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Document History:

Title: **Pregnancy Test – Beckman Coulter Icon® 25**

Site(s): **Shared Health Diagnostic Services – All**

Document #:	**110-10-14**	**Version #:**	**15**
Section:	**Clinical Biochemistry**	**Subsection:**	**General**

Written by:	T Dembinski	**Date:**	8 Dec 2019

Approved by: *(Approval on File)*	Laurel Thorlacius	**Date:**	12 April 2024
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#	Details of Revisions:	Approval:	Date:
1	New document	T Dembinski	8 Dec 2019
2	Inaccuracies detected in version 1 in procedural parts and interpretation (previous version said 50 IU/L, changed to 25 IU/L); re-formatted; appendices added; serum assay section added	L Thorlacius	08-DEC-2009
3	Redundant centrifugation processing step for urine samples removed (p.3 – 3rd bullet)	T Dembinski	30-AUG-2012
4	Centrifugation step re-instated to be consistent with current package insert procedure. Added worksheet to document Procedure control and test results.	H Malvern	15-JAN-2013
5	Added to procedure step 1 – instruction for Delphic "ICON" work list.	H Malvern	24-JAN-2013
6	Content of Doc# 110-50-07 and F110-50-07 are merged in this document, and the above are archived.	H Malvern	20-JAN-2015
7	Pg. 5 - Added comment under "REPORT FORMAT" re: male testing (as per memo from Dr. Thorlacius Apr/2015).	H Malvern	29-MAY-2016
8	Updated package insert references (pg.2). Under "Sample Collection Requirement" (pg.3), added instructions for handling urine specimens with visible precipitates. Revised the EPT program to use (pg.5)	T Dembinski	05-DEC-2016
9	Redesigned worksheet to prevent transcription errors (pg.10)	T Dembinski	10-FEB-2017
10	Removed the statement "Bloody urine samples are unsuitable for testing..." under Sample Collection Requirements (pg.4).	T Dembinski	05-APR-2017
11	Corrected kit storage temperature to 20-30 °C (pg.4). Converted patient and QC worksheets into forms.	L. Thorlacius	26 FEB-2017
12	Clarified EPT programs for all sites	H. Malvern	02-Jan-2018
13	Update urine QC material to qUAntify Plus (p.4). Added the "QC performed date" column to the QC Log sheet; removed Date Kit Was Opened column from QC log sheet	H Vakili	July 11, 2022
14	Removed a row from the Appendix 2 Table to allow the LIS labels to fit	H Vakili	Dec 12, 2022
15	Addition of qUAntify Advanced QC material to section 10.0	N Landry	12-APR-2024

1.0 REFERENCES:

1.1 Beckman Coulter Inc. Icon® 25 Product Instructions (kit insert) for Immunochemical Test for hCG in Urine and serum, July 2014
1.2 Wilcox AJ, Baird DD, Dunson D, McChesney R, Weinberg CR, Natural limits of pregnancy testing in relation to the expected menstrual period. JAMA 2001; 286: 1759-1761

2.0 INTRODUCTION:

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by syncytiotrophoblast cells of the developing placenta shortly after fertilization. hCG is also produced by syncytiotrophoblast precursor cells and by some types of tumors. It consists of alpha and beta subunits as do TSH, FSH and LH. It is the beta subunit which differentiates hCG from these other hormones. In early pregnancy hCG acts to sustain the corpus luteum beyond its normal lifetime. In this way estrogen and progesterone production (necessary for the maintenance of the endometrium) is continued allowing pregnancy to progress. Later in pregnancy the placenta becomes able to produce adequate amounts of these hormones with a reduction in hCG synthesis.

3.0 TESTING RATIONALE:

In normal pregnancy, hCG can be detected in both urine and serum as early as 7 to 10 days after conception. hCG concentrations of pregnant women begin to rise following implantation of the fertilized egg (blastocyst) in the uterine wall. hCG levels then continue to rise very rapidly, frequently exceeding 100 IU/L by the first missed menstrual period, peaking in the 100,000-200,000 IU/L range about 10-12 weeks into pregnancy. The practical reference point for the detection of pregnancy is the first day of a woman's missed period, i.e. the day on which she expects her next period to begin. The sensitivity of this pregnancy kit is approx. 25 mIU/L in urine or serum. One of the limitations for urine testing is the wide variability in urine concentration. A first morning urine (most concentrated) specimen is therefore the preferred urine specimen. HCG can also be determined in a serum or plasma sample by this qualitative test.

The use of serum/plasma hCG measurements in the diagnosis of ectopic pregnancy should be performed using a sensitive quantitative (typically with a sensitivity limit 3 IU/L, available in a laboratory setting) assay. Similarly, a sensitive and reproducible quantitative hCG assay is required for the monitoring of early pregnancy and trophoblastic tumors.

4.0 CLINICAL APPLICATION:

Confirmation of pregnancy

5.0 PRINCIPLE:

The ICON® 25 hCG Urine kit 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 detects hCG at concentrations of 25 IU/L or greater, and is standardized to the W.H.O. 3rd International Standard. The test utilizes a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG. The assay is conducted by adding urine (or serum) to the sample well of the test device and observing the formation of coloured lines. The sample migrates via capillary action along the membrane to react with the coloured conjugate.

Positive samples react with the specific antibody-hCG-coloured conjugate to form a coloured line at the test region of the membrane. Absence of this coloured line suggests a negative result. To serve as a procedural control, a coloured line will always appear at the control line region (coated goat anti-mouse antibodies) if the test has been performed properly.

6.0 MATERIALS / EQUIPMENT:

- Reagents / Consumables Provided:
  - Beckman Coulter ICON® 25 hCG Urine Kit, Product No. 43025
    - 25 individually wrapped test devices including pipettes
    - 2 additional disposable pipettes in a zip lock bag
    - Package insert

- Equipment Required
  - Sample collection container
  - Electronic timer

7.0 STORAGE AND STABILITY:

- Store kits at room temperature (20 – 30 °C) out of direct sunlight
- Stable until expiration date stated on each foil pouch
- Do not freeze.

8.0 SAMPLE REQUIREMENTS:

Urine Assay

- A urine sample must be collected in a clean and dry container **[N.B. urine samples collected with preservatives are unacceptable and must not be used]**

- 5mL urine (1 mL minimum) of urine is required. The first morning urine is optimal because it generally contains the highest concentration of hCG; however urine samples collected at any time of day are acceptable.

- Examine urine for precipitate. If no visible precipitates are observed proceed with testing. Urine specimens exhibiting visible precipitates must be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.

- Patient samples and controls must be brought to room temp (20 – 30°C) prior to testing.

- Urine specimens may be kept up to 8 hours at room temperature.

- Urine can be stored at 2 – 8°C up to 48 hours. Do not freeze specimens

Serum Assay

- Blood should be collected into a SST or plain vacutainer tube

- Separate serum as soon as possible to avoid hemolysis

- Use clear non-hemolyzed samples only

- Serum can be stored at 2 – 8°C up to 48 hours

9.0 SAFETY PRECAUTIONS:

Use appropriate (universal) precautions in the collection, handling, storage and disposal of the specimens and used kit contents. Discard used pipettes and test cassettes in a proper biohazard container.

10.0 QUALITY CONTROL:

Bench Quality Control: *Record QC data on attached 'Pregnancy Test Kit QC Log Sheet'.*

Pregnancy testing using Icon Test Kits in the lab or ward setting requires running quality control specimens to verify validity of test kit reagents and procedure. This must be done in compliance with the following standards. Two quality control levels (a negative and a positive) must be analyzed at least once per month and always after introducing a new lot of reagents. Control results must be retained for a minimum of two years.

1. The maximum analytical run length for pregnancy testing is 1 month. *An analytical run length defines the interval between evaluation of control results.*

2. All pregnancy testing **must** be controlled with a negative and a positive control. *This is a minimum acceptable standard.*

3. Quality control specimens must be analyzed at least once during an analytical run length prior to patient testing. *There is no requirement to run controls beyond the analytical run length if patient testing is not performed.*

4. Quality control specimens must be analyzed on each new lot of reagent prior to patient testing.

5. Procedural control must be evaluated and documented on the Pregnancy test worksheet for every patient test performed.

6. Quality control results must be dated, complied, available for review and retained for a minimum of two years.

Note:

Preferred QC material for Pregnancy testing:

- Urine Pregnancy test - Use Bio-Rad qUAntify Plus controls or Bio-Rad qUAntify Advanced controls
- Serum Pregnancy test – Use the Stanbio hCG Tri-Level controls (Negative & Low Positive 25mIU/ML)

External Proficiency Program:

Each site should subscribe to the CAP Urine Qualitative hCG - CMP Program

For the sites that perform serum pregnancy (Beckman Icon 25 Method) please enroll in the CAP "SERUM – HCG" code HCG. Five 1.0 mL serum specimens / 3 shipments/year.

11.0 PROCEDURE:

Follow the steps in the table below to perform the Beckman Coulter Icon® 25 Pregnancy Test

Step	Action
1	Compile or call up a test request work list as per site procedures (e.g. for DSM labs performing this test using Delphic LIS enter HPREG or ICON). Save the compiled or printed work list with results in a binder marked "Pregnancy Test", or as retrievable computer files.

2	Test cassettes and patient samples must be at room temperature (20 – 30°C) prior to testing
3	Remove the test cassette from the foil pouch and place it on a clean, dry, level surface
4	Label the cassette with the sample ID number or patient name
5	Using one of the disposable pipettes supplied, add 3 drops of urine (or serum) to the round sample well on the test cassette. The test cassette should not be moved again until the assay is complete and ready for interpretation
6	Set the timer for 3 minutes (urine assay) or 5 minutes (serum assay)
7	Read the reaction area and record the results when the time is up. **Do not interpret beyond set development time period**

12.0 INTERFERENCE:

For analytes or substances tested and found not to interfere in this assay, please refer to the package insert included with each kit.

13.0 INTERPRETATION OF RESULTS:

Positive	The appearance of any pink-to-purple line next to the letter 'T' in the Result Window, along with a pink-to-purple Procedural Control Line next to the letter 'C'
Negative	One pink-to-purple band appears in the Procedural Control region next to the letter 'C'
Invalid	No coloured ban appears in the control region next to the letter 'C'. **Repeat the test!**

When a positive result is obtained, the specimen contains at least 25 IU/L of ßhCG. This is indicative of early pregnancy.

14.0 REPORT FORMAT:

Report results as either **POSITIVE** or **NEGATIVE** on requisition, or slot enter results into LIS

Note – For urine pregnancy test requests in males:

Attach comment: This test is for the detection of pregnancy. Serum quantitative HCG should be used for other investigations.

15.0 LIMITATION OF THE PROCEDURE*:

1. Very dilute urine samples may not contain representative levels of hCG. If a pregnancy is still suspected (with a negative result), a first morning urine sample should be recollected 48 hours later and retested.

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 urine or serum sample should be collected 24 hours later and retested.

3. Very low levels of hCG (less than 50 IU/L) are present in urine and serum samples 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 sample collected 48 hours later.

4. This test reliably detects intact hCG up to 500,000 IU/L.

5. This test detects intact hCG only. This test does not reliably detect hCG degradation products, including free-beta subunits and beta-core fragment. Therefore, this test may show reduced reactivity in urine after 8 weeks gestation. This test should not be used to monitor trophoblastic disease or post-partum patients.

6. Quantitative assays used to detect hCG may detect hCG degradation products, and therefore may disagree with the results of the ICON 25 hCG test.

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

8. 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, which may cause false positive or false negative results.

9. This test provides a presumptive diagnosis for pregnancy. A confirmed pregnancy diagnosis should only be made by a physician after all clinical laboratory findings have been evaluated.

** extracted from: Beckman Coulter Inc. Icon® 25 Product Instructions (kit insert) for Immunochemical Test for hCG in Urine and serum, April 2004*

15.0 APPENDICES:

Appendix 1 – Lab Aid Sheet
Appendix 2 – Pregnancy Test Patient Worksheet
Appendix 3 – Pregnancy Test Kit QC Worksheet

Appendix I – Pregnancy Test – Beckman Coulter Icon® 25 (Lab-aid sheet)

- Abbreviated Test Procedure & Interpretation of Test Results
- Read complete SOP before running test

Test Procedure

Allow the test device, urine or serum sample and/or controls to equilibrate to room temperature prior to testing.

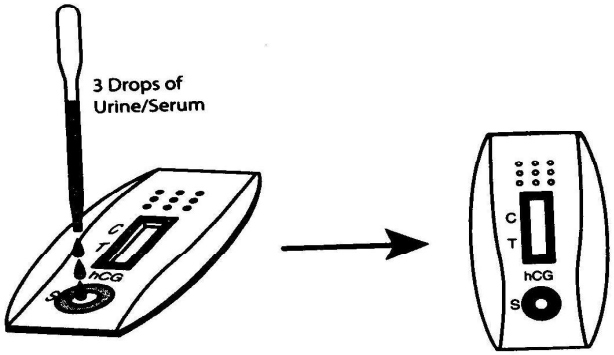
1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.

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.100 µL) to the sample well (S) of the test device, and then start the timer. Avoid trapping air bubbles in the sample well (s). See the illustration below.

3. Wait for the red line(s) to appear. It is important that the background is clear before the result is read.

    - **When testing a urine sample, read the results at 3 minutes**
    - **When testing a serum sample, read the results at 5 minutes**

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 3 minutes (urine sample) or after 5 minutes (serum sample).

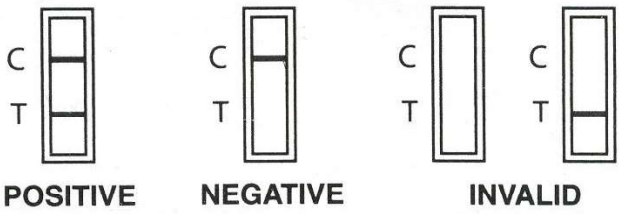


Interpretation of Test Results

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).

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 sample 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 device. If the problem persists, discontinue using the test kit immediately and contact Beckman Coulter Technical Marketing at 800-877-6242 or 650-845-3526



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

APPENDIX 2 – Pregnancy Test Patient Worksheet

SITE: _____

DATE	LIS LABEL (WITH PHN AND WARD)	Circle SAMPLE TYPE	Circle PROCEDURE CONTROL	Circle RESULT	COMMENTS	TECH INITIALS
		URINE SERUM	PASS FAIL	POS NEG		
		URINE SERUM	PASS FAIL	POS NEG		
		URINE SERUM	PASS FAIL	POS NEG		
		URINE SERUM	PASS FAIL	POS NEG		

Supervisor Review: _____ Date: _____

APPENDIX 3: Pregnancy Test Kit QC Log Sheet

SITE: _____

Date QC performed (dd/mm/yyyy)	**Pregnancy kit Lot # & Expiry date**	**Urine = U Serum = S**	Level 1 **Lot # and Expiry date**	Control Level 1 **Results:**	Level 2 **Lot # and Expiry date**	Control Level 2 **Results:**	**Comments**	**Tech Int.**

Supervisor Review: _____ Date: _____