

Procedures: Status hCG [®] Urine/Serum Combo
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Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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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.¹⁻⁴ 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 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 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}

The **Status hCG[™] Serum/Urine—One Step Pregnancy Test** is a rapid serum or urine test for confirming 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 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 the aid of a transfer pipette 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 in the test window and the other in the control window, indicates a positive result, while the absence of the line in the test window indicates a negative result.

SPECIMEN:**Urine Assay**

- Approximately 110 μL (0.11 mL) of serum or 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. However, randomly collected urine specimens may be used.
- Collect the urine specimen in a clean glass or plastic cup.
- As in many test systems, 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 inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying.
- 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 for 15 days. Frozen specimens must be completely thawed, thoroughly mixed and brought to room temperature. 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.

EQUIPMENT AND MATERIALS:**Materials:**

Provided in the kit:

The Status hCGTM 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 coated membrane and a pad with the mouse monoclonal IgG (anti-hCG)-dye conjugate in a protein matrix containing 0.1% sodium azide.
- Transfer pipette (disposable plastic pipettes).
- Instruction insert.

Storage Requirements:

Status hCGTM Serum/Urine—One Step Pregnancy Test kit should be stored at 2–30°C (36–86°F) in the sealed pouch.

QUALITY CONTROL:

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 LifeSign for technical assistance. The positive control will produce a positive result and the negative control will yield a negative result (control line only). Before using a new lot, 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 in the Control window can be considered an internal positive procedural control, i.e., a proper amount of sample is used; sample is added to the sample well, and not through the reading 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 LifeSign for technical assistance.
- A clear background in the Test Result Window (T) is considered an internal negative procedural control. If the test is performed correctly and the Status[®] hCG device is working properly, the background in the Test Result Window (T) should be clear, providing a distinct negative result.

PROCEDURE - STEPWISE:

A. For Serum or Plasma:

1. For each test, open one **Status hCG[™]** pouch, and label the Status device with the patient ID.
2. Holding the dropper in a vertical position, add 3 drops (110 µL) of sample into the sample well (S).
3. Read the result after 3 minutes, but within 5 minutes. **Negative results should be confirmed at 5 minutes.**

REPORTING RESULTS:

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

- a. Two strong pinkish-purple lines, one each in the test (T) and control (C) windows.
- b. One strong pinkish-purple line in the test window (T) and one light pinkish-purple line in the control window (C).
- c. One light pinkish-purple line in the test window (T) and one pinkish-purple colored line in the control window (C).

Negative: Only one pinkish-purple line, in the control window (C).

Notes on Results

Positive

A specimen containing a detectable level of hCG will generate a pinkish-purple line in the test window (T) within 3 minutes. The time required to generate the line is dependent on the hCG concentration in the sample. Some positive results can be read in as early as one (1) minute. To be interpreted as positive, the pinkish-purple line in the test window 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).

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 in the test window; rather, there may be a uniform background color over the membrane area. The control line in the control window should be clearly readable. **Negative results should be confirmed at 5 minutes.**

Inconclusive or Invalid Results

If there is no distinct pinkish-purple line visible either in the test window or in the test control window, 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 48 hours later. A control line should always appear; the absence of a pinkish-purple line in the control window means the test is invalid and should be repeated.

LIMITATIONS OF THE PROCEDURE:

- 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.
- A very early pregnancy containing an extremely low concentration of hCG 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.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 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.
- 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 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.

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ALTERNATIVE METHOD:

List the alternative method or reference laboratory to be used should the Status HCG kit become unavailable.

Alternative Method:

Reference Laboratory:

Name:
Address:
Phone:
Contact:

CRITERIA FOR REFERRAL OF SPECIMENS:

When referring specimens for outside testing, the following procedures are recommended:

1. Verify that the testing laboratory possess a valid CLIA certification authorizing performance of the referred test.
2. Follow laboratory guidelines for specimen shipment
3. Report results exactly as received (no alteration or revision either of results or interpretive information provided by the testing laboratory.).
4. Permit direct test report from the testing laboratory to the authorized person or entity that ordered the test.
5. Retain or be able to produce an exact duplicate of each testing laboratory's report.
6. Provide the name and the address where the test was performed and indicate this information on the test report.

TECHNICAL ASSISTANCE:

Technical assistance is available from the distributor of Status, Lifesign, LLC, Somerset, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. ET

1-800-525-2125 ext 102