Urine Beta hCG (pregnancy) testing with DXpress Reader

VERSION	2
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COPY	**2**
LOCATION OF COPIES	1. PoCT Office 2. Q-pulse

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0 ASSOCIATED DOCUMENTS

- Medical Devices Policy.
- Infection Control Policy.
- POC-EXT-22 Extract from training manual see appendix A.
- POC-EXT-16 BioSign hCG One Step Pregnancy Urine Test REF BSP -121 kit insert see appendix B.

1 PURPOSE OF THE EXAMINATION

Detection of raised human beta chorionic gonadotropin to diagnose pregnancy, missed abortion and ectopic pregnancy using the DXpress reader.

2 PRINCIPLE AND METHOD OF THE PROCEDURE(S) USED

2.1 Principle

High sensitivity testing based on an immunochemical sandwich assay, relying on the recognition and formation of specific antibody – hCG - antibody+dye complexes on a porous carrier. The membrane is coated with mouse anti-hCG in the Test Band region and goat anti-mouse in the Control Band region, and a monoclonal anti-hCG/colloidal gold conjugate is coated in the sample application region. When urine is added to the sample well it migrates along the membrane mixing with and carrying the coloured gold-conjugate. If the urine contains >25 mIU/mL of hCG then a detectable line will form in the Test Band region. If the level of hCG in the sample is less than 25 mIU/mL then no line will be detected, but in both cases a line must be detected at the Control Band region to demonstrate that test function is satisfactory. The DXpress reader uses image analysis to "read" the test cassette and compares the colour intensity of the lines against calibration values held in its memory and derives a qualitative result.

2.2 Method

The test device must not be used if the foil pouch containing it is damaged in any way e.g. there is a tear in it.
Once the pouch is opened the test must be used within 5-10 minutes or discarded and another opened.

Test Protocol

See attached manufacturer instruction in Appendix A if the reader should fail, tests can be performed manually as in Appendix B.
If uncertain of the result repeat the test ensuring that correct timing and sample application have been used in a well-lit environment. If possible get another person to view the result. If still inconclusive repeat the test 48hrs later ensuring a reasonably concentrated sample is used. If an urgent result is required, contact the Biochemistry Laboratory for advice.
Refer to kit insert attached for further details.

3 PERFORMANCE CHARACTERISTICS

See Performance characteristics section in Pack insert (Appendix C)

4 SAMPLE TYPE REQUIRED

A fresh (<2 hrs old) unpreserved (no additives) urine sample is appropriate for pregnancy testing but the first morning urine specimen is optimal for the detection of early pregnancy. The urine should not be too dilute.
Samples should be collected in a clean, dry container such as a foil bowl or plain universal.
All sample containers must be labelled before leaving the patient.

5 PATIENT PREPARATION

Explain procedure and gain consent where possible.

6 TYPE OF CONTAINER AND ADDITIVES

See 4.

7 EQUIPMENT AND REAGENTS REQUIRED

DXpress cassette reader
BioSign hcg One Step Pregnancy Urine Test Kit – BSP 12235 (the kit contains complete reagent components and materials to perform the test).

Printer paper (TPR24) or Labels (SPR24)

8 ENVIRONMENTAL AND SAFETY CONTROLS

Follow Trust's Infection Control Policy, Waste Policy and other relevant policies and procedures.

9 CALIBRATION PROCEDURES (METROLOGICAL TRACEABILITY)

Weekly calibration carried out by PoCT.

10 PROCEDURE

The procedure consists of adding the specimen to the sample well in the device, inserting the device into the DXpress Reader and following the instructions to get the result. (See APPENDIX A).

11 QUALITY CONTROL

Status Control kit 135-100A (Positive and Negative)
External WEQAS scheme.
System and cassette self checks.
Internal quality controls will be run periodically.
External quality controls are circulated bi monthly and should be run by user as patient tests.
System calibration will be performed by PoCT staff weekly.
Regular system checks need to be performed by selecting the following:

Main menu> Option 2 "Run QC" > Option 1"Self Check".

12 INTERFERENCES

Potentially interfering substances were prepared at the following concentrations containing either 0 or 25 mIU/mL hCG. These samples were tested with the BioSign® hCG Test. No interference was found (Table 4) at these concentration.

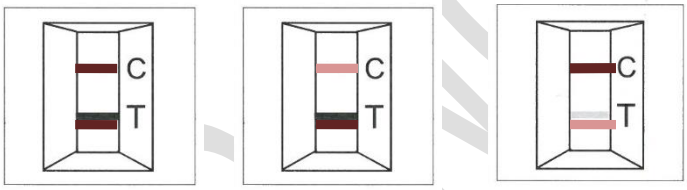
Table 4. Interfering Substances and Concentrations Tested

Acetaminophen	20 mg/dL
Acetylsalicylic Acid	20 mg/dL
Ampicillin	20 mg/dL
Ascorbic Acid	20 mg/dL
Atropine	20 mg/dL
Caffeine	20 mg/dL
Gentisic Acid	20 mg/dL
Phenothiazine	20 mg/dL
Phenylpropanolamine	20 mg/dL
Salicylic Acid	20 mg/dL
Tetracycline	20 mg/dL
Bilirubin	1 mg/dL
Glucose	2000 mg/dL
Hemoglobin	1 mg/dL
Ketones	100 mg/dL

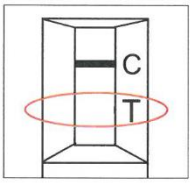
13 PRINCIPLE OF PROCEDURE FOR CALCULATING RESULTS

(Including, where relevant, the measurement uncertainty of measured quantity values)

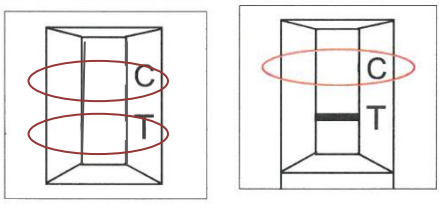
Positive: Two pinkish-purple lines, one each at the Test position (T) and at the Control position (C). The colour intensity of the test line varies with hCG concentration.



Negative: Only one pinkish-purple line at the Control position (C).



Invalid: No lines visible or a test line only. Repeat with a new cassette.



14 REFERENCE RANGES AND/OR CLINICAL DECISION VALUES

The test is capable of detecting hcg levels of 25 mlU/ml. hCG levels in normal pregnancy vary and hCG levels often exceed 100 mIU/mL by the first day of the missed menstrual period. The test is usually capable of detecting hCG by the first day of the missed menstrual period.

15 REPORTABLE INTERVAL OF THE TEST

See 14

16 INSTRUCTIONS FOR DETERMINING QUANTITATIVE RESULTS

N/A

17 ALERT AND/OR CRITICAL VALUES

(E.g. improbable ranges, impossible ranges, telephone trigger limits or results that could indicate a method/analyser failure).

18 LABORATORY CLINICAL INTERPRETATION

• An extremely low concentration of hCG during the early stage of pregnancy can give a negative result. In this case, testing of another specimen obtained at least 48 hours later is recommended.
• A false negative result could be possible in case of ectopic pregnancy due to the fact that the concentration of hCG level tends to be lower than those with a normal pregnancy.
• Low levels of hCG have been reported in non-pregnant females with no history of ectopic pregnancy, trophoblastic disease or germ-cell tumors. In the case of Borderline test results testing of another specimen obtained at least 48 hours later is recommended.
• The hCG level may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous abortion, or therapeutic abortion.
• The test is highly sensitive, and specimens which test positive during the initial days after conception may later be negative due to natural termination of the pregnancy. Natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall. Subsequent testing of a new urine sample after an additional 48 hours is recommended in order to confirm that the hCG level is rising as indicated in a normal pregnancy.

• Elevated hCG levels have been reported in patients with both gestational and nongestational trophoblastic diseases.8,9,10 The hCG of trophoblastic neoplasms is similar to that found in pregnancy, so these conditions, including choriocarcinoma and hydatidiform mole, should be ruled out before pregnancy is diagnosed.
• The physician should evaluate data obtained with this kit in light of other clinical information.

19 POTENTIAL SOURCES OF VARIATION

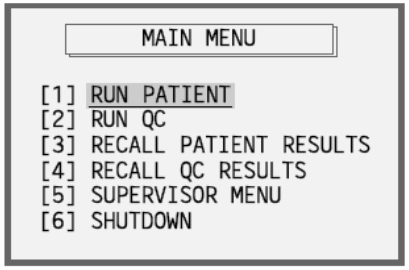
• Samples which contain excessive bacterial contamination or which have been subjected to repeated freezing and thawing should not be used because such specimens can give spurious results.
• Degradation of hCG in sample may occur by a certain protease during storage even at 4o C and give a negative test result.
• In rare occasions, persistent low levels of hCG present in men and in nonpregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.

20 REFERENCES (FOR THE ORIGINAL TEST METHOD)

1. Braunstein, G.D., Rasor, J., Adler, D., Danzer, H., and Wade, M.E. Serum Human Chorionic Gonadotropin Levels Throughout Normal Pregnancy, Am. J. Obstet. Gynecol. 1976; 126:678.
2. Krieg, A.F. Pregnancy Tests and Evaluation of Placental Function in: Clinical Diagnosis and Management by Laboratory Methods, 16th ed., Henry, J.B. (ed.) W.B. Saunders Co., Philadelphia, pp. 680, 1979.
3. See references on pack insert Q-Pulse POC-Ext-16.

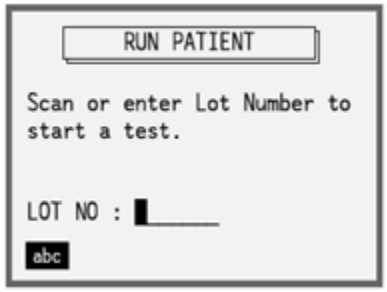
21 APPENDIX A

1



SELECT **[1] RUN PATIENT.**

2



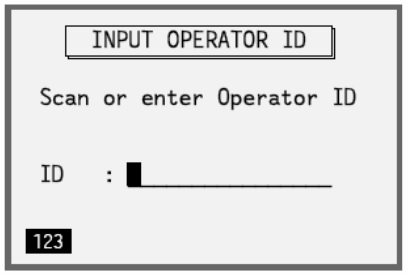
Scan the Lot Number barcode on the box or pouch (if available) or enter the Lot Number using the keypad.

3

Confirm the Product Name and Lot Number displayed on the screen. *SELECT* **OK** to go to the next step or **CANCEL** to re-enter the Lot Number.

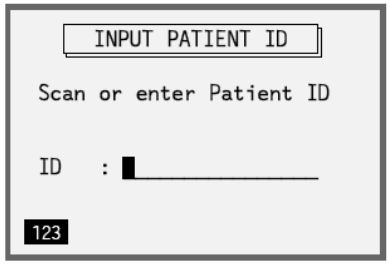
The reader will automatically check whether this Lot Number and associated calibration profile exist in the reader database.

4



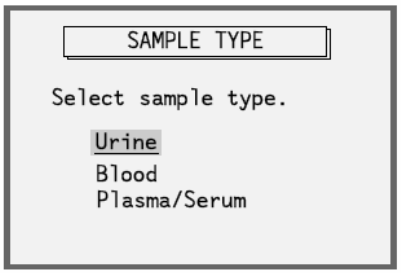
SCAN or *ENTER* the Operator ID (up to characters).

5



SCAN or *ENTER* the Patient ID(s) (up to 16 characters).

6

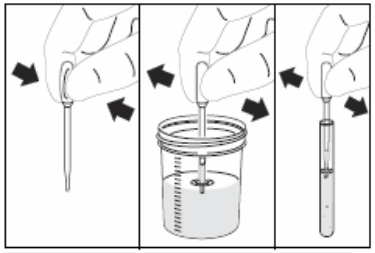


With Urine highlighted, as illustrated, press ENTER to select it

7 Take a test cassette out of the box and check if the foil pouch containing it is damaged in any way, if there is any sign of damage discard it and use another test cassette. Tear open the foil pouch and follow the steps below.
Once the pouch is opened the test must be used within 5-10 minutes or discarded and another opened.

8

Draw Sample Into Dropper

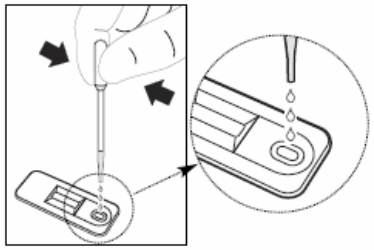


- Draw sample into dropper by first squeezing dropper bulb to create a vacuum, then dipping dropper end into the sample and then releasing dropper bulb.

  NOTE: Avoid introducing air bubbles into dropper.

9

Add Sample to Test Cassette

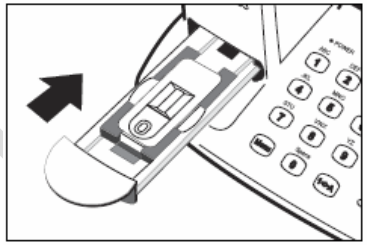


- Holding dropper vertically, squeeze the dropper bulb to dispense 3 full drops into test sample well.

  NOTE: Do not touch sample well or test cassette with the tip of dropper.

10

Insert Test Cassette Into Reader

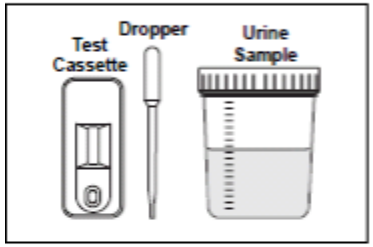
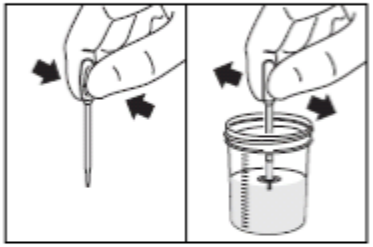
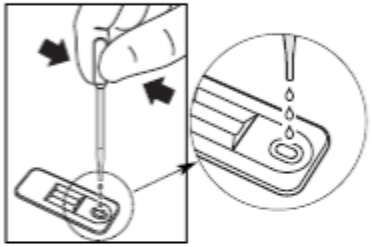
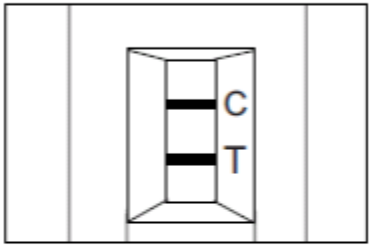


- IMMEDIATELY place test cassette into Reader tray and then close tray to start countdown (incubation) timer.

11 The result will be displayed on screen and printed on the tear off label.
Remove the used test cassette and dispose of it in the appropriate bin for biologically contaminated waste.
Ensure the cassette tray is shut to prevent damage/contamination.

APPENDIX B (MANUAL PROCEDURE)

Test Procedure - BioSign® hCG Urine Test

STEP 1	STEP 2	STEP 3	STEP 4
Collect Sample & Prepare Parts	Draw Sample Into Dropper	Add Sample to Test Cassette	Wait 3 Minutes & Read Result
			
▸ Collect urine sample. ▸ Check expiry date on test cassette pouch. ▸ Tear open pouch and remove test cassette and dropper. ▸ Write patient ID on test cassette and place on a flat surface.	▸ Draw sample into dropper by first squeezing dropper bulb to create a vacuum, then dipping dropper end into the sample and then releasing dropper bulb. NOTE: Avoid introducing air bubbles into dropper.	▸ Holding dropper vertically, squeeze the dropper bulb to dispense 3 full drops into test sample well. NOTE: Do not touch sample well or test cassette with the tip of dropper.	▸ Read result at 3-5 minutes. ▸ DO NOT read result after 5 minutes.

APPENDIX C

User Quality Control

Internal Control: Each **BioSign® hCG Test** device has a built-in control. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should appear at the C position, indicating an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the reagents at the Control line and the conjugate-color indicator are reactive. In addition, the clearing background in the Result window, by providing a distinct readable result, may be considered an internal negative procedural control. If background color appears in the Result window, which interferes with the result interpretation of the reader, then the result is invalid. If the problem persists, contact PBM for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested before using a new lot or a new shipment of kits as good laboratory testing practice and that users follow federal, state, and local guidelines for quality control requirements. For information on how to obtain controls, contact PBM Technical Services.

Expected Values

BioSign® hCG Test is capable of detecting hCG level of 25 mIU/mL (calibrated against the WHO 4th International Standard). HCG levels in normal early pregnant women vary and hCG levels often exceed 100 mIU/mL by the first day of the missed menstrual period.[1] The test is usually capable of detecting hCG by the first day of the missed menstrual period.

Performance Characteristics

Comparison Study

A total of 65 clinical samples were studied. These specimens were assayed with **BioSign® hCG Test** and a predicate device according to the respective test's protocol. The summary of the results is shown in Table 1.

Table 1. BioSign® vs. Predicate Device

		BioSign® hCG			
		Positive	Borderline	Negative	Total
Predicate Device	Positive	37	1	0	38
	Negative	0	1	26	27
	Total	37	2	26	65

Two samples gave discrepant results between **BioSign® hCG** and the predicate devices. These two samples contained hCG, but the amount of hCG present in these samples was less than 25mIU/mL. The **BioSign® hCG** gave borderline (indeterminate) results for these two samples, while the predicate device gave 1 positive and 1 negative result for these two samples.

All discrepant samples had less than 25 mIU/mL hCG. **BioSign® hCG Test** gave correct results (borderline or negative) for all these samples.

Sensitivity

To evaluate analytical sensitivity of **BioSign® hCG Test**, the following experiment was performed.

Pooled negative from non-pregnant people was spiked with hCG at several levels. Each level was tested 20 times with two lots. The result is summarized in Table 2.

This data supports that **BioSign® hCG Test** detects hCG concentrations equal to or greater than 25 mIU/mL (calibrated to the WHO 4th International Standard).

Table 2. BioSign® hCG Sensitivity Study

hCG (mIU/mL)	Percent Positive (N)
0	0
3	0
5	0
10	0
15	30 (6)
20	80 (16)
25	100 (20)
40	100 (20)

Precision Study

The precision of **BioSign® hCG Test** was determined by carrying out the test with hCG spiked into pooled negative samples. Four levels of hCG concentration were tested for three days with 2 lots and three DXpress readers. There were no significant differences between readers, between days or between lots. Table 3 shows the precision data combining all repeated tests.

Table 3. Summary of Precision Study Data

hCG Conc. (mIU/mL)	Total No. Tested	No. of Positive	No. of Borderline	No. of Negative	% Correct Results
0	180	0	0	180	100
5	180	0	3	177	98.3
25	180	179	1	0	99.4
40	180	180	0	0	100

Physicians' Office Laboratory Evaluation

Reproducibility of **BioSign® hCG** test results was evaluated at three physicians' office laboratories using a total of 120 blind control samples. Each panel consisted of five negative (–) samples, five at 5 mIU/mL, five at 25 mIU/mL, and five at 100 mIU/mL hCG. The results obtained at each site agreed 100% with expected results.

Specificity

The assay is free from interference with other commonly known homologous hormones when tested at the levels specified below.

Homologous Hormones	
hFSH	1000 mIU/mL
hLH	300 mIU/mL
hTSH	1000 µIU/mL

Interfering Substances

Potentially interfering substances were prepared at the following concentrations containing either 0 or 25 mIU/mL hCG. These samples were tested with the **BioSign® hCG Test**. No interference was found (Table 4) at these concentration.

Table 4. Interfering Substances and Concentrations Tested

Substance Added	Concentration Added
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	20 mg/dL
Ampicillin	20 mg/dL
Ascorbic Acid	20 mg/dL
Atropine	20 mg/dL
Caffeine	20 mg/dL
Gentisic Acid	20 mg/dL