Laboratory Name:	
Laboratory Address:	
Date of this packet:	
Insert Revision:	Sept. 25, 2013 / D

Alere™ hCG Cassette (25 mIU/mL) Test (40 Tests) Product No. 92217 Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. **Any modifications to this document are the sole responsibility of the Facility.**

CLIA Complexity: Waived

1. Intended Use

The **Alere™ hCG Cassette (25 mIU/mL)** test is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine to aid in the early detection of pregnancy.

2. Summary

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in urine as early as 7 to 10 days after conception.[1,2,3,4] hCG levels continue to rise very rapidly, frequently exceeding 100 mIU/mL by the first missed menstrual period,[2,3,4] and peaking in the 100,000-200,000 mIU/mL range about 10-12 weeks into pregnancy. The appearance of hCG in urine 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 **Alere™ hCG Cassette (25 mIU/mL)** test is a rapid test that qualitatively detects the presence of hCG at the sensitivity of 25 mIU/mL in urine. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of hCG in urine. At the level of claimed sensitivity, the **Alere™ hCG Cassette (25 mIU/mL)** test shows no cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at high physiological levels.

3. Test Principle

The **Alere™ hCG Cassette (25 mIU/mL)** test is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine 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 a urine specimen to the specimen well of the test cassette and observing the formation 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 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.

4. Specimen Collection/Treatment

Urine Assay:	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.
Specimen Storage:	Urine specimen may be stored at 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.
Handling Precautions:	All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

5. Reagents

A. Reagents and Materials Provided

Component	Content
Test cassettes	Contains mouse anti-beta hCG antibody conjugated to colloidal gold and goat anti-alpha hCG antibody coated on the membrane
Disposable pipettes	
Directional insert	

B. Materials Required But Not Provided

- Specimen collection container
- Timer

6. 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 beyond the expiration date.

7. Quality Control

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.

It is recommended that a positive hCG control (containing ≥ 25 mIU/mL hCG in urine) and a negative hCG control (containing "0" mIU/mL hCG) be evaluated to verify proper test performance with each new lot, each new shipment, monthly as a check on storage, each new untrained operator and as otherwise required by your lab internal quality system procedures.

8. Precautions

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.

- The test cassette should remain in the sealed pouch until use.
- 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.
- The test cassette should not be reused.

9. Test Procedure

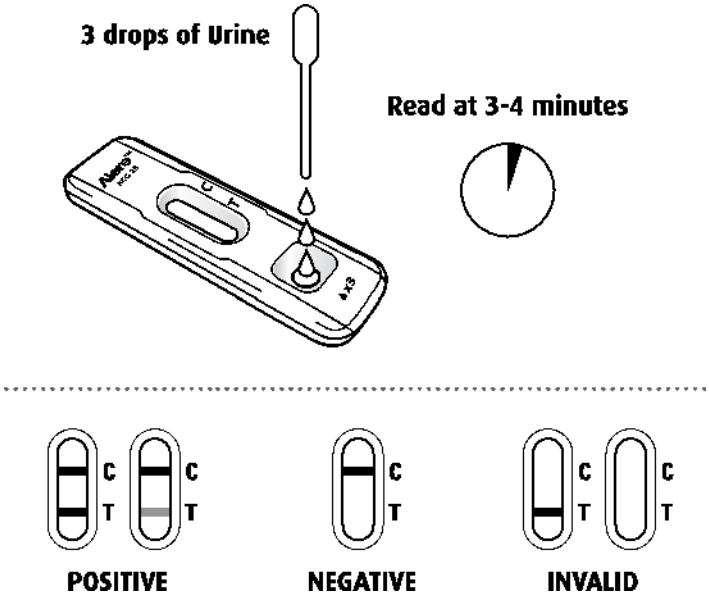
(Please refer to the illustration below.)

Allow the test cassette, urine, and/or controls 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 pipette vertically and transfer 3 full drops of urine (approx. 100 µL) to the specimen well (♦x3) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well. See the illustration.

3. Wait for the red line(s) to appear. **Read the result at 3-4 minutes. Do not interpret results after the appropriate read time.** It is important that the background is clear before the result is read.



10. Interpretation of Test Results

(Please refer to the illustration above.)

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: A sample hCG concentration below the cut-off level of this test might result in a weak line appearing in the test region (T) after an extended period of time. A line in the test region (T) seen after the read time could be indicative of a low hCG in the sample. If such results are seen, it is recommended that the test be repeated with a new sample in 48-72 hours or that an alternate confirmation method is used.

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 Alere Technical Support at 866-216-0094.

***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.

11. Limitations

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 urine specimen should be collected 48 hours later and tested.
3. Very low levels of hCG (less than 50 mIU/mL) are present in urine 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 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.[6,7] Therefore, the presence of hCG in urine specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.
6. 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.

12. Expected Values

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 Cassette (25 mIU/mL)** test has a sensitivity of 25 mIU/mL, and is capable of detecting pregnancy as early as 1 day after the first missed menses.

13. Performance Characteristics

Accuracy

A multi-center clinical evaluation was conducted comparing the results obtained using the **Alere™ hCG Cassette (25 mIU/mL)** test and another commercially available urine membrane hCG test. The study included 159 urine specimens: both assays identified 88 negative and 71 positive results. The results demonstrated 100% overall agreement (for an accuracy of > 99%) of the **Alere™ hCG Cassette (25 mIU/mL)** test when compared to the other urine membrane hCG test.

		Reference hCG Method	
		Positive	Negative
Alere™ Method	Positive	71	0
	Negative	0	88

Sensitivity and Specificity

The **Alere™ hCG Cassette (25 mIU/mL)** test detects hCG at concentration 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 hCG) specimens showed no cross-reactivity.

Interfering Substances

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

All substances listed in mg/dL unless otherwise noted.

Acetaminophen	20	Acetone	1,000
Acetylsalicylic Acid	20	Acetoacetic Acid	2,000
Ampicillin	20	Ascorbic Acid	20
Atropine	20	Albumin	2,000
ß-Hydroxybutyrate salt	2,000	Benzoylecgonine	10
Bilirubin	20	Brompheniramine	20
Caffeine	20	Cannabinol	10
Clomiphene	100	Cocaine	10
Codeine	10	Cholesterol	500
Creatine	20	Dextromethorphan	20
DMSO	5%	EDTA	80
Ephedrine	20	Ethanol	1%
Estriol	2	Estrone 3-Sulfate	10
Gentisic Acid	20	Glucose	2,000
Hemoglobin	1,000	Heroin	1
Ibuprofen	20	Methadone	10
Methamphetamine	10	Methanol	10%
Morphine	0.6	Oxalic Acid	40
Phenothiazine	20	Phenylpropanolamine	20
Pregnanediol	2	Salicylic Acid	20
Tetracycline	20	Triglycerides	1,200
Theophylline	20	Urea	2,000
Uric Acid	20		

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

14. References

1. Batzer FR. “Hormonal evaluation of early pregnancy”, *Fertil. Steril.* 1980; 34(1): 1-13
2. Catt KJ, ML Dufau, JL Vaitukaitis “Appearance of hCG in pregnancy plasma following the initiation of implantation of the blastocyte”, *J. Clin. Endocrinol. Metab.* 1975; 40(3): 537-540
3. Braunstein GD, J Rasor, H. Danzer, D Adler, ME Wade “Serum human chorionic gonadotropin levels throughout normal pregnancy”, *Am. J. Obstet. Gynecol.* 1976; 126(6): 678-681
4. Lenton EA, LM Neal, R Sulaiman “Plasma concentration of human chorionic gonadotropin from the time of implantation until the second week of pregnancy”, *Fertil. Steril.* 1982; 37(6): 773-778
5. Steier JA, P Bergsjo, OL Myking “Human chorionic gonadotropin in maternal plasma after induced abortion, spontaneous abortion and removed ectopic pregnancy”, *Obstet. Gynecol.* 1984; 64(3): 391-394
6. Dawood MY, BB Saxena, R Landesman “Human chorionic gonadotropin and its subunits in hydatidiform mole and choriocarcinoma”, *Obstet. Gynecol.* 1977; 50(2): 172-181

7. Braunstein GD, JL Vaitukaitis, PP Carbone, GT Ross “Ectopic production of human chorionic gonadotropin by neoplasms”, *Ann. Intern Med.* 1973; 78(1): 39-45.

Test Procedure Approval and Review Sheet

Prepared By:	
Date:	
Supervisor Review:	
Date:	
Laboratory Director or Designee Approval:	
Implementation Date:	
Supersedes Procedure Dated:	
Date Procedure Retired:	

Laboratory Director or Designee	Date Reviewed	Laboratory Director or Designee	Date Reviewed

Alere™ Urine hCG Cassette (25 mIU/mL) Verification Form

Account Name: _____

Address: _____

_____

Telephone: _____

Alere™ Urine hCG Cassette (25 mIU/mL) Lot #/Exp: _____

Date: _____

Supervisor Signature: _____

Record the results from reference samples below.

Record the Sample #, the **Alere™ Urine hCG Cassette (25 mIU/mL)** results, Tester's Initials, and any comments. After the **Alere™ Urine hCG Cassette (25 mIU/mL)** results have been recorded (positive or negative) then record the Expected Results (positive or negative).

Sample #	Expected Results	**Alere™ Urine hCG Cassette (25 mIU/mL)** Result	Tester's Initials	Comments

Alere™ Urine hCG Cassette (25 mIU/mL) Verification Form (continued)

Sample #	Expected Results	**Alere™ Urine hCG Cassette (25 mIU/mL)** Result	Tester's Initials	Comments

Review: _____ Date: _____

Laboratory Director Review and Approval for Clinical Use: _____

Date: _____

Alere™ Urine hCG Cassette (25 mIU/mL) External Quality Control

Name of Facility: _____

External QC testing is recommended:

- With each new lot, each new shipment, monthly as a check on storage, each new untrained operator
- When required by local, state, and/or federal regulations, accrediting groups, or your lab's Quality Control procedures

Date	**Alere™ Urine hCG Cassette (25 mIU/mL)** Kit Lot/Exp	Positive Ctrl Lot/Exp	Negative Ctrl Lot/Exp	Positive Result	Negative Result	Tester's Initials	Comments

Reviewed by: _____ Date: _____

Alere™ Urine hCG Cassette (25 mIU/mL) Quality Control and Patient Record

Lot Number _____ **Exp. Date** _____

Record the Date, Patient's Name, Patient Test Result, Internal Control Results and the performer's initials.

Positive Internal Control=the red line appearing at the "control line" position; Negative Internal Control=background color should be white to light pink.

Date	Patient Name	Patient ID Number	Patient Results	Are Internal Control Results Invalid or Valid?		Internal Control Results		Comments	Tech
				Invalid	**Valid**	**+**	**-**		

Reviewed By: _____ Date: _____

Quality Assessment Review Form and Checklist

These forms are used for periodical review of the patient testing process. These should be filed with the quality assessment records.

Quality Assessment Activity	**Comments**	**Date**	**Initials**
Patient Test Management: Evaluate criteria for specimen submission, handling, and rejection; test results requisitions and reporting, accuracy and reliability of reports.			
Quality Control: Assess calibration and control data, reference range verification, errors in reporting results, corrective actions taken with appropriate documentation records.			
Proficiency Testing: Review the effectiveness of corrective actions taken for unsatisfactory performance or failures.			
Comparison of Test Results: Review at least semi-annually comparative results for multiple methods, instruments, or site correlations when more than one procedure exists.			
Relationship of Patient Test Information to Test Results*:* Evaluate patient test reports for accuracy of patient information, test results, and normal ranges. Identify and evaluate results inconsistent with Patient's age, sex, diagnosis, and other test parameters.			
Personnel: Evaluate the effectiveness of policies and procedures for assuring employees competence of testing and reporting test results.			
Communications: Evaluate documented problems and corrective actions that occur between the laboratory and the authorized individual who orders or receives the test result.			
Complaint Investigation: Evaluate documented complaints and corrective actions.			
Quality Assessment Reviews with Staff: Document discussion with Staff regarding identified problems and corrective actions during the QA review.			

Corrective Action Form

Problem/Error	**Corrective Action**

Technologist: _____ Date: _____

Supervisor: _____ Date: _____

Laboratory Director: _____ Date: _____

TEMPERATURE LOG

Equipment: _____

Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: _____

Date	˚C	Initials	Adjustments	Date	˚C	Initials	Adjustments

Tips for Successful Proficiency Testing (PT) Performance

- Strictly follow the PT provider's storage or handling requirement ***before testing PT specimens***.
- Analyze PT specimens ***within the time frame*** provided by the PT provider.
- Contact the PT provider ***promptly*** when specimens are received damaged. You may be able to receive a replacement immediately.
- Avoid clerical error when filling out PT answer sheets. Be sure to ***enter the correct result next to the correct analyte*** on the answer form.
- Remember to identify the instrument or method you are using to perform your PT so you are ***graded among your peer group***.
- Make copies of all answer forms ***before submitting them*** to your PT provider.
- Please contact Technical Support at 877-441-7440 or Lateral.Flow.Support@alere.com for further information on proficiency providers.

Certification of Training

This is to verify that personnel responsible for running the **Alere™ Urine hCG Cassette (25 mIU/mL)** test at _____ have been thoroughly in-serviced on the test and the test procedure. This has included:

- **Review of the package insert**
- **Demonstration of the product assay**
- **Successful performance of the Alere™ Urine hCG Cassette (25 mIU/mL) test and interpretation of results**

Names of the personnel who have been trained with the **Alere™ Urine hCG Cassette (25 mIU/mL)** test and are responsible for reporting patient results:

PRINT NAME	SIGNATURE	DATE

Signature of Laboratory Director(s) responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Trainer Date

Testing Personnel Training Assessment

Test Method: Alere™ Urine hCG Cassette (25 mIU/mL)

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments / Corrective Actions
Observation of Test Performance:				
Patient Sample Preparation (if applicable)				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records:				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator:_____ Date: _____

Employee:_____

Alere™ Urine hCG Cassette (25 mIU/mL) Quiz

Name:_____

Date: _____

Circle T (True) or F (False) for each Question:

1.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test must be refrigerated at 2-8°C.	T	F
2.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test pouches may be opened one hour before the test is performed.	T	F
3.	Urine specimens may be refrigerated up to 48 hours prior to testing.	T	F
4.	Four drops of the specimen are added to the **Alere™ Urine hCG Cassette (25 mIU/mL)** test.	T	F
5.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test detection limit is 20 mIU/mL.	T	F
6.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test cassette should be at room temperature before performing a test.	T	F
7.	An **Alere™ Urine hCG Cassette (25 mIU/mL)** test cassette without a red line at the control region, and a red line at the test region, may be reported as positive.	T	F
8.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test must be read at three to four minutes.	T	F
9.	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test may be read at 20 minutes.	T	F
10.	If a red line is not visible at the control region, the **Alere™ Urine hCG Cassette (25 mIU/mL)** test result is invalid.	T	F

Alere™ Urine hCG Cassette (25 mIU/mL) Quiz Answer Key

	Answer Key	Explanation
1.	F	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test may be stored refrigerated or at room temperature 2-30°C.
2.	F	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test cassettes should remain stored in the sealed pouch until ready to use.
3.	T	Urine specimens may be refrigerated up to 48 hours prior to testing.
4.	F	Three drops of specimen should be added to the sample well using the kit pipette.
5.	F	The detection limit of the **Alere™ Urine hCG Cassette (25 mIU/mL)** test is 25 mIU/mL.
6.	T	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test cassette should be at room temperature prior to testing.
7.	F	If the red control line fails to appear, the test is invalid.
8.	T	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test must be read at 3 to 4 minutes.
9.	F	The **Alere™ Urine hCG Cassette (25 mIU/mL)** test must be read at 3 to 4 minutes.
10.	T	If the red control line fails to appear, the test is invalid.