

<b>Procedures:</b> Status hCG® Urine
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Prepared by	Date Adopted	Supersedes Procedure #

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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.<sup>1-4</sup> 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.<sup>1-4</sup> 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.<sup>5</sup> 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.<sup>6,7</sup>

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 elevated levels of hCG in 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:**

- 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. However, randomly collected urine specimens may be used.
- Collect the urine specimen in a clean glass or plastic cup without preservatives.
- As in many test systems, urine containing excessive bacterial contamination should not be used since spurious results may occur with such specimens.
- Bring specimens to room temperature (18–30°C) 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 hCG™—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.
- Disposable plastic droppers
- Package insert

**Storage Requirements:**

The **Status hCG™—One Step Pregnancy Test** kit should be stored at 2–30°C (36–86°F) in the original 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<sup>®</sup> 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<sup>™</sup>** pouch.
2. Holding the dropper in a vertical position, add 3 drops (150-200 µl) of sample into the sample well (S).
3. Read the result after 3 minutes, but within 10 minutes.

## REPORTING RESULTS:

**Positive:** Two pinkish-purple lines, one each in the test window (T) and in the control window (C). One 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 strong 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. 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 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). Note: For urine samples the high dose hook effect has been found to occur at approximately 500,000 mIU/mL. For samples with extreme concentration of hCG value, 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 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 visible.

### **Inconclusive or Invalid Results**

If there is no distinct pinkish-purple line visible in the 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. 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:**

- 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 caesarean section, spontaneous abortion, or therapeutic abortion.<sup>11</sup>
- 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.<sup>12</sup> 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.<sup>13</sup> 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.<sup>8,9,10</sup> 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 which 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 obtained for retesting.

#### REFERENCES:

1. Braunstein, G.D., Rasor, J., Adler, D., Danzer, H., and Wade, M.E. Serum Human Chorionic Gonadotropin Levels Throughout Normal Pregnancy. *Am. J. Obstet. Gynecol.* 1976; 126:678.
2. Krieg, A.F. Pregnancy Tests and Evaluation of Placental Function in: *Clinical Diagnosis and Management by Laboratory Methods*, 16th ed., Henry, J.B. (ed.) W.B. Saunders Co., Philadelphia, pp. 680, 1979.
3. Brody, S. and Carlstrom, G. Immunoassay of Human Chorionic Gonadotropin in Normal and Pathologic Pregnancy. *J. Clin. Endocrinol. Metab.* 1962; 22:564.
4. Hussa, R.O. Human Chorionic Gonadotropin, A Clinical Marker: Review of its Biosynthesis. *Ligand Review* 1981; 3:6.
5. Swaminathan, N. and Bahl, O.P. Dissociation and Recombination of the Subunits of Human Chorionic Gonadotropin. *Biochem. Biophys. Res. Commun.* 1970; 40:422.
6. Ross, G.T. Clinical Relevance of Research on the Structure of Human Chorionic Gonadotropin. *Am. J. Obstet. Gynecol.* 1977; 129:795.
7. Reuter, A.M., Gaspard, U.J., Deville, J-L., Vrindts-Gevaert, Y. and Franchimont, P. Serum Concentrations of Human Chorionic Gonadotrophin and its Alpha and Beta Subunits. 1. During Normal Singleton and Twin Pregnancies. *Clin. Endocrinol.* 1980; 13:305.
8. Morrow, C.P., et al. Clinical and Laboratory Correlates of Molar Pregnancy and Trophoblastic Disease. *Am. J. Obstet Gynecol.* 1977; 50:424-430.
9. Dawood, M.Y., Saxena, B.B., and Landesman, R. Human Chorionic Gonadotropin and its Subunits in Hydatidiform Mole and Choriocarcinoma. *Am. J. Obstet. Gynecol.* 1977; 50:172-181.
10. Braunstein, G.D., Vaitukaitis, J.L., Carbone, P.P., and Ross, G. T. Ectopic Production of Human Chorionic Gonadotropin by Neoplasms. *Ann. Inter. Med.* 1973; 78:39-45.
11. Steier, J.A., Bergsjø, P., and Myking, O.L. Human Chorionic Gonadotropin in Maternal Plasma After Induced Abortion, Spontaneous Abortion, and Removed Ectopic Pregnancy. *Am. J. Obstet. Gynecol.* 1984; 64:391-394.
12. Wilcox, A.J., Weinberg, C.R., O'Connor, J.F., Baird, D.D., Schlatterer, J.P., Canfield, R.E., Armstrong, E.G., and Nisula, B.C. Incidence of early loss of pregnancy. *N. Engl. J. Med.* 1988; 319:189-194.
13. Thorneycroft, I.H. When You Suspect Ectopic Pregnancy. *Diagnosis*, January: 67-82, 1976.