Design Verification

HumaPreg^{Urine}, HEXAGON hCG 1-Step^{Urine}

CONTENTS

1 Function

HumaPreg[Urine] and HEXAGON hCG1-Step[Urine] tests are chromatographic immunoassays for the early detection of human chorionic gonadotropin (hCG) in human urine specimen.
The membrane of the test device was coated with anti HCG antibodies on the test region and goat anti mouse IgG antibodies on the control region. During the test, urine specimen is allowed to react with the HCG monoclonal antibody-colloid gold conjugate, which was pre-dried on the test strip. The mixture then moves upward on the membrane chromatographically by capillary action. For a positive specimen, the conjugate binds to the HCG forming an antibody-antigen complex. This complex is captured by anti HCG forming an antibody-antigen complex on the test region (T) and produces a pink colour band when HCG concentration is equal to or greater than 25 IU/l. Absence of this coloured band in the test region suggests a negative result. To serve as a procedural control, a coloured band at the control region (C) will always appear regardless the presence or absence of HCG.

2 Analytical Sensitivity

To determine the sensitivity of the HumaPreg[Urine] and HEXAGON hCG1-STEP[Urine] tests HCG Antigen (lot: R10122001) was diluted in NBS solution at concentrations of 0 IU/L, 25 IU/L, 50 IU/L, 1000 IU/L, 6000 IU/L, 10 000 IU/L, 50 000 IU/L, 100 000 IU/L, 250 000 IU/L, 500 000 IU/L, 1 000 000 IU/L. Each concentration was tested with two devices according to test procedure.

The results of the sensitivity study are summarized as shown below, results at 5 minutes.

HumaPregUrine lot 15112027

	Test 1		Test 2	
Sample ID	TL-Referenz	Interpretation	TL-Referenz	Interpretation
0 IU/L	0	negative	0	negative
25 IU/L	5	positive	5	positive
50 IU/L	6	positive	6	positive
1 000 IU/L	9	positive	9	positive
6 000 IU/L	10	positive	10	positive
10 000 IU/L	10	positive	10	positive
50 000 IU/L	9	positive	9	positive
100 000 IU/L	8	positive	8	positive
250 000IU/L	8	positive	8	positive
500 000 IU/L	7	positive	7	positive
1 000 000 IU/L	7	positive	7	positive

Color scale: 0 – 4 negative, 5 – 10 positive

HEXAGON hCG1-STEP[Urine] lot 15112015

	Test 1		Test 2	
Sample ID	TL-Referenz	Interpretation	TL-Referenz	Interpretation
0 IU/L	0	negative	0	negative
25 IU/L	5	positive	5	positive
50 IU/L	6	positive	6	positive
1 000 IU/L	9	positive	9	positive
6 000 IU/L	10	positive	10	positive
10 000 IU/L	10	positive	10	positive
50 000 IU/L	9	positive	9	positive
100 000 IU/L	8	positive	8	positive
250 000IU/L	8	positive	8	positive
500 000 IU/L	7	positive	7	positive
1 000 000 IU/L	7	positive	7	positive

Color scale: 0 – 4 negative, 5 – 10 positive

Conclusion: The results show that HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} has the sensitivity of 25 IU/L. The detection range is from 25 to 1 000 000 IU/L. The strongest color signal is observed between 6 000 and 10 000 IU/L- After 10000 IU/L, it starts to decline but is still clearly visible at 1 000 000 IU/L.

To further determine the analytical sensitivity of the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} tests, 25 devices per control level were tested for each of the 3 different levels of controls with different hCG concentrations. For this, hCG negative urine was spiked with standard material (14.82 IU/ampule) calibrated to the HCG international standard NIBSC07/364.

Name	concentration	Control lot number
negative	0 IU/l	Q11021501
weak positive	25 IU/l	Q11021502
positive	500 IU/l	Q11021503

The results of the sensitivity study are summarized as shown below, results at 5 minutes.

Lot: H11040902

Control level	Expected results	No. of tested	No. of negative	No. of positive	% agreement
negative	N	25	25	0	100
weak positive	P	25	0	25	100
positive	P	25	0	25	100

Lot: H11040803

Control level	Expected results	No. of tested	No. of negative	No. of positive	% agreement
negative	N	25	25	0	100
weak positive	P	25	0	25	100
positive	P	25	0	25	100

Lot: H11042804

Control level	Expected results	No. of tested	No. of negative	No. of positive	% agreement
negative	N	25	25	0	100
weak positive	P	25	0	25	100
positive	P	25	0	25	100

Conclusion: The analytical sensitivity of the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} tests is 25IU/l.

3 Interference

To evaluate the potential interfering compounds known to appear in urine specimens, each compound was tested in triplicates by using HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} test. The following substances were added to hCG free and 25 mIU/ml hCG spiked urine samples.

Lot: H11042902

Substance	Conc.	Negative	Weak positive	positive	Interfere (Y/N)
Acetaminophen	20mg/dL	N	P	P	N
Acetysalicylic acid	20mg/dL	N	P	P	N
Ascorbic acid	20mg/dL	N	P	P	N
Atropine	20mg/dL	N	P	P	N
Caffeine	20mg/dL	N	P	P	N
Gentisisc acid	20mg/dL	N	P	P	N
Glucose	2.0g/dL	N	P	P	N
Hemoglobin	1.0mg/dL	N	P	P	N

Conclusion: None of the substances at the concentration tested interfered with the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} assays.

4 Analytical Specificity

Potential specificity substances: hLH, hFSH and hTSH were added into hCG negative urine (hCG concentration at 0 IU/L, urine group 1), hCG weak positive urine (hCG concentration at 25 IU/L, urine group 2) and hCG positive urine (hCG concentration at 500 IU/L, urine group 3) respectively. The concentrations of substances in urine are: 300 IU/L hLH, 1000 IU/L hFSH and 1000 mIU/L hTSH. One lot was used to detect the above three urine samples. The test were performed according to the package insert.

Name	Concentrations	Control lot number
negative	0 IU/l	Q11021501
weak positive	25 IU/l	Q11021502
positive	500 IU/l	Q11021503

Lot: H11040902

Substance	Conc.	Urine group 1	Urine group 2	Urine group 3	Interfere (Y/N)
hLH	300IU/L	N	P	P	N
hFSH	1000IU/l	N	P	P	N
hTSH	1000mIU/l	N	P	P	N

Conclusion: The specificity of the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} assays were determined from cross-reactivity studies with known amounts of Luteinizing Hormone (hLH), Follicle Stimulation Hormone (hFSH) and Thyroid Stimulation Hormone (hTSH). Negative results were obtained from all tests conducted with 300 IU/L hLH, 1000 IU/L hFSH, 1000 mIU/L hTSH and negative hCG specimen.

5 Precision

To evaluate the random error of visual interpretation, precision study is performed by testing one lot of HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} tests with different concentrations of controls (negative, weak positive and positive) in a period of 21 consecutive working days, interpreted by 3 persons independently.

Sample preparation
Two devices HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} per control level, were tested for each of the three different levels of controls.

Name	Concentrations	Control lot number
negative	0 IU/L	Q11021501
weak positive	25 IU/L	Q11021502
positive	500 IU/L	Q11021503

The results of the precision study are summarized as shown below, results at 5 minutes.

Person #1: Lot H11040902

Control level	Expected results	No. of Tested	No. of Negative	No. of Positive	% Agreement
Negative	N	42	42	0	100
Weak Positive	P	42	0	42	100
Positive	P	42	0	42	100

Person #2: Lot H11040902

Control level	Expected results	No. of Tested	No. of Negative	No. of Positive	% Agreement
Negative	N	42	42	0	100
Weak Positive	P	42	0	42	100
Positive	P	42	0	42	100

Person #3: Lot H11040902

Control level	Expected results	No. of Tested	No. of Negative	No. of Positive	% Agreement
Negative	N	42	42	0	100
Weak Positive	P	42	0	42	100
Positive	P	42	0	42	100

Conclusion: For visually interpreted HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} tests the study has shown a very good reproducibility of negative, weakly positive and positive results (between run precision).

6 Specimen stability

To evaluate the specimen stability of control specimen, HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} were tested with the control specimens in the collection tube after the following storage conditions:

- Frozen (-20 °C) for 7 days.
- Refrigerator 2-8 °C for 7 days

The results of the specimen stability study are summarized as shown below.
Concentrations of tested controls are:

Name	Concentrations	Control lot number
Negative	0 IU/L	Q11021501
Weak positive	25 IU/L	Q11021502
Positive	500 IU/L	Q11021503

Frozen (-20 °C) for 7 days

Control level	Expected results	No. of tested	No. of Negative	No. of Positive	% agreement
Negative	N	4	4	0	100
Weak positive	P	4	0	4	100
Positive	P	4	0	4	100

Refrigerator 2 – 8 °C for 7 days

Control level	Expected results	No. of tested	No. of Negative	No. of Positive	% agreement
Negative	N	14	14	0	100
Weak positive	P	14	0	14	100
Positive	P	14	0	14	100

Conclusion: Compared with initial test results recorded at day 1, results from all other days are identical with day 1, therefore, the control specimens are stable in the specific time range in this report.

7 Real-time stability

Real time stability study was performed at room temperature (10 – 30 °C) for 24 months.

Duplicates were performed on each control level. The table lists the testing schedule and the acceptance criteria.

Temperature	Testing period	Testing schedule	Expected results		
			Negative	Weak Positive	Positive
RT	24 months	Every month	N	P	P
Acceptance %			100	90	100

Product Lot Number List

HumaPreg^{Urine} / HEXAGON hCG1-STEP^{Urine}	Date of Manufacture
H11040902	Apr. 09, 2011
H11041803	Apr. 18, 2011
H11042804	Apr. 22, 2011

Control Level	Lot
Negative	Q11021501
Weak Positive	Q11021502
Positive	Q11021503

Three lots of HumaPreg^{Urine} and HEXAGON HCG1-STEP^{Urine} were manufactured to conduct this stability study. The devices were manufactured and stored at room temperature (10 – 30°C). Duplicate tests were performed on each control level. Batch information, testing conditions and acceptance criteria are summarized in the previous tables.

Pouches devices were stored at room temperature (10 -30°C) and tested on month 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24 in duplicates. Devices were tested in accordance with the product instructions for use with the controls described above. Read result visually at 5 minutes.

Product name	Lot ID	control	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
HumaPreg^{Urine} / HEXAGON HCG1-STEP^{Urine}	Lot 1	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
	Lot 2	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
	Lot 3	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P

Product name	Lot ID	control	Month 13	Month 14	Month 15	Month 16	Month 17	Month 18	Month 19	Month 20	Month 21	Month 22	Month 23	Month 24
HumaPreg^{Urine} / HEXAGON HCG1-STEP^{Urine}	Lot 1	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
	Lot 2	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
	Lot 3	Negative	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
		Weak positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P
		positive	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P	P/P

Conclusion: HumaPreg^{Urine} and HEXAGON HCG1-STEP^{URINE} has met the QC acceptance criteria for the time period of twenty four months when stored at room temperature (10 – 30°C). No change of the performance was observed and documented. Shelf-life of this product is thus proved to last for at least 24 months.

8 Clinical Evaluation

For the clinical evaluation of the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} test two hundred and eighty nine (289) confirmed samples were selected by two different hospitals. The results of the HumaPreg^{Urine} and HEXAGON hCG1-STEP^{Urine} have been compared with a commercially available test (BlueCross® One Step HCG Urine Pregnancy Test, lot 20110302).

Test results:

Hospital A
Lot: H11032807

		Reference		Total
		Positive	Negative	
HumaPreg^{Urine}	Positive	42	1	43
HEXAGON hCG1-STEP^{Urine}	Negative	0	136	136
Total		42	137	179

Hospital B
Lot: H11032807

		Predicate Kit		Total
		Positive	Negative	
HumaPreg^{Urine}	Positive	39	0	39
HEXAGON hCG1-STEP^{Urine}	Negative	0	71	71
Total		39	71	110

The positive and negative agreement are summarized in the tables below.

		Predicate Kit		Total
		Positive	Negative	
HumaPreg^{Urine} HEXAGON hCG1-STEP^{Urine}	Positive	81	1	82
	Negative	0	207	207
Total		81	208	289

Diagnostic sensitivity and diagnostic specificity were calculated according to:

$$\text{Sensitivity} = \frac{\text{true positive results}}{\text{true positive results + false negative results}} \times 100\ [\%]$$

$$\text{Specificity} = \frac{\text{true negative results}}{\text{true negative results + false positive results}} \times 100\ [\%]$$

Conclusion: The clinical sensitivity of the HumaPreg^{Urine} and HEXAGON hCG 1-STEP^{Urine} test is 100%.

The clinical specificity of the HumaPreg^{Urine} and HEXAGON hCG 1-STEP^{Urine} is 99.5%.