PREGNANCY TESTING
URINE AND SERUM

CLIA: Waived test (Urine)
     Non-waived test (Serum: moderate complexity)

Materials and Equipment: McKesson Medilab hCG Combo kit (McKesson Medical): store at room temperature (15 to 30°C)
                         Urine HCG controls (qUAntify, BioRad, Irvine, CA)
                         Serum HCG controls (Stanbio Laboratory, Boerne, TX)
                         Pipette (located in kit)
                         Timer
                         Specimen collection container

Specimen: Handle patient specimens as potentially infectious. Wear gloves and a lab coat.
          URINE--First morning specimen is preferable for testing (generally contains the highest concentration of hCG). Specimen must be collected in a clean, dry container. Specimens may be stored at 2-8°C for up to 48 hours. SPECIMEN MUST BE AT ROOM TEMPERATURE PRIOR TO TESTING. If sample is very cloudy and has visible precipitates, centrifuge, and test clear aliquot.
          SERUM--Collect blood specimen by venipuncture. Allow blood to clot twenty minutes and centrifuge for 10-15 minutes. Serum may be refrigerated at 2-8°C for up to 48 hours, or frozen at -20°C for up to 3 months. Do not freeze and thaw sample repeatedly. Grossly hemolyzed samples should not be used. SPECIMEN MUST BE AT ROOM TEMPERATURE PRIOR TO TESTING.

Principle: The intended use for this kit is the rapid detection of human chorionic gonadotropin (hCG) in serum and urine specimens. The test kit is used to obtain a visual, qualitative result sensitive to 25 mIU/ml in urine and serum.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placenta shortly after fertilization. HCG can be detected as early as 7 days following conception. At the time of the first missed menstrual period, hCG concentration is about 100 mIU/ml.

Medilab hCG Combo Pregnancy Test is a chromatographic immunoassay utilizing monoclonal antibodies to detect the presence of hCG. The immunologic specificity of the test kit virtually eliminates cross-reactivity interference from the structurally related glycoprotein hormones FSH, LH, AND TSH at physiological levels.

During testing, the urine or serum specimen is allowed to react with the colored antibody particles that have been coated with anti-beta hCG monoclonal antibody. The mixture then moves upward on the membrane via capillary action. For a positive result, a red-colored band with a specific antibody-HCG-antibody complex will form on the membrane in the test band region. Absence of a red-colored band in the test band region indicates a negative result. A red-colored band at the control region will always appear and serves as a procedural control. The appearance of a colored band in the control region is an indication of proper test performance.
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Procedure: Urine

1. Collect urine in clean, dry container.
2. Specimens collected at any time of day may be used; however, the first morning urine generally contains the highest concentration of hCG. See above for storage information.
3. Allow urine specimen and test kit, in the original pouch, to warm to room temperature (20-30°C) before testing. Gloves and lab coat should be worn for protection.
4. Immediately prior to testing, open kit and remove test device and pipette. Place device on clean, flat surface. Check expiration date; do not test beyond this date.
5. Label test device with patient or control identification.
6. Thoroughly mix urine or control specimen.
7. Holding pipette in VERTICAL position, draw the urine sample into the pipette and dispense 3 full drops into the sample well. Use a separate test device and pipette for each patient or control.
8. Set timer for 3 minutes.
9. Observe color development of colored lines in test and control regions. Positive results may be observed as soon as 40 seconds. To confirm negative results, the complete reaction time of 3 minutes is required. Do not interpret after 10 minutes.
10. Log patient result and quality controls (if done) on the appropriate log and enter into Orchard Harvest LIS.

Procedure: Serum

1. Collect venous blood via venipuncture in a serum separator tube (SST).
2. Allow to clot for twenty minutes, and centrifuge for 10 minutes. Serum may be refrigerated at 2-8°C for up to 48 hours, or frozen below -20°C for up to 1 year. Do not freeze and thaw sample repeatedly. Grossly hemolyzed samples should not be used. SPECIMEN MUST BE AT ROOM TEMPERATURE PRIOR TO TESTING.
3. Allow serum specimen and test kit, in the original pouch, to warm to room temperature (20-30°C) before testing. Gloves and lab coat should be worn for protection.
4. Immediately prior to testing, open kit and remove test device and pipette. Place device on clean, flat surface. Check expiration date; do not test beyond this date.
5. Label test device with patient or control identification.
6. Holding pipette in VERTICAL position, draw the serum sample into the pipette and dispense 3 full drops into the sample well. Use a separate test device and pipette for each patient or control.
7. Set timer for 3 minutes.
8. Observe color development of colored lines in test and control regions. Positive results may be observed as soon as 40 seconds. To confirm negative results, the complete reaction time of 3 minutes is required. Do not interpret after 10 minutes.
9. Log patient result and quality controls (if done) on the appropriate log and enter into Orchard Harvest LIS.
Interpretation of Results:

1. **NEGATIVE**: one red-colored band in the control region; **no** band in the test region.
2. **POSITIVE**: two red-colored bands in the test and control regions; a band in the test region may be any shade of red. Any questionable result may be resolved by ordering two quantitative hCG levels, two days apart (i.e. ectopic pregnancy or missed abortion).
3. **INVALID RESULT**: A total absence of red-colored bands in both regions or a missing control band is an indication of a procedural error or reagent deterioration. Repeat procedure using a new test device. If problem persists, call Technical Service at (866)288-7653.

<table>
<thead>
<tr>
<th>POSITIVE RESULT</th>
<th>NEGATIVE RESULT</th>
<th>INVALID RESULT</th>
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Quality Control:

1. **Urine**: positive and negative controls (Bio-Rad qUAntify urine control system) should be performed with each **new box** of test devices.
2. **Serum**: positive (low or high) and negative controls (Stanbio serum controls) are run **daily** with patients. Control results are logged on the Pregnancy Testing Log.
3. **Internal controls**: a colored band appearing in the control region is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the results window is considered an internal negative procedural control. If the test has been performed correctly and reagents are working properly, the background will clear to give a distinct result.

Limitations:

1. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by a physician after all clinical and laboratory findings have been evaluated.
2. A negative result may be due to a very dilute urine specimen (as indicated by a low specific gravity) or a specimen collected too early in the term of the pregnancy. If pregnancy is suspected, a first morning urine should be obtained from the patient 48 hours later and tested.
3. A number of conditions other than pregnancy including trophoblastic disease and certain nontrophoblastic neoplasms cause elevated levels of hCG. The test is not intended to diagnose conditions other than pregnancy.
4. Immunologically interfering substances such as those used in antibody therapy treatments may invalidate the test result.
Expected Values:

Healthy men and healthy non-pregnant women do not have detectable hCG by this method. HCG levels of 50-250 mIU/ml can be reached on the day of the first missed menstrual period. HCG levels peak about 8-10 weeks after the last menstrual period and then decline to lower values for the remainder of the pregnancy. Following delivery, hCG levels rapidly decrease and usually return to normal within days.

Sensitivity and Specificity:

1. McKesson Medical-Surgical Combo Pregnancy Test detects hCG concentration in the urine and serum equal to or greater than 25mIU/ml as indicated by a visually detectable red-colored band in the test region.
2. No cross-reaction was observed with LH, FSH, or TSH.

References:

2. Vaughn, Gail, Understanding & Evaluating Common Laboratory Tests, pp. 627 – 628, Appleton & Lange, Stamford, CT, 1999