New Vision Medical Laboratories DACC, ESUC, WSUC, and Institute for Orthopaedic Surgery Technical Procedure		Procedure Number: nvml.urg.t002d Procedure Initiated: 10/1/2019

Pregnancy, Urine and Serum hCG

1. PRINCIPLE:

The Medline hCG Combo Pregnancy Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in serum or urine to aid in the early detection of pregnancy. The test utlilizes 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 specimen to the specimen well of the cassette and observing the formulation 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 lines 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 performed properly.

2. CLINICAL SIGNIFICANCE:

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in both urine and serum as early as 7 to 10 days after conception. hCG levels continue to rise very rapidly, frequently exceeding 100mIU/mL by the first missed menstrual period, and peaking in the 100,000-200,000mIU/mL range about 10-12 weeks into pregnancy. The appearance of hCG in both urine and serum soon after conception, and its subsequent rapid rise in concentration during early gestational growth, make it an excellent marker for the early detection of pregnancy.

The Medline hCG Combo Pregnancy Test Cassette is a rapid test that qualitatively detects the presence of hCG at the sensitivity of 10 mIU/ml is serum and 20 mIU/ml in urine.

3. SPECIMEN:

A. COLLECTION AND PROCESSING:

Urine – 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.

Serum- Blood should be collected aseptically into a clean tube without anticoagulants. (SST or red top). Separate the serum from blood as soon as possible to avoid hemolysis. Use clear-non-hemolyzed specimens when possible.

Specimen Storage – Serum or urine specimen may be stored at 36-46° F (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.

B. REJECTION:
Unlabeled specimens will be rejected.

C. STORAGE AND PRESERVATION:
Store as packaged in the sealed pouch at 36-86°F (2-30°C). The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

4. REAGENTS, STANDARDS, AND CONTROLS:

A. PREPARATION:
Test device containing mouse anti-beta hCG antibody conjugated to colloidal gold and goat anti-alpha hCG antibody coated on a membrane.

B. CONTROL PROCEDURE:
INTERNAL CONTROLS:
The appearance of a Red Control Line in the C region of the device 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 CONTROLS:
Negative and positive controls will be performed when each kit is opened and monthly as a check on continued storage conditions per package insert. Results will be recorded into the laboratory computer system as a kit validation. See procedure below.

5. EQUIPMENT:

A. INSTRUMENTS: NA

6. PROCEDURE:

A. PERFORMANCE:
Allow the test device and urine specimen and/or controls to equilibrate to room temperature 59-86° F (15-30°C) prior to testing.

1. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine or serum (approx. 100µL) to the specimen well (S) of the test device, and then start the timer. Avoid trapping air bubbles in the specimen well.
3. Read the result at 3-4 minutes when testing a urine specimen or at 5-6 minutes when testing a serum specimen. It is important that the background is clear and a control line is present before the result is read.
   ***Do not interpret the result after the appropriate time*.**

B. INTERPRETATION OF 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 (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.*
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 likely reasons for control line failure. Repeat analysis with a new test device. If the

problem persists, discontinue using the test kit immediately. Contact the POC office for further instructions.

C. FAILURE/REMEDIAL ACTION:

If QC results are unacceptable (either internal or external); repeat test with new test device. Do not report patients. Contact the POC office for further instructions if needed.

7. EXPECTED RESULTS:

A. REPORTABLE RANGE:

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 Medline hCG Combo Pregnancy Test Cassette has a sensitivity of 10 mIU/ml is serum and 20 mIU/ml in urine and is capable of detecting pregnancy as early as 1 day after the first missed menses.

8. REPORTING RESULTS:

A. NORMAL VALUES:

Results will be recorded on the kit testing patient log sheet and entered into the LIS.

9. PROCEDURAL NOTES:

A. BACKUP FOR INOPERABLE SYSTEM:

Send to main laboratory for testing.

B. REFERAL OF SPECIMENS: N/A

C. SUBMISSION/HANDLING OF REFERRAL SPECIMENS:

Submit properly labeled and ordered specimen to main lab for testing.

10. LIMITATIONS AND INTERFERING SUBSTANCES:

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 serum or urine specimen should be collected 48 hours later and tested.

3. Very low levels of hCG (less than 50mIU/mL) are present in urine and serum 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 serum or urine specimen collected 48 hours later.

4. This test reliably detects intact hCG up to 500,000 mIU/mL. it does not reliably detect hCG degradation products, including free-beta hCG and beta core fragments. Quantitative assays used to detect hCG may detect hCG degradation products and therefore may disagree with the results of this rapid test.

5. 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. Therefore, the presence of hCG in

serum or urine specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.

6. 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.

7. 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.

11. METHOD VALIDATION:

August 2019

12. REFERENCES:

hCG Combo Pregnancy Test Cuvette (Serum/Urine) product insert, 4/1/2015

13. POLICY APPROVAL:

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Scott M Wendroth MD - Laboratory Medical Director 8-30-2019