Laboratory Name:	
Laboratory Address:	
Date of this packet:	

Sure-Vue® Serum/Urine hCG-STAT 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.**

This is a Waived Complexity test for Urine, and Moderately Complex for Serum.

I. Intended Use

The Sure-Vue® Serum/Urine hCG-STAT is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine and serum to aid in the early detection of pregnancy.

II. Test Principle

The Sure-Vue® Serum/Urine hCG-STAT is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine and serum 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 serum or urine specimen to the specimen well of the test device 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.

III. Specimen Collection/Treatment

A. Specimen:	Acceptable: Urine and serum specimens Unacceptable: Specimens from other sources.
B. Collection Container:	Urine: Clean, dry collection container. A first morning specimen is preferred; however, urines collected any time of day may be used. Serum: Collect aseptically into a clean tube without anticoagulants.
C. Specimen Storage:	Urine and serum samples may be stored at 2°-8°C up to 48 hours. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.
D. Handling Precautions:	Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.

IV. Reagents and Equipment

A. Reagents and Materials Provided

- Test Devices
- Disposable specimen droppers
- Package insert

B. Reagents and Materials not Provided

- Specimen collection container
- Timer

C. Storage and Stability

Store as packaged in the sealed pouch at 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.

V. Quality Control

Internal Procedural Controls

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.

External Procedural Controls

It is recommended that a positive hCG control and a negative hCG control be evaluated to verify proper test performance. Urine controls should be used when testing urine. It is recommended that federal, state, and local guidelines be followed.

When correct control results are not obtained, do not report patient results. Contact Technical Services at 877-441-7440.

VI. Precautions

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test device 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 device should be discarded in a proper biohazard container after testing.
- The test device should not be reused.

VII. Test Procedure

Specimen Collection and Handling:

To collect urine specimen:

- 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.

To collect serum specimen:

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

Urine and serum 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.

Test Procedure:

Allow the test device, urine or serum, and/or controls to equilibrate to room temperature (15-30°C) 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 specimen well of the test device, and then start the timer. Avoid trapping air bubbles in the specimen well.
3. Wait for the red line(s) to appear. **Read the result at 3-4 minutes when testing a urine specimen, and at 5-6 minutes when testing a serum specimen. Do not interpret the results after the appropriate read time.** It is important that the background is clear before the result is read.

VIII. 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).

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. 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 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 call 1-877-441-7440, Option 2 for Technical Assistance.

***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.

IX. 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 the 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. 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 for 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.

X. 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.

Sure-Vue® Serum/Urine hCG-STAT has a sensitivity of 10 mIU/mL in serum and 20 mIU/mL in urine and is capable of detecting pregnancy as early as 1 day after the first missed menses.

XI. Performance Characteristics

Accuracy

A multi-center clinical evaluation was conducted comparing the results obtained using the Sure-Vue® Serum/Urine hCG-STAT to another commercially available urine membrane hCG test. The study included 100 specimen and both assays identified 50 negative and 50 positive results. The results demonstrated a 100% overall agreement (for an accuracy of >99%) of the Sure-Vue® HCG Urine STAT when compared to the other Urine membrane hCG test.

Sensitivity and Specificity

The Sure-Vue® Serum/Urine hCG-STAT detects hCG at a concentration of 10 mIU/mL or greater in serum and 20 mIU/mL or greater in urine. 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 (10 mIU/mL in serum and 20 mIU/mL hCG in urine) specimens showed no cross-reactivity.

Interfering Substances

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

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

Clomiphene	100	Heroin	1	Uric acid	20

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

XII. 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
8. Sure-Vue Serum/HCG Urine STAT Package Insert

Test Procedure Review Sheet

Laboratory Name:	
Laboratory Address:	
Date of this packet:	

Supervisor	Date Reviewed	Supervisor	Date Reviewed

Sure-Vue® Serum/Urine hCG-STAT Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Sure-Vue® Serum/Urine hCG-STAT Lot #: _____

Date: _____

Supervisor Signature: _____

Record the results from reference samples below.

Record the Sample #, the Sure-Vue® Serum/Urine hCG-STAT results, Tester's Initials, and any comments. After the Sure-Vue® Serum/Urine hCG-STAT results have been recorded (positive or negative) then record the Expected Results (positive or negative).

Sample #	Expected Results	Sure-Vue® Serum/Urine hCG-STAT Result	Tester's Initials	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Sure-Vue® Serum/Urine hCG-STAT Validation Form

Sample #	Expected Results	Sure-Vue® Serum/Urine hCG-STAT Result	Tester's Initials	Comments
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Reviewed By: _____

Sure-Vue® Serum/Urine hCG-STAT Quality Control

Name of Facility: _____

Use this coversheet with each new shipment.

Sure-Vue® Serum/Urine hCG-STAT Card Kit Lot# _____ Expiration Date_____

Date Received_____ Received By Whom_____

	Date	Kit Positive Control	Kit Negative Control	Performer's Initials
1st week open date				
2nd week (if applicable)				
3rd week (if applicable)				
4th week (if applicable)				
5th week (if applicable)				
6th week (if applicable)				

Reviewed by: _____ Date: _____

Sure-Vue® Serum/Urine hCG-STAT Quality Control and Patient Record

Lot Number _____ Exp. Date _____

Alere recommends that external positive and negative controls be run for each new lot.

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 clear within 10 minutes.

Date	Patient Name	Patient ID Number	Patient Results	Internal Control		Comments	Tech
				+	-		

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.

Date/ Initials	**Quality Assessment Activity**	**Comments**
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

Laboratory Performer: _____ Date: _____

Laboratory Director: _____ Date: _____

Tips for Successful 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.

Certification of Training

This is to verify that personnel responsible for running the Sure-Vue® Serum/Urine hCG-STAT 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 Sure-Vue® Serum/Urine hCG-STAT Test and interpretation of results**

Names of the personnel who have been trained with the Sure-Vue® Serum/Urine hCG-STAT Test and are responsible for reporting patient results:

PRINT NAME	SIGNATURE	DATE

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

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Trainer 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

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

TEMPERATURE LOG

Equipment: Lab Refrigerator

Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: 2-8°C

Date	°C	Initials	Adjustments	Date	°C	Initials	Adjustments

Test Procedure Review

Supervisor	Date Reviewed	Supervisor	Date Reviewed

Sure-Vue® Serum/Urine hCG-STAT
Competency Assessment

We have provided you with a written quiz that can be administered to all testing personnel as part of their competency assessment.

A Competency Assessment Checklist has been created that can be used to verify and document that all areas of competency for the Sure-Vue® Serum/Urine hCG-STAT have been evaluated.

Sure-Vue® Serum/Urine hCG-STAT Quiz Answer Key

1.	F	The SureVue® hCG Serum/Urine STAT kit may be stored at refrigerated or at room temperature (2-30°C).
2.	F	The SureVue® hCG Serum/Urine STAT test cassettes should remain stored in the pouch until ready to test.
3.	T	The urine and serum specimens may be refrigerated up to 48 hours prior to testing.
4.	F	Three drops of the specimen should be added to the sample well using the kit pipette.
5.	F	The detection limit of the SureVue® hCG Serum/Urine STAT kit is 10 mIU/mL for serum and 20 mIU/mL for urine.
6.	T	The SureVue® hCG Serum/Urine STAT 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 SureVue® hCG Serum/Urine STAT test may be read at 3 minutes for urine specimens and 5 minutes for serum specimens.
9.	F	The SureVue® hCG Serum/Urine STAT test should not be read after 4 minutes for urine and 6 minutes for serum.
10.	T	If the red control line fails to appear, the test is invalid**.**

Testing Personnel Competency Assessment

Test Method: Sure-Vue® Serum/Urine hCG-STAT Test

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Actions
Observation of Test Performance:				
Patient 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:_____

Sure-Vue® Serum/Urine hCG-STAT Quiz

Name:_____
Date: _____

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

1.	The Sure-Vue® Serum/Urine hCG-STAT cassette must be refrigerated at 2-8°C.	T	F
2.	The Sure-Vue® Serum/Urine hCG-STAT test pouches may be opened 1 hour before the test is performed.	T	F
3.	Urine and serum sample may be refrigerated up to 48 hours prior to testing.	T	F
4.	Four drops of the specimen are added to the Sure-Vue® Serum/Urine hCG-STAT test.	T	F
5.	The Sure-Vue® Serum/Urine hCG-STAT test detection limit is 20 mIU/mL for both serum and urine specimens.	T	F
6.	The Sure-Vue® Serum/Urine hCG-STAT test cassette should be at room temperature before performing a test.	T	F
7.	A Sure-Vue® Serum/Urine hCG-STAT 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 Sure-Vue® Serum/Urine hCG-STAT test may be read at 3 minutes for urine specimens and 5 minutes for serum specimens.	T	F
9.	The Sure-Vue® Serum/Urine hCG-STAT test may be read at 20 minutes	T	F
10.	If a red line is not visible at the control region, the Sure-Vue® Serum/Urine hCG-STAT test result is invalid.	T	F