Status hCG Serum/Urine
One-Step Pregnancy Test
For Professional In Vitro Use
Rapid Immunoassay for the Qualitative Detection of Human Chorionic Gonadotropin in Serum or Urine
For the Early Detection of Pregnancy
LifeSign, LLC

Intended Use
Status hCG Serum/Urine One-Step Pregnancy Test is a simple immunoassay for the detection of human chorionic gonadotropin (hCG) in serum or urine for the early confirmation of pregnancy.

Summary and Principle
Human chorionic gonadotropin is a 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 the urine and serum soon after conception and its rapid rise in concentration make it an excellent marker for confirmation of pregnancy. The hormone becomes 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 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. The alpha subunit is structurally similar to other human pituitary glycoprotein hormones, whereas the beta (ß) subunit confers unique biological and immunological specificity to the molecule. The Status hCG Serum/Urine One-Step Pregnancy Test is a rapid serum or urine test for detecting hCG.

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

Specimen Collection and Preparation
Urine Assay:
- For optimal early detection of pregnancy, a first morning urine specimen is preferred; however, the test generally contains the highest concentration of 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.

Serum Assay:
- Remove the serum from the clot as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used. Specimens containing particulate matter may give a positive test result and may require the specimens be clarified by centrifugation prior to testing.
- If refrigerated, bring sera to room temperature (18–30°C) prior to testing.

Storage:
- Do not freeze. Solutions 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 specimen should be refrigerated 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. 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. Avoid repeated freezing and thawing.

If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of biologicals. If urine specimens are used, 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 reactivity with urine or serum specimens.

- Allow specimens and the Status hCG Serum/Urine One-Step Pregnancy Test device to stand at room temperature for at least 30 minutes prior to testing.
- Label the Status device with the patient name or control number.
- Allow the dropper to fill with sample. Holding the dropper in a vertical position, add 3 drops of sample into the Sample Well (S).
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the Status 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 a Status hCG Serum/Urine pouch, and label the Status device with the patient ID.

STEP 2
Hold 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 at the Test Position (T) and at the Control Position (C). Each of the following indicates a positive test result.

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

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

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. The control line at the Control Position should be clearly readable.

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

Note: If there is no distinct pinkish-purple line visible at the Control Position, the test is inconclusive. If there is a suspected procedural error made by the user, the result should be considered inconclusive. It is recommended that in this case the test be repeated or a fresh specimen be obtained and tested. A Control line should always appear. The absence of a pinkish-purple line at the Control Position means the test is invalid and should be repeated with a new test device.

Limitations

- As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse IgG antibodies (HAMA) in the sample. Similarly, specimens from patients who have been routinely exposed to animals or animal serum products may contain heterophile antibodies which may cause erroneous results.
- 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 these conditions, including choriocarcinoma and hydatidiform mole, may 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, another specimen should be obtained at least 48 hours later and tested.
- The hCG level may remain detectable for several weeks after normal delivery, delivery by cesarean section, spontaneous abortion, or therapeutic abortion.

The HCG level in the case of spontaneous abortion may be very low and may not be detectable for several days to a few weeks. The test is highly sensitive, and specimens which test positive during the initial days after confirmation 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 or serum 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.
- High levels of hCG may exist in certain pregnancies and pathological conditions (e.g., choriocarcinoma and hydatidiform mole). This may weaken the signal line.

The physician should evaluate the data obtained from this kit in light of other clinical information.

Samples which contain excessive bacterial contamination or have not 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 tested.

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.

User Quality Control

Internal Control: Each Status hCG Serum/Urine 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 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 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 LifeSign for technical assistance at 1-800-506-2125.

Expected Values

Status hCG Serum/Urine One-Step Pregnancy Test is capable of detecting hCG levels of 25 mIU/mL (WHO 3rd International Standard). 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. The test is usually capable of detecting hCG by the first day of the missed menstrual period.

Performance Characteristics

Clinical Evaluation—Urine Assay
A total of 247 blind clinical urine samples were studied. These specimens were assayed with Status hCG Serum/Urine One-Step Pregnancy Test and Tandem® Icon™ II according to the package inserts (Table 1). Thirty-six (36%) samples are from menopausal women.

<table>
<thead>
<tr>
<th></th>
<th>Tandem® Icon™ II</th>
<th>Status hCG</th>
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<tbody>
<tr>
<td>Positive</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Negative</td>
<td>133</td>
<td>133</td>
</tr>
<tr>
<td>Menopausal</td>
<td>Not Determined</td>
<td>Not Determined</td>
</tr>
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</table>

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

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

Clinical Evaluation—Serum Assay
A total of 425 clinical serum samples were studied. These specimens were assayed with Status hCG Serum/Urine One-Step Pregnancy Test and Tandem® Icon™ II according to the package inserts. The results demonstrate 100% relative sensitivity, 99% relative specificity and 99.5% overall accuracy (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Tandem® Icon™ II</th>
<th>Status hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>215</td>
<td>215</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>215</td>
</tr>
</tbody>
</table>

Overall Accuracy: 99.5%
Relative Sensitivity: 100%
Relative Specificity: 99%

Physicians’ Office Laboratory Evaluation (Proficiency Study)
Reproducibility of Status hCG 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 5 negative (-), 5 low positive (25 mIU/mL hCG), 5 moderate positive (50 mIU/mL hCG), 5 high positive (500 mIU/mL hCG) samples. The results obtained at each site agreed 100% with expected results and with predicate tests compared in parallel, obtained at each site agreed 100% with expected results and with predicate tests compared in parallel.

Sensitivity- Urine Assay
Standard controls (calibrated to the WHO 3rd International Standard ranging from 5 mIU/mL to 40 mIU/mL in urine) were included in 20 replicates. The results confirmed sensitivity of 25 mIU/mL.
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 reactivity with serum or urine specimens.

- Allow specimens and the Status hCG Serum/Urine one-step pregnancy test device to stand at room temperature for at least 30 minutes prior to testing.
- Label the Status device with the patient name or control number.
- Hold the dropper in a vertical position, and add 3 drops of sample into the Sample Well (S).
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the Status 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 Serum/Urine pouch, and label the Status device with the patient ID.

STEP 2
Hold 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 at the Test Position (T) and at the Control Position (C). Each of the following indicates a positive test result.

- The hCG level in the case of spontaneous abortion may be very low and may later be below the level of detection of the assay. Natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall.12 Subsequent testing of a new urine or serum 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 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 signal line.
- The physician should evaluate the data obtained from 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 specimens collected after consumption of a large amount of fluids may contain a lower hCG concentration. If such a urine specimen is negative, a first morning specimen should be obtained and retested.
- 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.14

Limitations
- As with any assay employing mouse antibodies, the possibility of cross-reactivity exists. 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 hCG level in the case of spontaneous abortion may be very low and may later be below the level of detection of the assay.
- 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.
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- There are occasions when persistent low levels of hCG present in men and in nonpregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.

User Quality Control

Internal Control: 
Each Status hCG Serum/Urine one-step pregnancy test device has a built-in control. The Control line is an internal positive procedural control. A distinct pinkish-purple Control line should appear at C position indicating an adequate sample volume. If no pinkish-purple line appears at the Control Position, the test is considered inconclusive. If there is a suspected procedural error made by the user, the result should be considered inconclusive. It is recommended that in this case the test be repeated or a fresh specimen be obtained and tested. A Control line should always appear. The absence of a pinkish-purple line at the Control Position means the test is invalid and should be repeated with a new test device.

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Expected Values

Status hCG Serum/Urine one-step pregnancy test device is capable of detecting hCG levels of 25 mIU/mL (WHO 3rd International Standard). 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. The test is usually capable of detecting hCG by the first day of the missed menstrual period.

Performance Characteristics

Clinical Evaluation-Urine Assay
A total of 247 blind clinical urine samples were studied. These specimens were assayed with Status hCG Serum/Urine one-step pregnancy test and Tandem® icon™ II according to the package inserts (Table 1). Thirty-six (36) samples are from menopausal women. The data demonstrate the excellent correlation between Status hCG Serum/Urine one-step pregnancy test and Tandem® icon™ II. The clinical accuracy and sensitivity of the two tests are found comparable. Overall Accuracy: 100% Relative Sensitivity: 99% Relative Specificity: 100%

Clinical Evaluation—Serum Assay
A total of 425 blind clinical serum samples were studied. These specimens were assayed with Status hCG Serum/Urine one-step pregnancy test and Tandem® icon™ II according to the package inserts. The results demonstrate 100% relative sensitivity, 99% relative specificity and 99.5% overall accuracy (Table 2).

Sensitivity- Urine Assay
Standard controls (calibrated to the WHO 3rd International Standard ranging from 5 mIU/mL to 40 mIU/mL in urine) were tested in 20 replicates. The results confirmed sensitivity of 25 mIU/mL.
Potential Interfering Substances
Potential interfering substances were prepared at the following concentrations in both urine and serum which contain either 0 or 25 mIU/mL hCG. These samples were tested with the Status hCG Serum/Urine (Table 4).

<table>
<thead>
<tr>
<th>Substance Added</th>
<th>Concentration Added in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs:</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Ampicillin Sulphate</td>
<td>20 µg/mL</td>
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<tr>
<td>Atropine</td>
<td>20 µg/mL</td>
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<tr>
<td>Caffeine</td>
<td>20 µg/mL</td>
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<tr>
<td>Gentamicin</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Gentamicin Sulphate</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>20 µg/mL</td>
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<tr>
<td>Erythromycin</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Hemoglobin</td>
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<tr>
<td>Histamine</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Triglycerides</td>
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</tbody>
</table>

Homologous Hormones:

<table>
<thead>
<tr>
<th>Substance Added</th>
<th>Concentration Added in Serum</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>

Summary and Principle
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.\(^1\)\(^2\) 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 confirming pregnancy. The hormone concentration becomes detectable in both urine and serum as early as 7 to 10 days after conception.\(^3\) 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.\(^4\) The alpha subunit is structurally similar to other human pituitary glycoprotein hormones, whereas the beta (ß) subunit confers unique biochemical and immunological specificity to the molecule.\(^5\)\(^6\) The Status hCG Serum/Urine One-Step Pregnancy Test is a rapid serum or urine test for detecting hCG. The test is a solid-phase, two-site immunometric assay in which a monoclonal antibody is coated on a nitrocellulose membrane and a pad with the mouse monoclonal IgG (anti-hCG) conjugate in a protein matrix containing 0.1% sodium azide. Reagents and Materials Provided

Status hCG Serum/Urine One-Step Pregnancy Test kit contains enough reagents and materials to perform all the tests. Status device. Test device containing the polyclonal anti-hCG coating and a pad immunometric assay in which a monoclonal IgG (anti-hCG) conjugate in a protein matrix containing 0.1% sodium azide.

References