HUMAN CHORIONIC GONADOTROPIN

By Monoclonal and Polyclonal Antibody Technique

# PRINCIPLE

Human Chorionic Gonadotropin (hCG) is a placental glycoprotein hormone that can be detected in serum and urine and is the basis for testing to confirm pregnancy. The hCG hormone is secreted by the developing placenta after fertilization and doubles approximately every 2.2 days during the 1st trimester. (1) Detectable levels start at 5 mIU/mL during the first week of gestation and rise to 100,000 mIU/mL at 2 to 3 months. A slower rise may be associated with high risk abortions. (2) Values decline between 10% and 15 % of peak concentrations during the 2nd and 3rd trimesters. (3) The Sure-Vue Urine hCG Kitis a rapid qualitative test that can detect hCG in urine samples. The test utilizes a combination of monoclonal and polyclonal antibody reagents to selectively detect elevated levels of hCG in urine. The assay is conducted by the addition of specimen into the test device sample well and watching for the formation of colored lines. The specimen migrates via capillary action along the membrane and reacts with the colored conjugate. A positive specimen reacts with the hCG-specific antibody colored conjugate and forms a colored line in the T (test) window. Absence of this colored line suggests a negative result. To serve as a control for the procedure, a colored line in the C (control) window will always appear regardless of the presence or absence of hCG.

# SPECIMEN COLLECTION AND PREPARATION

**Urine Collection**: Any urine sample is appropriate for hCG testing, but the first morning void is optimal because it generally contains the highest concentration of hCG. Very dilute specimens may not contain a representative quantity of hCG. The specimen should be collected in a clean, dry container and should be clearly labeled with the patient's name and SSN.

A urine specimen containing visible precipitates should be filtered, centrifuged, or allowed to settle (obtaining clear aliquots) before testing. If a very turbid sample is obtained, it should be sent to the laboratory for centrifugation and testing or allowed to settle before testing. Samples should be pipetted from the clear supernatant. Gross hematuria may prevent an accurate reading of test results by masking the positive line.

**Cause for Rejection:**

* Gross hematuria
* Urine samples that have been stored at room temperature for more 1 hour
* Urine samples that have been stored at 2-8°C for more than 72 hrs

**PRECAUTIONS**

* For *In-Vitro* diagnostic use only.
* The test device should be discarded in a proper biohazard container after testing.
* Do not use the kit beyond the expiration date.
* The test device should remain in the sealed pouch until ready for use.

# STORAGE AND STABILITY

* The test kit is to be stored at room temperature (15-30°C) for the duration of the shelf-life.
* The test device must remain sealed in the pouch until ready for use.
* Urine specimens may be store at 2-8°C for up to 72 hrs prior to testing

# MATERIALS PROVIDED

# Test device containing monoclonal mouse-hCG colored conjugate and hCG antibody coated on a membrane.

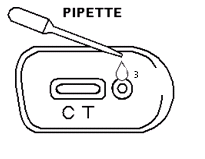
* Disposable specimen dropper.

**TESTING PROCEDURE**

The pouch must be at room temperature before opening to avoid condensation of moisture on the membrane. Allow specimen and/or external controls to reach room temperature prior to testing.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with patient or control identification.

2. Dispense 3 drops (approx. 0.11mL) of specimen into the round sample well (see illustration below).



Wait for colored lines to appear.

3. **Read** urine results at **3 minutes**. Positive results may be observed in as short as 30 seconds depending on the hCG concentration. The presence of the control line is not indicative of the test being completed. Wait the entire 3 minutes for the completion of the test.

# INTERPRETATION OF RESULTS

**Negative Result**

The test is negative if a red colored line only appears in the C (Control) window.

**Positive Result**

The test is positive if one red colored line appears in the T (test) window and one red colored line appears in the C (control) window. Any red colored line in the T (test) window should be considered positive. Colored lines may be lighter or darker than each other. Specimens with hCG levels near the threshold of the test may develop color (faintly) after the 3 minute reading for urine. In such cases, another test should be performed with a new specimen in 48-72 hours or laboratory serum hCG should be ordered. The initial test should be considered negative. A line that appears after 10 minutes should be ignored.

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If a colored line does not appear in the C (control) window, the test is invalid. A laboratory serum hCG should be ordered.

**Invalid Test Results**

The test is invalid if no colored line appears in the C (control) window even if a colored line appears in the T (test) window.

# EXPECTED RESULTS

Negative results are expected in healthy non-pregnant women and healthy men. Healthy pregnant women have hCG present. The amount will vary with gestational age and between patients. Sure-Vue Urine hCG Kit can detect hCG levels as low as 25 mIU/ml in urine.

Results should be documented by entering patient result and internal QC result directly into VISTA or using the glucometer OTE (Other Test Entry) option. See Attachment A for more information on using the OTE option.

# QUALITY CONTROL

The built-in (internal) controls must develop appropriately with each test or the test is not reportable and must be repeated. The appearance of a Control Line in the C region of the device is a positive procedural control. Correct procedural technique, specimen flow and device performance is confirmed when a colored line appears in the C (control) area of the membrane. A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. If a more intensely red background color appears, the test should be repeated, as it may interfere with the ability to read the test result.

External Quality Control must be performed on each new shipment for each lot number of test kits received in the shipment and monthly thereafter to verify proper storage conditions and proper performance of the test cartridge. The Sure-Vue Urine hCG Control Set contains a Negative, a Low Positive (25 mIU/mL), and a High Positive (250 mIU/mL) control. Each level should be tested and evaluated for acceptability before using the test kit for patient testing. Record results on the QC Log. Store the Sure-Vue Urine hCG Control Set at 2-8°C. Allow controls to come to room temp before testing, and then return to 2-8°C for storage until the manufacturer’s expiration date. Reference the manufacturer’s package insert for any additional information.

# LIMITATIONS

Shortly after implantation, very low levels of hCG are present in urine. False negative results may occur when the levels of hCG are below the sensitivity level of the test. When pregnancy is suspected, a fresh serum sample should be sent to the main laboratory or a first morning urine specimen should be collected 48 hours later and tested.

Elevated hCG concentrations unrelated to pregnancy have been reported in patients with some types of cancer (trophoblastic disease, choriocarcinoma, embryonal cell carcinoma and islet cell tumors.) Positive results should be interpreted in conjunction with other clinical and laboratory data. Detectable levels of hCG may remain several weeks following a normal pregnancy, delivery by caesarean section, or following a spontaneous or therapeutic abortion. Ectopic pregnancies may produce very low levels of hCG. A negative test does not exclude an ectopic pregnancy. If this condition is suspected, further testing using a quantitative test may be desirable. Approximately one-third of all conceptions end in natural termination. This may produce positive results when testing early in the pregnancy, followed by negative results after the natural termination. This test provides a presumptive diagnosis for pregnancy. Physicians should evaluate all clinical and laboratory findings before making a definitive diagnosis. A viscous specimen may exhibit a slower flow rate, requiring more time for the test to be completed. A high dose "hook effect" may occur when the intensity of the sample line decreases as the concentration of hCG increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the sample line. The test is designed to be a qualitative test only and does not correlate directly to quantitative hCG tests. The intensity of color in a positive line should not be evaluated as "quantitative or semi-quantitative". Sensitive immunoassays may demonstrate false positive results with specimens containing heterophilic antibodies. Assays may also exhibit false-positive or false negative results with specimens containing human anti-mouse antibodies. If the qualitative interpretation is inconsistent with the clinical evaluation, results should be confirmed by an alternate hCG method such as the serum assay available in the main laboratory.

# PERFORMANCE CHARACTERISTICS

**Accuracy**

A multi-center clinical evaluation was conducted comparing the results obtained using the Sure-Vue Urine hCG to another commercially available urine membrane hCG test. The study included 159 urine specimens: both assays identified 88 negative and 71 positive results. The results demonstrated a 100% overall agreement (for an accuracy of >99%) of the Sure-Vue Urine hCG when compared to the other urine membrane hCG test. **Method**

**Sensitivity and Specificity**

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

**Interfering Substances**

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

Acetaminophen 20mg/mL

Caffeine 20mg/mL

Acetylsalicylic Acid 20mg/mL

Gentisic Acid 20mg/mL

Ascorbic Acid 20mg/mL

Glucose 2g/dL

Atropine 20mg/mL

Hemoglobin 1mg/dL

Bilirubin (urine) 2mg/dL

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

# REFERENCES

* Sure-Vue® Urine hCG Package Insert; Fischer Scientific Company.

Note: For technical assistance, contact Ancillary Testing personnel or Fisher Scientific Technical Support, ph 1-888-727-3315.

Attachment A

Entering HCG Results into the Accu-chek Inform via OTE Option:

1. Power the meter on.
2. Scan/Enter Operator ID.
3. With the Main Menu displayed, press the **Patient Test** button to display the Patient Test Menu.
4. Press the **Other Patient Tests** button to access the list of Other Patient Tests.
5. Press the **Pregnancy** button.
6. Scan/Enter the Patient ID. Press the next button to display the Date Tested screen.
7. If the **date tested** is the current day, press the next button to default to the current date and advance to the Time Tested screen. If the date tested is a date other than the current day, enter the date tested and press the next button.
8. If the **time tested** is the current time, press the next button to default to the current time and advance to the Control Bar screen. If the time tested is not the current time, enter the time tested and press the next button.
9. The **Pregnancy Kit Lot** screen will pop up next. Manually key in the lot number or scan the lot number barcode provided by the Ancillary Testing Coordinator.
10. The kit **Expiration Date** screen will pop up next. Manually enter the kit expiration date. Format: mm/dd/yy
11. The **Control Bar** screen will pop up after entering the time. If the internal QC was acceptable, press the acceptable button. If the internal QC was not acceptable, patient results should NOT be reported.
12. The **Result screen** will pop up after entering the QC results. Press the “positive” or “negative” button, whichever is appropriate.

NOTE: If you enter incorrect information during individual steps, you may select the “back” button to edit the information.

1. Once the result is entered, the Patient Result screen is displayed. To change the results, press **Results**. To enter comments, press **Comments** to display the Add Comments screen. Comments are not required.
2. Review information. If any information needs changed, press the appropriate button to display that choice and edit the information.
3. If information is correct, or once edits are complete, press the next button, power off the meter, and place the meter in the base to download.