

BioSign® RC hCG

Serum/Urine Pregnancy Test

For Professional *In Vitro* Diagnostic Use Only

Rapid Immunoassay for the Qualitative Detection of Human Chorionic Gonadotropin in Serum or Urine

For the Early Detection of Pregnancy

PBM

Catalog No.	BSP-122-35	35 Test Kit
	BSP-122-10	10 Test Kit

Intended Use

BioSign® RC hCG Serum/Urine Test is a simple immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in serum or urine for the early confirmation of pregnancy. This test is intended to be used with the DXpress™ Reader only, and is not for visual reading.

Summary and Explanation

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placental trophoblastic cells shortly after the implantation of fertilized ovum in the uterine wall.^{1,4} The primary function of hCG is to maintain the corpus luteum during early pregnancy. The appearance of hCG in both the urine and serum soon after conception and its rapid rise in concentration make it an excellent marker for confirmation of pregnancy. The hormone can be detected in both urine and serum as early as 7 to 10 days after conception.^{1,4} The concentration of hCG continues to rise rapidly, frequently exceeding 100 mIU/mL by the first missed menstrual period and peaking in the 100,000–200,000 mIU/mL range by 10 to 12 weeks into pregnancy. The hormone is comprised of two non-covalently bound dissimilar subunits containing approximately 30% carbohydrate by weight.⁵ The alpha subunit is structurally similar to other human pituitary glycoprotein hormones, whereas the beta (β) subunit confers unique biological and immunological specificity to the molecule.^{6,7}

Principle

The **BioSign® RC hCG Serum/Urine Test** is a rapid serum or urine test for detecting hCG qualitatively. The test employs a solid-phase, chromatographic immunoassay technology to detect elevated levels of hCG in serum or urine with a high degree of sensitivity. In the test procedure, sample is added to the sample well with a transfer pipette. If hCG is present in the specimen, it will react with the conjugate dye, which binds to the antibody on the membrane to generate a colored line. The DXpress™ Reader interprets the test result automatically by comparing the intensity of the test line to the preset cutoff value. The hCG levels greater than or equal to 25 mIU/mL are reported as positive. Samples containing less than 25 mIU/mL are reported as either negative or borderline.

Reagents

The **BioSign® RC hCG Serum/Urine Test** kit contains complete reagent components and materials to perform the test.

Materials Provided

- **BioSign® RC hCG Serum/Urine Test:** Each device contains mouse monoclonal and goat anti-hCG antibodies
- Disposable dropper
- Package insert

Materials Required but Not Provided

- Specimen cup
- DXpress™ Reader

Materials Recommended but Not Provided

- Timer
- External positive and negative controls

Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different product lots and do not use beyond the expiration date.
- The test device should remain in its sealed pouch until ready for use.

Storage and Stability

BioSign® RC hCG Serum/Urine Test kit is to be stored at 2°C to 30°C (35°F to 86°F) in the sealed pouch.

Specimen Collection and Preparation

- Approximately 110 µL of sample is required for each test.
- Specimens containing particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying.
- Frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing by allowing the specimens to stand at room temperature for at least 30 minutes.

Serum sample

- Remove serum from the clot as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used.

Urine sample

- For optimal early detection of pregnancy, a first morning urine specimen is preferred since it generally contains the highest concentration of hCG of the day. However, randomly collected urine specimens may be used.
- Collect the urine specimen in a clean glass or plastic cup.

Specimen Storage

- If testing will not be performed immediately, the specimens should be refrigerated (2°C to 8°C) for up to 72 hours prior to assay.
- For prolonged storage, specimens should be frozen and stored below -20°C. Frozen specimens must be completely thawed, thoroughly mixed before using. Avoid repeated freezing and thawing.
- If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of etiologic agents.

Procedure

Test Procedure Summary

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.

Procedural Notes

- Allow the dropper to fill with sample without air bubbles.
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the **BioSign® RC** device, and the specimen dispenser following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

Using DXpress™ Reader

For complete instructions, including installation and start up, refer to the

DXpress™ Reader User Manual. Operators must consult the DXpress™ Reader User Manual prior to use and become familiar with the processes and quality control procedures.

Performing Self Check

Each time the DXpress™ Reader is turned on, Self Check is automatically performed and the operator may then proceed to Calibration QC. If the DXpress™ Reader is left on or in power save mode, the operator should perform Self Check daily, as follows:

From the Main Menu, select: [2] RUN QC
Then select [1] SELF CHECK

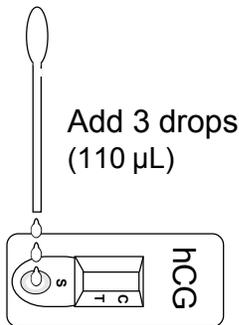
Self Check takes about 15 seconds. PASS or FAIL results will be displayed/printed when testing is completed. All Self Check items should pass before testing patient samples.

Note: Perform Calibration QC in accordance with your laboratory procedures. Consult the DXpress™ Reader User Manual for more information.

Testing Patient Samples

Patient samples may be tested using the DXpress™ Reader Scheduler mode, as described below. To use other modes (batch mode or read-now mode) consult the DXpress™ Reader User Manual.

1. Open the pouch and remove the test device.
 - Write the patient ID on the test device.
 - Place the test device on a level surface.
2. Enter test information in the DXpress™ Reader:
 - From the Main Menu, select [1] RUN PATIENT.
 - Scan the lot number barcode.
 - Confirm test device information and lot number as displayed on the screen and press ENTER.
 - Scan or enter the Operator ID.
 - Scan or enter the Patient ID.
 - Select sample type: Urine or Serum.
 - From the Incubation Time window, select SCHEDULER.



3. Add 3 drops of patient sample to the sample well of the test device while holding the dropper vertically. Press ENTER on the DXpress™ Reader (internal clock will start).
4. Place the test device in the Reader tray, and push in to close the tray.
5. After 5 minutes of incubation the DXpress™ Reader will automatically display/print the results.
 - At this point the test device may be removed and appropriately discarded.

Interpretation of Results

Positive

The instrument will automatically determine the result as positive if the test line intensity is greater than or equal to 25 mIU hCG/mL and confirm that the control line intensity meets the minimum requirement.

Negative

The instrument will automatically determine the result as negative if the test line intensity is less than 25 mIU hCG/mL and confirm that the control line is present (valid test).

Borderline Results

Samples reported as borderline are considered indeterminate and the operator is advised to repeat the test with a new specimen obtained 48-72 hours later.

Invalid

The instrument will automatically determine if a procedural error has occurred by confirming that the control line is not present (invalid test).

If the result is invalid, the sample should be retested with a new device. If the problem persists, contact your local distributor of LifeSign.

Limitations

- As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Similarly, specimens from patients who have routinely been exposed to animals or to animal serum products may contain heterophile antibodies which may cause erroneous results. • Samples reported as borderline are considered indeterminate and the operator is advised to repeat the test in 48-72 hours.
- 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.¹¹
- Low levels of hCG have been reported in non-pregnant females with no history of ectopic pregnancy, trophoblastic disease or germ-cell tumors.¹² 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.¹⁴
- 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.¹⁵ 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.
- 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 4° C and give a negative test result.

- In rare occasions, persistent low levels of hCG present in men and in non-pregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.^{12, 13}
- This test is intended to be used with DXpress™ Reader. Do not interpret the result visually.

User Quality Control

Internal Control: Each **BioSign® RC Serum/Urine 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® RC hCG Serum/Urine Test is capable of detecting hCG level of 25 mIU/mL (calibrated against the WHO 4th International Standard) in urine or serum. 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 116 clinical serum samples and clinical urine samples were studied. These specimens were assayed with **BioSign® RC hCG Serum/Urine Test** and a predicate device according to their respective protocols. The summary of the results is shown in Table 1 and Table 2.

Table 1. BioSign® RC vs. Predicate Device with Serum Specimens

		BioSign® RC hCG Serum/Urine			
		Positive	Borderline	Negative	Total
Predicate Device	Positive	25	5	1	31
	Negative	0	0	20	20
	Total	25	5	21	51

Table 2. BioSign® RC vs. Predicate Device with Urine Specimens

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

In serum sample testing, 6 samples showed discrepant results between the **BioSign® RC** test and the predicate device. All 6 of the discrepant samples contained hCG but the amount of hCG present in these samples was less than 25mIU/mL. The predicate device gave positive results for these 6 discrepant samples. The **BioSign® RC hCG Serum/Urine Test** gave borderline results for 5 of the 6 discrepant samples and a negative result for 1 of the 6 discrepant samples.

In urine sample testing, two samples gave discrepant results between the two devices. These two samples contained hCG, but the amount of hCG present in these samples was less than 25mIU/mL. The **BioSign® RC** 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® RC hCG Serum/Urine Test** gave correct results (borderline or negative) for all these samples.

Sensitivity

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

Pooled negative urine or serum 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 3.

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

Table 3. BioSign® RC™ Sensitivity Study with urine or serum samples

hCG (mIU/mL)	Percent Positive (N)	
	Urine	Serum
0	0	0
3	0	0
5	0	0
10	0	0
15	30 (6)	35 (7)
20	80 (16)	85 (17)
25	100 (20)	100 (20)
40	100 (20)	100 (20)

Precision Study

The precision of **BioSign® RC hCG Serum/Urine 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 4 shows the precision data combining all repeated tests.

Table 4. Summary of Precision Study Data

Serum

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	0	180	100
25	180	180	0	0	100
40	180	180	0	0	100

Urine

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® RC** test results was evaluated at three physicians' office laboratories using a total of 120 blind control samples. The control panels were prepared in serum or urine. 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	Urine	Serum
hFSH	1000 mIU/mL	1000 mIU/mL
hLH	300 mIU/mL	300 mIU/mL
hTSH	1000 µIU/mL	1000 µIU/mL

Interfering Substances

Potentially interfering substances were prepared at the following concentrations in both serum and urine containing either 0 or 25 mIU/mL hCG. These samples were tested with the **BioSign® RC hCG Serum/Urine Test**. No interference was found (Table 5) at these concentration.

Table 5. Interfering Substances and Concentrations Tested

Substance Added	Concentration Added	
	in Urine	in Serum
Acetaminophen	20 mg/dL	20 mg/dL
Acetylsalicylic Acid	20 mg/dL	20 mg/dL
Ampicillin	20 mg/dL	20 mg/dL
Ascorbic Acid	20 mg/dL	20 mg/dL
Atropine	20 mg/dL	20 mg/dL
Caffeine	20 mg/dL	20 mg/dL
Gentisic Acid	20 mg/dL	20 mg/dL
Phenothiazine	20 mg/dL	20 mg/dL
Phenylpropanolamine	20 mg/dL	20 mg/dL
Salicylic Acid	20 mg/dL	20 mg/dL
Tetracycline	20 mg/dL	20 mg/dL
Bilirubin	1 mg/dL	15 mg/dL
Glucose	2000 mg/dL	2000 mg/dL
Hemoglobin	1 mg/dL	125 mg/dL
Ketones	100 mg/dL	-

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Symbols Key

	Lot Number		Transfer Pipette
	Expiration Date		CE Mark
	Store at 2-30°C		Authorized Representative
	Do Not Reuse		Pregnancy Test
	For <i>in vitro</i> Diagnostic Use		Contents
	Catalog Number		Test Device
	Manufacturer		Consult Instructions For Use
	Instructions For Use		
	Contains sufficient for <n> tests		

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