Laboratory: _____ Date Implemented: _____

Address: _____

_____

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Sure-Vue® Urine hCG Strip Laboratory Procedure

I. Test Principle

The Sure-Vue® Urine hCG Strip is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine to aid in the early detection of pregnancy. The test utilizes a combination of antibodies including mouse monoclonal anti-hCG antibodies and goat polyclonal anti-hCG antibodies to selectively detect elevated levels of hCG. The assay is conducted by immersing the test strip in a urine specimen and observing the formation of colored lines. The specimen migrates via capillary action along the membrane to react with the colored conjugate.

Positive specimens react with the specific colored antibody conjugates and form a colored line at the test line region of the membrane. Absence of this colored line suggests a negative result. To serve as a procedural control, a colored line will always appear at the control line region if the test has been performed properly.

II. Specimen Collection/Treatment

A. Specimen:	Urine Specimen.
B. Collection Container:	Clean and dry container.
C. Specimen Storage:	Urine may be stored refrigerated at 2-8° C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20° C.
D. Handling Precautions:	Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.

III. Reagents and Equipment

A. Reagents and Materials Provided

- Test Strips
- Package insert

B. Materials Required but not Provided

- Specimen collection container
- Timer

C. Storage and Stability

Store as packaged in the closed canister at 2°-30°C. The test strip is stable through the expiration date printed on the closed canister. The test strip must remain in the closed canister until use, and is stable 90 days after opening the canister. **DO NOT FREEZE.** Do not use beyond the expiration date.

D. Quality Control

Internal Procedural Controls

Internal procedural controls are included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative background control. If the test is working properly, the background in the result area should be white to light pink and not interfere with the ability to read the test result.

External Quality Control Testing

It is recommended that a positive hCG control (containing 25-250 mIU/mL hCG) and a negative hCG control (containing "0" mIU/mL hCG) be evaluated to verify proper test performance. It is recommended that federal, state, and local guidelines be followed.

External Quality Control Testing

Remedial Actions

When correct control results are not obtained, do not report patient results. Contact Technical Services at 800-637-3717.

E. Precautions

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test strip should remain in the closed canister until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test strip should be discarded in a proper biohazard container after testing.

IV. Test Procedure

Specimen Collection and Handling:

- A urine specimen must be collected in a clean and dry container.
- A first morning urine specimen is preferred since it generally contains the highest concentration of hCG; however, urine specimens collected at any time of the day may be used.
- Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.
- Urine specimens may be stored at 2 - 8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

Test Procedure:

Allow the test strip, urine, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1. Bring the canister to room temperature before opening it. Remove the test strip from the closed canister and use it as soon as possible. Immediately close the canister tightly after removing the required number of test strip(s). Record the initial opening date on the canister. Once opened, the remaining test strip(s) are stable for 90 days only.
2. With arrows pointing toward the urine specimen, immerse the test strip vertically in the urine specimen for at least 5 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip.
3. Place the test strip on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 3 minutes. It is important that the background is clear before the result is read.

Note: A low hCG concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 10 minutes.

V. Interpretation of Test Results

POSITIVE:* Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).

***NOTE:** The intensity of the red color in the test line region will vary depending on the concentration of hCG present in the specimen. However, neither the quantitative value nor the rate of increase in hCG can be determined by this qualitative test.

NEGATIVE: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and contact Technical Support at (800) 637-3717.

VI. Limitations

1. Very dilute urine specimens, as indicated by a low specific gravity, may not contain representative levels of hCG. If pregnancy is still suspected, a first morning urine specimen should be collected 48 hours later and tested.
2. False negative results may occur when the levels of hCG are below the sensitivity level of the test. When pregnancy is still suspected, a first morning urine specimen should be collected 48 hours later and tested.
3. Very low levels of hCG (less than 50 mIU/mL) are present in urine specimen shortly after implantation. However, because a significant number of first trimester pregnancies terminate for natural reasons,[5] a test result that is weakly positive should be confirmed by retesting with a first morning urine specimen collected 48 hours later.
4. A number of conditions other than pregnancy, including trophoblastic disease and certain non-trophoblastic neoplasms including testicular tumors, prostate cancer, breast cancer, and lung cancer, cause elevated levels of hCG.[6,7] Therefore, the presence of hCG in urine specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.
5. This test provides a presumptive diagnosis for pregnancy. A confirmed pregnancy diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

VII. Expected Values

Negative results are expected in healthy non-pregnant women and healthy men. Healthy pregnant women have hCG present in their urine and serum specimens. The amount of hCG will vary greatly with gestational age and between individuals.

The Sure-Vue Urine hCG has a sensitivity of 25 mIU/mL, and is capable of detecting pregnancy as early as 1 day after the first missed menses.

VIII. Performance Characteristics

Accuracy

A multi-center clinical evaluation was conducted comparing the results obtained using the Sure-Vue Urine hCG Strip to another commercially available urine membrane hCG test. The study included 150 urine specimens: both assays identified 72 negative and 78 positive results. The results demonstrated a 100% overall agreement (for an accuracy of $\geq$ 99%) of the Sure-Vue Urine hCG when compared to the other urine membrane hCG test.

Reference hCG Method

		Positive	Negative
Sure-Vue Urine hCG Strip	Positive	78	0
	Negative	0	72

Sensitivity and Specificity

The Sure-Vue Urine hCG Strip detects hCG at a concentration of 25 mIU/mL or greater. The test has been standardized to the W.H.O. Third International Standard. The addition of LH (300 mIU/mL), FSH (1,000 mIU/mL), and TSH (1,000 µIU/mL) to negative (0 mIU/mL hCG) and positive (25 mIU/mL hCG) specimens showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to hCG negative and positive specimens.

Acetaminophen	20 mg/mL	Caffeine	20 mg/mL
Acetylsalicylic Acid	20 mg/mL	Gentisic Acid	20 mg/mL
Ascorbic Acid	20 mg/mL	Glucose	2 g/dL
Atropine	20 mg/mL	Hemoglobin	1 mg/dL
Bilirubin	2 mg/dL		

None of the substances at the concentration tested interfered in the assay.

IX. References

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2. Catt KJ, ML Dufau, JL Vaitukaitis. “Appearance of hCG in pregnancy plasma following the initiation of implantation of the blastocyte.” *J. Clin. Endocrinol. Metab*. 1975; 40(3): 537-540
3. Braunstein GD, J Rasor, H. Danzer, D Adler, ME Wade. “Serum human chorionic gonadotropin levels throughout normal pregnancy.” *Am. J. Obstet. Gynecol*. 1976; 126(6): 678-681
4. Lenton EA, LM Neal, R Sulaiman. “Plasma concentration of human chorionic gonadotropin from the time of implantation until the second week of pregnancy.” *Fertil. Steril.* 1982; 37(6): 773-778
5. Steier JA, P Bergsjo, OL Myking. “Human chorionic gonadotropin in maternal plasma after induced abortion, spontaneous abortion and removed ectopic pregnancy.” *Obstet. Gynecol*. 1984; 64(3): 391-394
6. Dawood MY, BB Saxena, R Landesman. “Human chorionic gonadotropin and its subunits in hydatidiform mole and choriocarcinoma.” *Obstet. Gynecol.* 1977; 50(2): 172-181
7. Braunstein GD, JL Vaitukaitis, PP Carbone, GT Ross. “Ectopic production of human chorionic gonadotropin by neoplasms.” *Ann. Intern Med*. 1973; 78(1): 39-45
8. Sure-Vue Urine hCG Strip Package Insert

Test Procedure Review

Supervisor	Date Reviewed	Supervisor	Date Reviewed