P-5116-C

BioSign® hCG

New One Step Pregnancy Test

For Professional *In Vitro* Diagnostic Use

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

For the Early Detection of Pregnancy

PBM

CLIA Complexity: Waived
CDC Analyte Identifier Code: 9642
CDC Test System Identifier Code: 49200

Stock No.	BSP-121	35 Test Kit
	BSP-121-10	10 Test Kit

Intended Use

BioSign® hCG—One Step Pregnancy Test is a simple immunoassay for the Qualitative Detection of Human Chorionic Gonadotropin (hCG) in Urine for the Early Detection of Pregnancy.

Summary and Principle of Procedure

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placental trophoblastic cells shortly after the fertilized ovum is implanted 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 urine and serum soon after conception, and its rapid rise in concentration make it an excellent marker for pregnancy. The hormone level may become detectable 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 30,000-100,000 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 subunit confers unique biological and immunological specificity to the molecule.[6,7]

The **BioSign® hCG—One Step Pregnancy Test** is a rapid test for detecting pregnancy. The test is a solid-phase, two-site immunometric assay in which a combination of monoclonal and polyclonal antibodies is used to selectively detect hCG in urine with a high degree of sensitivity. In the test procedure, sample is added to the sample well using a dropper and sample is allowed to soak in. 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. Presence of two colored lines, one at the Test position and the other at the Control position, indicates a positive result, while the absence of the line at the Test position indicates a negative result.

Reagents

The **BioSign® hCG—One Step Pregnancy Test** kit contains enough reagents and materials to perform all the tests.

- **BioSign® hCG devices.** Test device containing the polyclonal anti-hCG coated membrane and a pad with the mouse monoclonal IgG (anti-hCG)-dye conjugate in a protein matrix containing 0.1% sodium azide.
- **Disposable plastic droppers**
- **Package insert**

Precautions

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

Storage and Stability

The BioSign® hCG—One Step Pregnancy Test kit should be stored at 2–30°C (36–86°F) in the original sealed pouch.

Specimen Collection and Preparation

- Approximately 150 µl of urine sample is required for each test.
- For optimal early detection of pregnancy, a first morning urine specimen is preferred since it generally contains the highest concentration of hCG on a given day. However, randomly collected urine specimens may be used.
- Collect the urine specimen in a clean glass or plastic cup without preservatives.
- Urine containing excessive bacterial contamination should not be used since spurious results may occur with such specimens.
- Bring specimens to room temperature prior to testing. 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.

Specimen Storage

- If testing will not be performed immediately, the specimens should be refrigerated (2–8°C) for up to 24 hours.
- For prolonged storage, specimens may be frozen and stored below –20°C. 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. Add sodium azide to a concentration of 0.1% as a preservative and ship by the quickest means possible.

Procedure

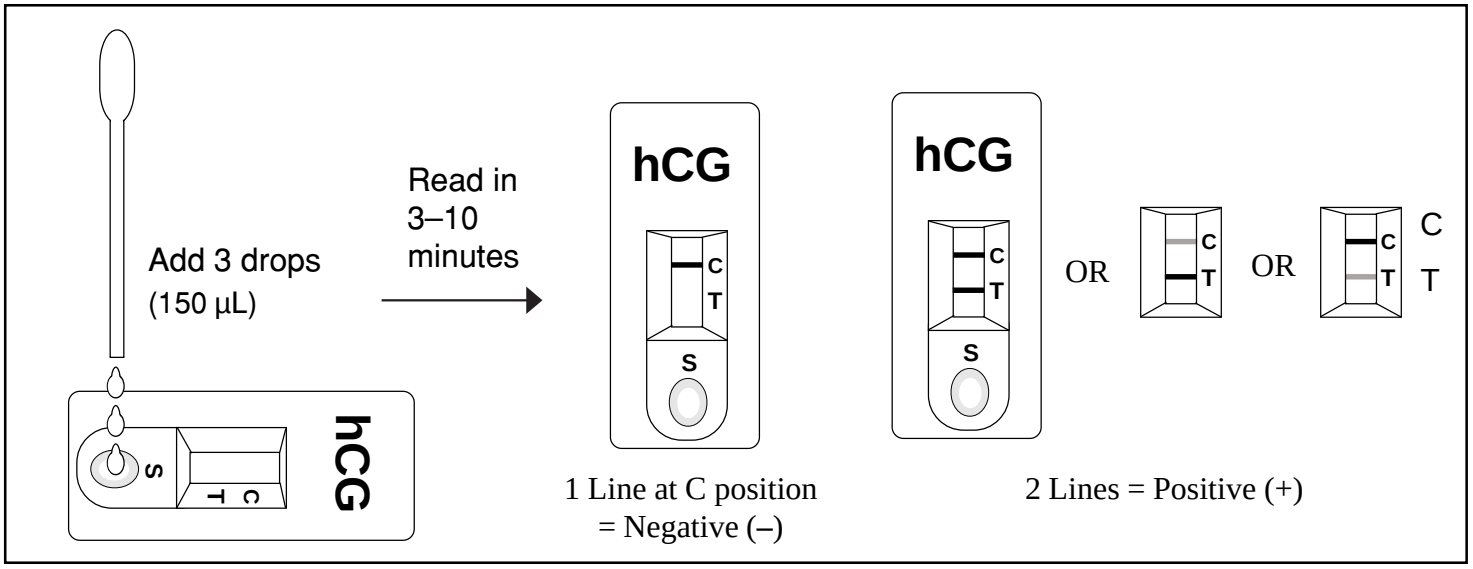
Test Procedure Summary

The procedure consists of adding the specimen to the sample well in the device and watching for the appearance of colored lines on the membrane.

Procedural Notes

The instructions below must be followed to achieve optimal test results.

- Before opening the pouch, the **BioSign® hCG—One Step Pregnancy Test** device must be allowed to stand at room temperature for at least 30 minutes prior to testing.
- Label the **BioSign® hCG** device with the patient name or control number.
- Fill the dropper with the sample without air bubbles.
- Handle all specimens as if capable of transmitting disease.



- After testing, dispose of the **BioSign® hCG** device, and the dropper following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

Materials Provided

- **BioSign® hCG** devices
- Disposable droppers

Materials Required But Not Provided

- Timer
- Specimen cup

Test Protocol

1. For each test, open one **BioSign® hCG** pouch.
2. Holding the dropper in a vertical position, add 3 drops (150 µl) of sample into the sample well (S).
3. Read the result after 3 minutes, but within 10 minutes.

Results

How to Read the Test

Positive: Two pinkish-purple lines, one each at the Test position (T) and at the Control position (C). One of the following indicates a positive test result:

a. Two strong pinkish-purple lines, one each at the Test (T) and Control (C) positions.
b. One strong pinkish-purple line at the Test position (T) and one light pinkish-purple line at the Control position (C).
c. One light pinkish-purple line in the Test position (T) and one strong pinkish-purple colored line at the Control position (C).

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

Notes on Results

Positive

A specimen containing a detectable level of hCG will generate pinkish-purple lines at the Control position (C) and at the Test position (T) within 3 minutes. The time required to generate the line is dependent on the hCG concentration in the sample. Positive results may be detected in as early as one (1) minute, depending on the hCG concentration. To be interpreted as positive, the pinkish-purple line at the Test position should be clearly distinguishable from the background color of the membrane. In strong positive tests, the color intensity of the Control line (C) may be much lighter than that of the Test line (T). Note: The high dose hook effect has been found to occur at approximately 500,000 mIU/mL. For samples with extremely high concentration of hCG, the higher the hCG concentration, the lighter the color band at the test region may become.

Negative

In the absence of hCG, or in the case that the hCG concentration is below the detection limit of the test, there will be no apparent line at the Test position; rather, there may be a uniform background color over the membrane area. The Control line at the Control position should be clearly visible.

Inconclusive or Invalid Results

If there is no distinct pinkish-purple line visible at the Control position, the test is inconclusive. The Control line should always appear. If there is a suspected procedural error, the result should be considered inconclusive. It is recommended that in these cases the test be repeated with a new test device.

Limitations

- 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.
- The hCG level may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous miscarriage, or therapeutic abortion.[11]
- 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.[12] 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.
- The concentration of hCG may be very low in the case of ectopic pregnancy.[13] A suspected ectopic pregnancy may be further evaluated by a physician.
- In addition to 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.

- Very high levels of hCG may exist in certain pregnancies and pathological conditions (e.g., choriocarcinoma and hydatidiform mole). This may weaken the test line.
- As is true with any diagnostic procedure, the physician should evaluate data obtained by using this kit in light of other clinical information.
- Samples which contain excessive bacterial contamination or have been subjected to repeated freezing and thawing should not be used because such specimens can give spurious results.
- Urine samples with low specific gravity may not contain representative levels of hCG. If such a sample is negative or weakly positive, a first morning specimen should be used for retesting.

User Quality Control

- Control standards are not provided with this kit; however, it is recommended that controls be tested at regular intervals as good testing practice and whenever there is any doubt about the interpretation of the test result. It is recommended that a positive control which is near the sensitivity limit of the assay be used for assay control. For information on how to obtain controls, contact PBM for technical assistance. Before using a new lot of kit, a quality control test using the positive and negative control should be conducted to confirm the expected Q.C. results and the validity of the assay. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
- The control line at the Control position can be considered an internal procedural control, i.e., a proper amount of sample is used; sample is added to the sample well, and not through the Result Window; and the reagent system worked properly. A distinct pinkish-purple control line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.
- A clear background in the Result Window is considered an internal negative procedural control. If the test is performed correctly and the **BioSign® hCG** device is working properly, the background in the Result Window should be clear, providing a distinct negative result.

Expected Values

BioSign® hCG—One Step Pregnancy Test is capable of detecting hCG level of 15 mIU/mL (WHO 3rd International Standard, previously known as the 1st I.R.P). The levels of hCG in normal early pregnant women vary. However, hCG levels often exceed 100 mIU/mL by the first day of the missed menstrual period.[1] The test is usually capable of confirming pregnancy by the first day of the missed menstrual period.

Performance Characteristics

Clinical Evaluation

A total of 247 blind clinical urine samples were studied. These specimens were assayed with **BioSign® hCG—One Step Pregnancy Test** and Tandem® Icon® II according to the package inserts (Table 1). Thirty-six (36) samples are from menopausal women.

Table 1

BioSign® hCG—One Step Pregnancy Test vs. Tandem® Icon® II

Test Result (# of Samples)

	Tandem® Icon® II	**BioSign® hCG**
Positive (+)	78	78
Negative (–)	133	133
Menopausal	Not Determined	36 (Negative, –)

Relative Sensitivy	100%
Relative Specificity	100%
Overall Agreement	100%

The data demonstrate the excellent correlation between **BioSign® hCG—One Step Pregnancy Test** and Tandem® Icon® II. The clinical accuracy and sensitivity of the two tests were found to be comparable.

Physicians' Office Laboratory Evaluation (Proficiency Study)

Reproducibility of **BioSign® hCG** test was evaluated at three physicians' offices using a total of 60 blind control samples. The panels consisted of 5 negative (–), 5 low positive (25 mIU/mL hCG), 5 moderate positive (200 mIU/mL hCG), and 5 high positive (500 mIU/mL hCG) samples. The results obtained at each site agreed 100% with expected results.

Sensitivity

Standard controls (calibrated to the WHO 3rd International Standard) ranging from 5 mIU/mL to 50 mIU/mL were tested in 5 replicates. The results confirmed the sensitivity of 15 mIU/mL in 3 to 4 minutes and 25 mIU/mL in 2 minutes assay time (Table 2).

Table 2. Sensitivity–Example

BioSign® hCG—One Step Pregnancy Test Sensitivity and Assay Time

	Standards (hCG, mIU/mL)					
hCG concentration	5	10	15	20	25	50
Time required for Positive Signal	7'	4'	2'40"	2'20"	1'50"	58"

Specificity

Thirty-six urine specimens collected from menopausal women were studied. Specimens from menopausal women are known to interfere frequently with pregnancy tests due to cross-reactivity with other gonadotropin hormones such as Leutenizing hormone. These specimens were assayed with **BioSign® hCG—One Step Pregnancy Test**. All 36 specimens were found negative.

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

Homologous Hormones:

hFSH	1000 mIU/mL
hLH	500 mIU/mL
hTSH	1000 µIU/mL

Other Interfering Substances

At the level of claimed sensitivity, **BioSign® hCG—One Step Pregnancy Test** showed no interference when the following potentially interfering substances, both endogenous and exogenous, were added to urine samples which had hCG levels of 0 and 25 mIU/mL (Table 3).

Table 3. Potentially Interfering Substances added to Urine and Tested with the BioSign® hCG—One Step Pregnancy Test

Substance Added	Concentration in Urine
Drugs:	
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
Urinary Analytes:	
Bilirubin	2 mg/dl
Glucose	2000 mg/dl
Hemoglobin	25 mg/dl
Ketones	100 mg/dl
Albumin	2000 mg/dl

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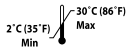
Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
	"Use By" date in year-month-day format
	Temperature Limitation
	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
PIP	Transfer Pipette
IFU	Instructions for Use
TEST PREG	Pregnancy Test

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