VA Salt Lake City Health Care System, MC 113
500 Foothill Drive, Salt Lake City, UT 84148

Document Management Number: SOP-0949
Document Title: Alere hCG Urine Only Procedure

Principle:

The Alere™ hCG Combo Cassette test is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine or serum 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 adding urine or serum specimen to the specimen well of the test cassette and observing the formation of colored lines. The sample migrates via capillary action along the membrane to react with the colored conjugate. Positive samples 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.

Specimen:

Urine specimens 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 samples may be stored 2°-8°C up to 48 hours.

All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. The test cassette should be discarded in a proper biohazard container after testing.

NOTE: Hand hygiene should be observed before and after interacting with each patient. Gloves are to be worn when handling specimens and while completing all testing. Gloves should be changed between patients.

Reagents and Supplies:

Reagents and Materials provided with each kit:

- Test Cassettes (mouse monoclonal anti-hCG antibodies and goat polyclonal anti-hCG antibodies)
- Disposable sample droppers
- Package insert
- Procedure Card

Materials required but not provided:

- Specimen collection container
- Timer

Storage and Stability:

Store as packaged in the sealed pouch at 2°- 30°C. The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE. Do not use tests beyond the manufacturer's expiration date.**

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.

The control must be acceptable before reporting out any patient results. The control result is also documented with each patient result.

If unexpected results are seen when running the controls, review the Directions for Use, Interpretation of Results and Limitations sections and repeat the test with another cassette. If the problem persists, discontinue use of the test kit immediately, and contact the Ancillary Testing Coordinator at extension 1595. The Ancillary Testing Coordinator will contact Technical Services.

External Quality Control Testing:

External Liquid Quality Controls are used to assure that the reagents are performing properly and that the analyst is correctly performing the test procedure. External Quality Control is performed by the Ancillary Testing Coordinator for the main hospital facility prior to using a new shipment or lot number of hCG assay and monthly, the Positive Control and Negative Control must be tested and shown to yield the expected results. Upon observing the expected results, the kit is ready for use with patient specimens.

External Liquid Quality Control will also be performed by the nursing staff at each Community Based Outpatient Clinics (CBOC) with each new lot/shipment and also monthly. The positive and negative controls must be tested and shown to yield the expected results before patient testing can begin.
See the *Alere™ hCG External QC Procedure* for full details.

Patient Testing:
Ensure Test Cassettes are labeled correctly; with the patients Full Name, and Full SSN.

Allow the test cassette and urine specimen to equilibrate to room temperature (15°-30°C) prior to testing.

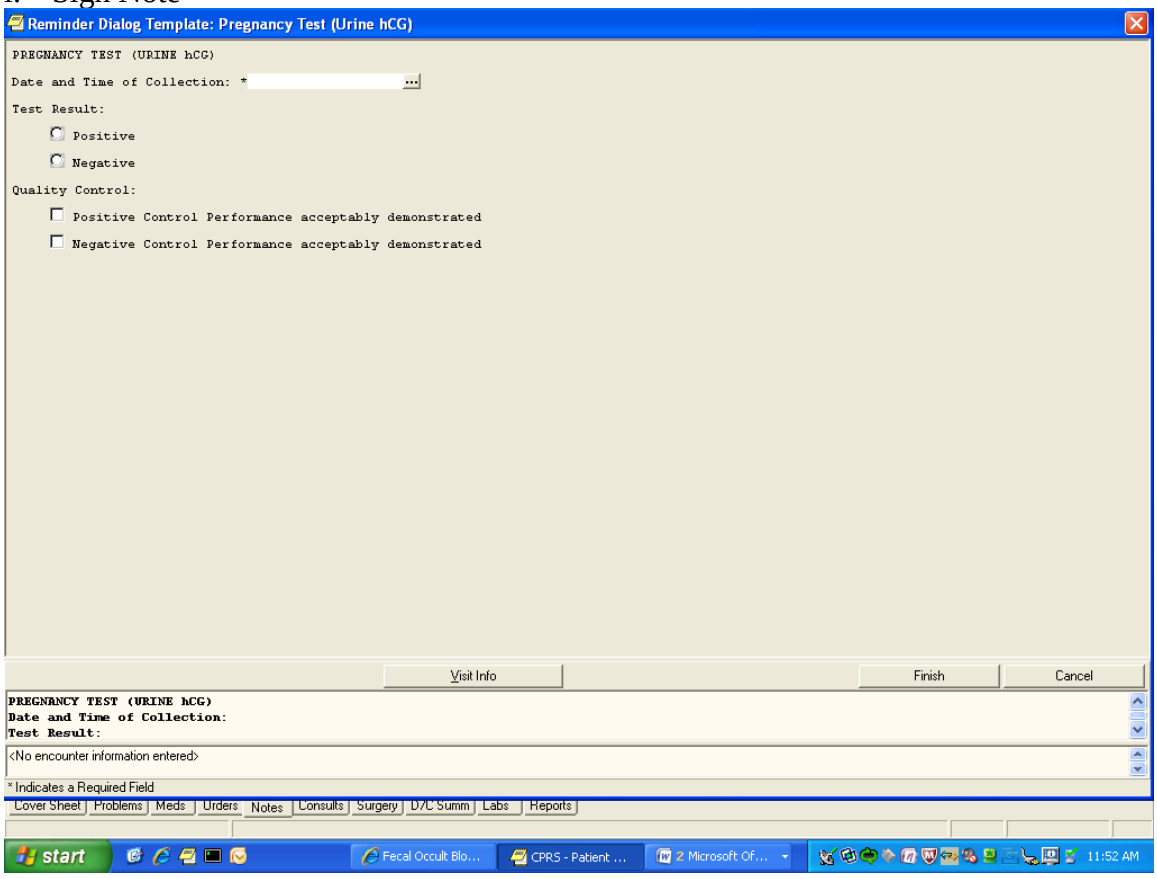
1. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx. 100µL) to the specimen well (S) of

the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S).

3. Wait for the red line(s) to appear. Read the result at 3 minutes when testing a urine specimen. It is important that the background is clear before the result is read.

Record results in CPRS.

a. Log into CPRS.
b. Click "NOTES" along bottom tab.
c. Click "Templates" on left hand side.
d. Click "Shared Templates".
e. Click on "Ancillary Testing"
f. Double click on "Pregnancy Test (Urine hCG)" Reminder Dialog
g. Select Location, Click "OK".
h. Select Progress Note Title, Click "OK"
i. Fill out reminder dialog, see example.
j. Click "Finish".
k. Select Primary Provider
l. Sign Note



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.

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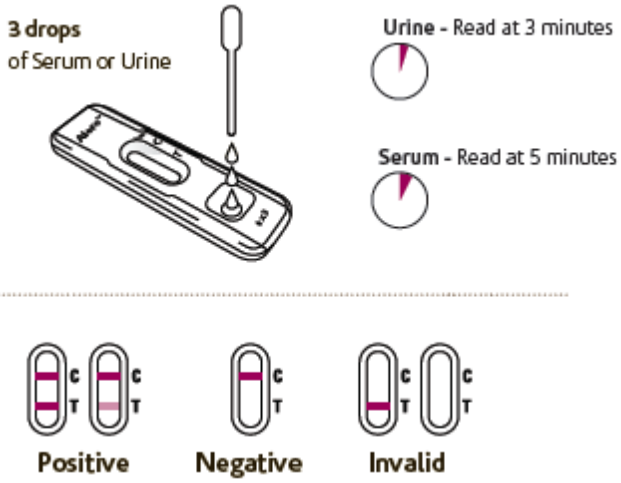
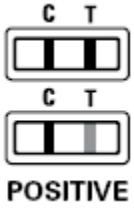


Interpretation of Results:

- **POSITIVE:** One line should be in the control region (C) and another line should be in the test region (T). Any shade of red color in the test line region (T) should be considered positive. **Two distinct red lines appear**.



- **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 cassette. If the problem persists, discontinue using the test kit immediately and contact the Ancillary Testing Coordinator at ext. 1595. A serum sample may be sent to the laboratory for a serum hCG.

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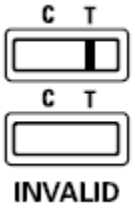

Note: The intensity of the red color in the test line region (T) will vary depending on the concentration of hCG present in the specimen. However, neither the quantitative value nor the rate of increase in hCG can be determined by this qualitative test.

Expected Results:

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 Alere™ hCG Combo Cassette test has a sensitivity of 25 mIU/mL and is capable of detecting pregnancy as early as 1 day after the first missed menses.

Interfering Substances:

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

Acetaminophen	20 mg/dL	Caffeine	20 mg/dL
Acetylsalicylic Acid	20 mg/dL	Gentisic Acid	20 mg/dL
Ascorbic Acid	20 mg/dL	Glucose	2 g/dL
Atropine	20 mg/dL	Hemoglobin	1 mg/dL
Bilirubin (serum)	40 mg/dL	Bilirubin (urine)	2 mg/dL
Triglycerides (serum)	1200 mg/dL		

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

Performance Characteristics:

Accuracy: A multi-center clinical evaluation was conducted comparing the results obtained using the Alere™ hCG Combo Cassette and another commercially available serum/urine membrane hCG test. The urine study included 159 specimens and both assays identified 88 negative and 71 positive results. The serum study included 73 specimens and both assays identified 51 negative and 21 positive and 1 inconclusive results. The results demonstrated 100% overall agreement (for an accuracy of > 99%) of the Alere™ hCG Combo Cassette when compared to the other Cassette membrane hCG test.

Sensitivity and Specificity: The Alere™ hCG Combo Cassette detects hCG at concentrations 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) specimens showed no cross-reactivity.

Limitations:

- 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.
- 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 or serum specimen should be collected 48 hours later and tested.
- Very low levels of hCG (less than 50 mIU/mL) are present in urine and serum specimen shortly after implantation. However, because a significant number of first trimester pregnancies terminate for natural reasons, a test result that is weakly positive should be confirmed by retesting with a first morning urine or serum specimen collected 48 hours later.
- 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 or serum specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.
- As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the specimen. Specimens from patients who have received preparations of monoclonal antibodies for diagnosis or therapy may contain HAMA. Such specimens may cause false positive or false negative results.
- 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.

References:

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- Dawood MY, BB Saxena, R Landesman "Human chorionic gonadotropin and its subunits in hydatidiform mole and choriocarcinoma." *Obstet. Gynecol.* 1977; 50(2): 172-181
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- Alere™ hCG Combo Cassette Package Insert.

Revision History

Section	Change	Date	Requester	Approver
Header	Update header	02/02/2017	Hung Ho	E. Hart MD
Font, Size	New Time Roman, 12	02/02/2017	Hung Ho	E. Hart MD