	G8	-	G8	G8	+	G9	G8	+
Lot 3	1	G6	G8	-	G8	G8	+	G9	G8	+
	2	G6	G8	-	G8	G8	+	G9	G8	+
	3	G6	G8	-	G8	G8	+	G9	G8	+
	4	G6	G8	-	G8	G8	+	G9	G8	+
	5	G6	G8	-	G8	G8	+	G9	G8	+
	6	G6	G8	-	G8	G8	+	G9	G8	+
	7	G6	G8	-	G8	G8	+	G9	G8	+
	8	G6	G8	-	G8	G8	+	G9	G8	+
	9	G6	G8	-	G8	G8	+	G9	G8	+
	10	G6	G8	-	G8	G8	+	G9	G8	+

Conclusion:

There was no significant difference in the variability among the 3 lots.