The 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. It is intended for use by laboratories and health care professionals to enhance the early detection of pregnancy by allowing the urine to be tested at any time during pregnancy.

### 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. 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. The hormone is comprised of two non-covalently bound dissimilar subunits containing approximately 30% carbohydrate by weight.

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 sit in the test device for 10 minutes. If hCG is present in the sample, 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

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

### Specimen Collection and Preparation

- 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.
- Urine containing excessive bacterial contamination should not be used.
- Do not interchange materials from different product lots and do not use expired material.
- Do not use specimens that are over 2 hours old.
- Do not use urine samples that contain diabetics, pregnant women, or women undergoing hormonal therapy.

### Storage and Stability

The BioSign hCG — One Step Pregnancy Test kit should be stored at 2−8°C (36−46°F) in the original sealed pouch.

### Procedure

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 specimen without air bubbles.
- Handle all specimens as if capable of transmitting disease.
Add 3 drops

Read at 3–5 minutes

1 Line at C position = Negative (−)

2 Lines = Positive (+)

• 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 of sample into the sample well (S).
3. Read the result at 3–5 minutes. Do not interpret the results after 5 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 at 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 3–5 minutes. The time required to generate the line is dependent on the hCG concentration in the sample. Positive results may be detected 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 brighter the hCG concentration, the lighter the color band at the test region may appear.

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
• Elevated hCG levels have been reported in patients with both gestational and non-gestational trophoblastic diseases. 138-140 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.

• 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, by caesarean section, spontaneous abortion, or therapeutic abortion.141

• The hCG level in the case of spontaneous abortion may be very low and eventually decrease. 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 pregnancy. Natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall.142 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. A suspected ectopic pregnancy may be further evaluated using a quantitative hCG assay.

• Very high levels of hCG may exist in certain pregnancies and pathological conditions (e.g., choriocarcinoma and hydatidiform mole). This may weaken the intensity of test line.

• 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.

• Urine samples collected after consumption of a large amount of fluids may contain a lower hCG concentration. If such a sample is negative, a first morning specimen should be obtained and retested.

• In rare occasions, persistent low levels of hCG present in men and non-pregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.142

User Quality Control
Internal Control: Each BioSign® hCG—One Step Pregnancy 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 C position indicating an adequate sample volume is used, the sample and reagent are working on the membrane, and the test reagents at the Control line and the conjugate-color indicator are reactive. In addition, the clearing background in the Result window is considered as an additional procedural control by providing a distinct readable result. This may be considered an internal negative procedural control. If background color appears in the Result window which interferes with your ability to read the test result and obscure the formation of the control band, your result may be 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 kit 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 PBMs Technical Services.

Expected Values
BioSign® hCG—One Step Pregnancy Test is capable of detecting hCG level of 25 mIU/mL in urine (calibrated against the WHO 3rd 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. The test is usually capable of detecting hCG 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

<table>
<thead>
<tr>
<th>Test Result (# of Samples)</th>
<th>BioSign® hCG</th>
<th>Tandem® Icon II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (+)</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>133</td>
<td>133</td>
</tr>
<tr>
<td>Menopausal</td>
<td>Not Determined</td>
<td>36 (Negative, −)</td>
</tr>
</tbody>
</table>

Relative Sensitivity 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, 3.5 low positive (25 mIU/mL), 5 moderate positive (200 mIU/mL), 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 40 mIU/mL, were tested in 5 replicates. The results confirmed the sensitivity of 25 mIU/mL at 3–5 minute assay time.

Specificity
Thirty-six urine specimens collected from menopausal women were studied. Specimens from menopausal women are known to interfere with pregnancy tests due to cross-reactivity with other gonadotropin hormones such as Luteinizing 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 hormones when tested against the levels specified below (Table 2).

Table 2. Homologous Hormones: hFSH 1000 mIU/mL, hLH 1000 mIU/mL, hTSH 1000 mIU/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

<table>
<thead>
<tr>
<th>Substance Added</th>
<th>Concentration in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs:</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Acetylcholinic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20 mg/dl</td>
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</table>