DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCM STANDING OPERATING PROCEDURE June 10, 2002

URINE PREGNANCY TEST BY TANDEM "ICON" II HCG

IMPORTANT NOTE: Although the Tandem Icon II may not be the product available for your use, most currently used products will be similar in principle and general procedures. However, you MUST follow the manufacturer's procedural instructions found on the product insert. The following procedures may apply only in a general manner for products other than the Tandem Icon II.

1. PRINCIPLE:

a. Urine tests for confirming pregnancy are based on detecting elevated levels of human chorionic gonadotropin (HCG), a hormone, which the placenta begins to produce in increasing, amounts about 10 days after fertilization. The TANDEM ICON HCG uses monoclonal antibody technology.

b. The ICON HCG assay detects HCG in urine by using two types of antibodies that react with two different regions of an HCG molecule. The first antibody is contained in the Test Zone of the ICON II membrane; it captures and immobilizes any HCG molecules present when urine comes in contact with the coated membrane. The second antibody to HCG is chemically linked to the enzyme alkaline phosphatase and binds to another site on the immobilized HCG molecules. The HCG molecules are "sandwiched" between these antibodies at the Test Zone. During a wash step, enzyme-linked antibodies not bound to immobilized HCG molecules are removed, so that the only enzyme remaining on the Test Zone is incorporated in the HCG sandwiches. To detect this remaining enzyme, a color developer is added that becomes blue in the presence of the enzyme. The intensity of blue color is directly proportional to the amount of enzyme that was specifically bound to the membrane, proportional to the amount of HCG present in the specimen.

c. Each TANDEM ICON II HCG assay incorporates a unique feature, the Positive Reference Zone. A test result is interpreted by comparing the color development found at the Test Zone with that observed at the Positive Reference Zone. Color development produced at the Positive Reference Zone has been adjusted to a level so that specimens containing 50 mIU HCG/mL yield an equivalent or darker color. The Positive

Reference Zone serves as an internal assay control as it turns blue only if the reagents were added correctly and were performing properly.

2. SPECIMEN COLLECTION AND STORAGE:

a. Specimen of choice is a first morning urine specimen; random samples are acceptable -- at least 100 *ul* of urine is required.

b. Urine specimen should be collected in urine collection containers without preservatives.

c. Turbid specimen should be centrifuged at 1000-x g for 3 minutes, and supernatant tested.

d. Urine specimen must reach room temperature before testing.

e. If specimen cannot be assayed immediately, it can be stored at 2-8°C for up to 48 hours. For longer storage, thimerosal, an antibacterial preservative, should be added.

3. REAGENTS AND MATERIALS:

a. Test Cylinder: Membrane-linked antibody for immobilizing HCG-50.

b. Bottle A (Antibody Conjugate): Enzyme-linked antibody for sandwiching HCG; contains mouse monoclonal IgG conjugated to bovine alkaline phosphatase, protein matrix, and O.l % sodium azide.

c. Bottle B (Substrate Reagent): Color developing solution for detecting sandwich formation (light-sensitive; store in a dark area), contains O.l % sodium azide.

d. Bottle C (Wash Concentrate): For removing unbound enzyme-linked antibody, contains O.l % sodium azide.

e. Transfer pipettes.

f. Insert.

g. Reagent station.

h. TANDEM ICON II HCG Control Set (Urine): A positive and negative control will be performed each shift that patient samples are tested. Results will be documented on appropriate sheet. Must be purchased separately.

4. QUALITY CONTROL:

a. Good laboratory practices include the use of control specimens to ensure proper kit performance. Negative and positive urine controls containing HCG at concentrations of 0, 5 and 50 *mIU/ml* are available from Hybritech (Catalog #9106).

b. Both a positive and negative quality control test may be performed using the same test cylinder by first using the negative control specimen to perform procedural steps A through E, and then performing the same steps using a positive control specimen. This technique should only be used for quality control purposes where specimen values are known.

WARNING: Do not reuse a test cylinder for multiple patient samples. Although the membrane does not turn blue after use with a negative sample, this indicates that the concentration of HCG in the sample was less than 50 *mIU/ml*; it does not indicate the HCG concentration was zero *mIU/ml*. Therefore, subsequent assay of another patient sample in the same cylinder may produce false positive results because of the additive response of low levels of HCG

5. PRECAUTIONS:

ICON II HCG is intended for in vitro diagnostic use. Do not mix components from different kit lots. Certain reagents in this kit contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

6. STORAGE AND STABILITY:

a. ICON II HCG Test Cylinders, liquid reagents, and wash concentrate should be stored in the refrigerator at 2-8°C. If stored in this manner, reagents are stable until expiration date on kit.

b. Liquid reagents (Conjugate Bottle A and Substrate Bottle B) may be brought to room temperature once a day and returned to the refrigerator at the end of the shift without altering the stability of the reagents. Substrate Reagent (Bottle B) should be colorless; if it has turned blue, it must be replaced.

c. ICON II Test Cylinders may be stored at room temperature (25°C) for two months. Record the new expiration date on the exterior of the kit. At the end of the two months, all reagents should be considered expired even if longer dating appears on the kit label. For example, if the kit has 12 months dating and you elect to store the test cylinders at room

temperature, at the end of two months the entire kit is expired even though 10 months of dating appears on the kit.

d. Bring all liquid reagents and test cylinders to room temperature. Thoroughly mix each liquid reagent by gentle agitation or swirling.

e. ICON II Test Cylinders should be appropriately labeled with patient or control identifications using a soft felt tip pen or glass marking pencil.

f. Use a new transfer pipette for each specimen or control.

7. PROCEDURAL NOTES: The following procedural notes should be read carefully to ensure the best results with ICON II HCG.

a. Dispensing specimens and reagents.

(1) The specified amounts of reagents, sample, and wash are optimal. The test procedure is relatively tolerant of small variations in these amounts.

(2) The specimen tubes have been specifically designed to deliver drop sizes appropriate for ICON II HCG testing and should not be substituted except by precision pipetting.

(3) Precision pipetting may be substituted according to the following volumes:

FLUID	VOLUME
Urine specimens	50.0 *ul*
Bottle A (Antibody Conjugate)	150.0 *ul*
Wash Solution	2.0 *ml*
Bottle B (Substrate Reagent)	150.0 *ul*

(4) Specimens must be pipetted using new, unused transfer pipettes to prevent HCG carryover from high level samples.

(5) Do not allow the tip of Bottle A (Antibody Conjugate) or of Bottle B (Substrate Reagent) to touch the membrane surface. Do not interchange the caps of Bottle A and Bottle B. Pipettes and reagent bottles should be held perpendicularly to cylinder membrane from a height equal to top rim of the ICON cylinder.

(6) Direct flow of wash solution toward inner wall of test cylinder -- do not dispense directly onto filter membrane.

(7) Do not reuse test cylinders.

b. Incubation tubes.

(1) The ICON HCG Assay will field the stated sensitivity at a room temperature of 74°F (23°C). Lower temperatures will result in less color development at the end of the 2-minute incubation period.

(2) The waiting times indicated in the testing procedure are optimal. However, except for the Substrate Step 4, these times may be extended to as long as 5 minutes without significantly affecting results.

(3) Very high concentrations of HCG will give positive results in less than 2 minutes of substrate development (typically 30 seconds for levels over 1000 mIU/mL).

c. Turbid specimens. Turbidity or particulate matter in specimens slows down the rate at which fluids pass through the membrane. If flow stops, centrifuge the specimen to clarify and retest in a fresh test cylinder.

d. Very low HCG levels. ICON HCG is sufficiently sensitive to measure the very low levels of HCG (below 50 *mIU/ml*) that occur shortly after implantation. Because a percentage of these pregnancies normally terminate for natural reasons, subsequent testing may show a disappearance in the HCG level. Accordingly, very faint blue test results may require subsequent testing of a new urine specimen to confirm that HCG levels are rising and that a normal pregnancy is occurring. If results are inconclusive, test new specimen after 48 hours.

e. Batch processing. If several specimens are to be tested during one run, run each test step for all specimens and controls before proceeding to the next step. For batch testing, do not run more than 10 cylinders in one batch.

8. PROCEDURE: To perform ICON HCG, the following steps are all carried out at room temperature. Before proceeding, carefully read Section VII entitled Procedural Notes.

a. Before testing, dipstick for specific gravity. If specific gravity is ≤ l.O05 and no blue color develops, then add this comment to report: Specific gravity < l.O05, results may be invalid; please resubmit.

b. Dispense 5 drops of urine samples onto the center of the ICON HCG membrane; wait for complete drainage of sample through the membrane prior to the next reagent addition (usually 5-10 seconds).

c. Dispense 3 drops from Bottle A (Antibody Conjugate) onto the center of the ICON HCG membrane; wait for at least one minute to ensure complete drainage and antibody reaction.

d. Dispense Wash Solution onto the membrane, filling the cylinder up to the ridged fill line; wait for complete drainage of Wash Solution through the membrane prior to the next reagent addition (usually 15-30 seconds).

e. Dispense 3 drops from Bottle B (Substrate reagent) onto the center of the ICON HCG membrane; wait for 2 minutes for results to develop.

f. Stop the color development by filling the cylinder up to the fill line with Wash Solution.

g. With the indicator mark (P) facing you, observe color development at Test Zone and Positive Reference Zone.

9. RESULTS:

a. A specimen that does not produce a circular blue spot in the Test Zone is negative. A circular blue spot in Test Zone is positive for HCG, regardless of color intensity. If the Test Zone is darker than the Reference Zone, there is > 50 *mIU* HCG/*ml*; if the Test Zone is lighter than the Reference Zone, there is < 50 *mIU* HCG/*ml*.

b. For test to be valid, the positive Reference Zone must appear as a discernible blue spot within two minutes after addition of substrate. Absence of color in Positive Reference Zone indicates an invalid test.

c. If a light blue color develops over the entire membrane, inadequate washing has occurred. Random blue specks on a membrane should not be interpreted as a positive result.

10. LIMITATIONS:

Very dilute urine specimens, as indicated by low specific gravity, may not contain representative urinary HCG concentrations. While intrauterine or ectopic pregnancy is the most likely reason for the presence of HCG in urine, elevated urinary HCG concentrations unrelated to pregnancy have been reported in patients diagnosed as having chorio-carcinoma, hydatidiform mole, and some nontrophoblastic malignancies including testicular tumors, prostatic cancer, breast cancer, and lung cancer. The presence of HCG in urine as determined using

ICON HCG should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

11. REFERENCES:

a. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods. 18th ed., Philadelphia: W. B. Saunders Co, 1991.

b. Tandem Icon II HCG Package Insert, Hybritech.