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 Explanation
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 early hormone level may become detectable in both urine and serum as early as 7 to 10 days after conception.5-7 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 contains only unique biological and immunological specificity to the molecule.6,7

The Status 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 and Materials Provided
Status hCG One-Step Pregnancy Test kit contains enough reagents and materials to perform all the tests.

- Status device
- Test device with 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 dropper
- Package insert

Materials Provided
- Timer
- Specimen cup
- Latex gloves

Storage and Stability
Status hCG One-Step Pregnancy Test kit should be stored at 2–30°C (36–86°F) in the sealed pouch.

Specimen Collection and Preparation

Urine Assay:
- For optimal early detection of pregnancy, a first morning urine specimen is preferred since it generally contains the highest concentration of human hCG. 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 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 at 2–8°C for up to 48 hours. Bring specimens to room temperature prior to testing.
- 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. For urine samples, add sodium azide to a concentration of 0.1% as a preservative and ship by the quickest means possible.

Procedures

Procedural 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 Status hCG One-Step Pregnancy Test device must be allowed to stand at room temperature for at least 30 minutes prior to testing.
- Label the Status hCG device with the patient name or control number.
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the Status hCG device, and the dropper
following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

**Test Procedure**

**STEP 1** For each test, open one Status hCG pouch, and label the Status device with the patient ID.

**STEP 2** Holding the dropper in a vertical position, add 3 drops of sample into the Sample well (S).

**STEP 3** Read the results at 3–5 minutes. Do not interpret the results after 5 minutes.

**Interpretation of Results**

Positive

Two pinkish-purple lines, one each in the Test Position (T) and in the Control Position (C). Each of the following indicates a positive test result:

- Elevated hCG levels have been reported in patients with both gestational and nongestational trophoblastic diseases. The hCG of trophoblastic neoplasms is similar to that found in pregnancy, so the test results may be positive for pregnancy and nongestational trophoblastic disease. The physician should evaluate data obtained with this kit in light of other clinical information.

- Only one pinkish-purple line, at the Control Position (C).

- 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.

- 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 in non-pregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.

**Limitations**

- Elevated hCG levels have been reported in patients with both gestational and nongestational trophoblastic diseases. The hCG of trophoblastic neoplasms is similar to that found in pregnancy, so the test results may be positive for pregnancy and nongestational trophoblastic disease. The physician should evaluate data obtained with this kit in light of other clinical information.

- 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 recognized 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.

- 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.

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- 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.

**Negative**

- Only one pinkish-purple line, at the Control Position (C).

- 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.

- 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 in non-pregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.

**Limitations**

- Elevated hCG levels have been reported in patients with both gestational and nongestational trophoblastic diseases. The hCG of trophoblastic neoplasms is similar to that found in pregnancy, so the test results may be positive for pregnancy and nongestational trophoblastic disease. The physician should evaluate data obtained with this kit in light of other clinical information.

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- 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.

**NOTE:** 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 (T). To avoid results to be considered inconclusive, it is recommended that in these cases the test be repeated with a new test device.

**User Quality Control**

**Internal Control:** Each Status hCG One-Step Pregnancy Test device has a built-in control. The Control line is an internal procedural control. A distinct reddish-purple Control line should appear at Control (C) position indicating an adequate sample volume is used.

**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 practice. For information on how to obtain controls, contact LifeSign's Technical Services.

**Expected Values**

**Status hCG One-Step Pregnancy Test** is capable of detecting hCG level of 25 mIU/mL in urine/calibrated against the WHO 3rd International Standard. The hCG levels in normal early pregnancy 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**

**Clinical Evaluation**

A total of 247 blind clinical urine samples were studied. These specimens were assayed with Status hCG One-Step Pregnancy Test and Tandem® Icon™ II. The clinical accuracy and sensitivity of the two tests are found comparable.

**Physicians’ Office Laboratory Evaluation (Proficiency Study)**

Reproducibility of Status hCG14 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 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 frequently with pregnancy tests due to cross-reactivity with other gonadotropin hormones such as Luteinizing hormone. These specimens were assayed with Status 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 (Table 2).
**Test Procedure**

**STEP 1**
For each test, open one Status hCG pouch, and label the Status device with the patient ID.

**STEP 2**
Holding the dropper in a vertical position, add 3 drops of sample into the Sample well (S).

**STEP 3**
Read the results at 3–5 minutes. Do not interpret the results after 5 minutes.

**Interpretation of Results**

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

- **OR**

| (examples of positive results) |

**Negative**
Only one pinkish-purple line, at the Control Position (C).

- **OR**

| (examples of negative results) |

**NOTE:** 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 (T); rather, there may be a uniform background color over the membrane area. The Control line at the Control Position (C) should be clearly visible.

**Invalid**
A distinctive colored line at the Control Position (C) should always appear. The test is invalid if no Control line forms.

- **OR**

| (examples of invalid results) |

**NOTE:** 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 subtle, faint pinkish-purple color at the Control Position, 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.12–16 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.
- 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 abortion, or therapeutic abortion.11
- 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 the pregnancy. Natural resorptions in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall.17 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.12 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 samples 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.
- 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 in non-pregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.14,15

**User Quality Control**

Internal Control: Each Status hCG One-Step Pregnancy Test device has a built-in control. The Control line is an internal procedural control. A distinct reddish-purple Control line should appear at C position indicating an adequate sample volume is used, and the sample and reagent are wicking 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 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 LifeSign’s Technical Service assistance at 1-800-526-2125.

**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 LifeSign’s Technical Services.

**Expected Values**

**Status hCG** One-Step Pregnancy Test is capable of detecting hCG level of 25 mIU/mL in urine (calibrated against 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.

**Performance Characteristics**

**Clinical Evaluation**
A total of 247 blind clinical urine samples were studied. These specimens were assigned with Status hCG One-Step Pregnancy Test and Tandem® Icon™ II according to the package inserts (Table 1). Thirty-six (36) samples are from non-pregnant women.

**Table 1 (Urine Assay)**

<table>
<thead>
<tr>
<th>Status hCG One-Step Pregnancy Test vs. Tandem® Icon™ II with Urine Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Result (# of Samples)</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Menopausal Not Determined</td>
</tr>
</tbody>
</table>

**Overall Accuracy:** 100%
**Relative Sensitivity:** 100%
**Relative Specificity:** 100%

The data demonstrate the excellent correlation between Status hCG One-Step Pregnancy Test vs. Tandem® Icon™ II. The clinical accuracy and sensitivity of the two tests are found comparable.

**Physicians’ Office Laboratory Evaluation**

1. **Proficiency Study**
Reproducibility of Status hCG18 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 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. Samples from menopausal women are known to interfere frequently with pregnancy tests due to cross-reactivity with other gonadotropin hormones such as Leutening hormone. These specimens were assayed with Status 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 (Table 2).

**Table 2**

<table>
<thead>
<tr>
<th>Homologous Hormones: Status hCG</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>hFSH</td>
<td>1000 mIU/mL</td>
</tr>
<tr>
<td>LH</td>
<td>500 mIU/mL</td>
</tr>
<tr>
<td>hTSH</td>
<td>1000 mIU/mL</td>
</tr>
</tbody>
</table>

**Other Interfering Substances**
At the level of claimed sensitivity, Status 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**

<table>
<thead>
<tr>
<th>Possibly Interfering Substances added to Urine and Tested with the Status hCG One-Step Pregnancy Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance Added</td>
</tr>
<tr>
<td>Drugs: Acetaminophen</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
</tr>
<tr>
<td>Atropine</td>
</tr>
<tr>
<td>Caffeine</td>
</tr>
<tr>
<td>Gastric Acid</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Phenylpropionate</td>
</tr>
<tr>
<td>Salicilic Acid</td>
</tr>
<tr>
<td>Tetracycline</td>
</tr>
<tr>
<td>Urinary Analytes: Bilirubin</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Ketones</td>
</tr>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>Homologous Hormones: hFSH</td>
</tr>
<tr>
<td>hLH</td>
</tr>
<tr>
<td>hTSH</td>
</tr>
</tbody>
</table>
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 concentration of hCG continues to rise rapidly, frequently exceeding 100 mL/U/L by the first missed menstrual period and peaking in the 30,000–100,000 mL/U/L range by 10 to 12 weeks into pregnancy. The hormone is comprised of two non-covalently bound disulfur subunits containing approximately 30% carbohydrate by weight. 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.

The Status 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 and Materials Provided
- Status hCG One-Step Pregnancy Test kit contains enough reagents and materials to perform all the tests.
- Status hCG device with the patient name or control number.
- Handle all specimens as if capable of transmitting disease.
- Avoid repeated freezing and thawing.
- If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transport of etiologic agents. For urine samples, add sodium azide to a concentration of 0.1% as a preservative and ship by the quickest means possible.

Procedures
- 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.
- If testing will not be performed immediately, the specimen should be refrigerated (2–8°C) for up to 48 hours. Bring specimens to room temperature prior to testing.
- For prolonged storage, specimens may be frozen and stored below −20°C. Avoid repeated freezing and thawing.
- Do not interchange materials from different product Lot’s and do not use beyond the expiration date.
- The Status hCG One-Step Pregnancy Test device should remain in its sealed pouch until ready for use.
- Before opening the pouch, the Status hCG One-Step Pregnancy Test device must be allowed to stand at room temperature for at least 30 minutes prior to testing.
- Label the Status hCG device with the patient name or control number.
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the Status hCG device, and the dropper.