PROCEDURE MANUAL

Lab Name: **Procedure #:**

Procedure: CLIA Complexity: WAIVED

Prepared By	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10). Prepared in accordance with the guidelines recommended by the Clinical and Laboratory Standards Institute, Wayne, PA 19087; CLSI Document GP2-A2.

Clarity Diagnostics LLC provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory

CLIA Complexity: WAIVED

INTENDED USE

The Clarity Pregnancy One Step Rapid Test (Cassette) is an in vitro diagnostic visual qualitative immunochromatographic assay designed for the rapid determination of human chorionic gonadotropin (hCG) in urine to aid in the detection of pregnancy. For professional use and OTC self test use.

SUMMARY AND EXPLANATION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced in pregnancy that is made by the developing embryo after conception and later by the syncytiotrophoblast. In normal pregnancy, hCG can be detected in urine as early as 1-2 weeks after conception. hCG levels continue to rise very rapidly, usually exceeding 100 mIU/mL by the first missed menstrual period, and peaking in the 100,000-200,000 mIU/mL range about 10-12 weeks into pregnancy. Early pregnancy testing, in general, is based on the detection or measurement of hCG. The Clarity Pregnancy One Step Rapid Test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of hCG in urine. Positive specimens react with the specific antibody-hCG-colored conjugate to form a colored line at the test line region of the membrane. Absence of this colored line suggests a negative result. At the level of claimed sensitivity, the Clarity Pregnancy One Step Rapid Test shows no cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at high physiological levels.

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PRINCIPLE OF THE TEST

Human chorionic gonadotropin (hCG) is a hormone, produced by the developing placenta shortly after the conception and secreted into the urine. The pregnancy test contains antibodies which specifically react with this hormone.

Capillary action carries the specimen to migrate along the membrane. When hCG in the sample reaches the Test Zone region of the membrane, it will form a colored line. Absence of this colored line suggests a negative result.

To serve as a procedure control, a colored line will appear at the control zone region, if the test has been performed properly.

REQUIRED MATERIAL - PROVIDED

1. One pregnancy test pouch, containing a cassette, dropper, and desiccant. The desiccant is for storage purposes only, and is not used in the test procedures.
2. One package insert.

REQUIRED MATERIAL – NOT PROVIDED

1. Clock or timer.
2. Specimen collection containers.
3. Gloves.

WARNINGS AND PRECATIONS

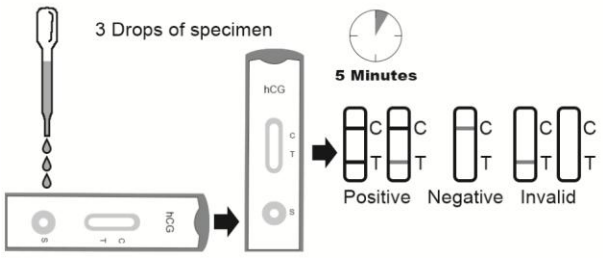
1. This test is designed for "*in vitro diagnostic*" use.
2. Read instructions carefully before using this test.
3. This kit is for external use only. Do not swallow.
4. Do not use the test kit beyond the expiration date.
5. Do not use the kit if the pouch is punctured or is not well sealed.
6. Keep out of the reach of children.
7. Urine specimens may be infectious; Insure proper handing and discard all used devices according to local regulations.
8. The test is for single use only. Do not reuse it.

COLLECTION AND STORAGE OF SPECIMENS

Any urine specimen is appropriate for pregnancy testing but the first morning urine specimen is optimal because of its highest concentration of HCG. Urine should be collected in a clean container prior to testing.

ASSAY PROCEDURE

1. Remove a Testing Device and dropper from the foil pouch by tearing at the notch then place it on a level surface, and use it as soon as possible.
2. Holding the Sample Dropper vertically, add exactly 3 drops of urine specimen to the sample well marked S.
3. Read results at 5 minutes and no later than 10 minutes.



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READING TEST RESULTS

Positive (pregnant):
Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T). This means you are probably pregnant.

Negative (not pregnant):
One red line appears in the control region (C).No apparent red or pink line appears in the test region (T).This means you are probably not pregnant.

Invalid:
The result is invalid if no red line appears in the control region (C), even if a line appears in the test region (T). You should repeat the test with a new strip.

NOTE: If the test line is weak, it is recommended that the test be repeated in 48 hours.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. You should not rely on results of pregnancy tests alone to determine clinical status.
2. If a urine sample is too dilute (ie, low specific gravity) it may not contain a representative level of hCG. If pregnancy is still suspected, another urine specimen should be collected 48 hours later and tested.
3. Low concentration of hCG in a very early pregnancy can give a negative result. In this case, another specimen should be obtained at least 48 hours later and tested.
4. Elevated levels of hCG can be caused by a few conditions other than pregnancy. Therefore, the presence of hCG in urine specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.
5. Certain health conditions, such as an ovarian cyst or ectopic pregnancy (pregnancy outside the uterus), can cause a false or irregular results.
6. Prescribed treatments containing hCG may cause false results.
7. The test is for early detection of pregnancy only.
8. Positive results from very early pregnancy may later prove negative due to natural termination of the pregnancy.
9. Elevated levels of hCG have been found in patients with gestational and non-gestational trophoblastic disease. Where appropriate, these conditions should be ruled out before a diagnosis of pregnancy is reached.
10. Abnormal pregnancy (e.g. ectopic) cannot be distinguished from normal pregnancy based on hcg measurements alone.
11. Negative results obtained before the time of the expected period maybe false negative results.
12. Drugs which contain hcg (such as Pregnyl, Profasi, Pergonal, APL) can give false positive result.

EXPECTED RESULTS

Negative results are expected in healthy non-pregnant women. Healthy pregnant women have hCG present in their urine specimens. The amount of hCG will vary greatly with gestational age and between individuals. The Clarity Pregnancy One Step Rapid Test has a sensitivity of 25 mIU/mL.

PERFORMANCE CHARACTERISTICS

Detection limit:

The sensitivity of the device was tested by spiking 81 negative urine serum samples (from non-pregnant females or males) with varying concentrations (15, 20, 25, 30 and 35 mIU/mL) of hCG (Genway, Catalog: 20-783-70022). The result is traceable to the WHO International Standard 4th of HCG (25 mIU/mL). The test shows a cut- off of 25mIU/mL of HCG. Results are summarized below:

		Result	
		+	-
HGC concentration (mIU/mL)	15	0	81
	20	3	78
	25	37	44
	30	80	1
	35	81	0

Linearity/assay reportable range:

Hook effect was evaluated by spiking high hCG concentrations (50 IU/mL, 100 IU/mL, 200 IU/mL, 300 IU/mL and 500 IU/mL) into negative urine samples and evaluating the test result line of 3 lots. Results indicate that for urine samples with HCG > 300 IU/mL, hook effect can be found.

Potential interference and cross reactors:

An interference study was carried out by adding known amounts of potential interfering substances to urine samples that contains 5 and 50 mIU/mL of hCG, and evaluated the test result lines. No interference was observed for the following compounds at the concentrations added: Acetaminophen 21.16 mg/dL; Acetylsalicylic acid 64.8 mg/dL; Ascorbic acid 6340 µg/dL; Atropine 926.1 µg/dL; Caffeine 62140.4 µg/dL; Tetracycline 1777.6 µg/dL; Ampicillin 5.94 mg/dL; Bilirubin 18.71 mg/dL; Hemoglobin 2.0 g/L; Glucose 1000 mg/dL; and Protein 120 g/L and Albumin 60 g/L.
No cross-reactivity was observed for urine samples up to the following concentrations: LH (300 mIU/mL), FSH (500 mIU/mL), TSH (200 miU/mL) and β-Core HCG (200 µg/mL).
Potential interfering factors of sample conditions and testing environment (sample pH, sample SG and testing temperature) were also evaluated. No influence was found in the studied range.

Potential interference and cross reactors:

Reproducibility of the device was evaluated by testing negative urine samples spiked with hCG (to 15 mIU/mL, 20 mIU/mL, 25 mIU/mL, 30 IU/mL and 35 IU/mL hCG) run 27 times within run with 3 lots of test strips (total N = 81). The results are summarized below:

		Site						Total	
		1		2		3			
		+	-	+	-	+	-	+	-
hCG concentration (mIU/mL)	15	0	27	0	27	0	27	0	81
	20	0	27	1	26	2	25	3	78
	25	11	16	13	14	13	14	37	44
	30	26	1	27	0	27	0	80	1
	35	27	0	27	0	27	0	81	0

Accuracy

A clinical trial was conducted in 3 sites, using specimens from one hundred and sixty-seven (167) women enrolled to participate in the study. The study was designed to demonstrate correlation between the Clarity Pregnancy One Step Rapid Test and another Home Pregnancy test. Study participants were

asked to retain urine samples and provide them to the study coordinator, which are divided into 5 panels based on their clinical situations:

Panel	Clinical situations	Number of Participants
A	Female patients not pregnant and are age 18-40	30
B	Premenopausal patients not pregnant and are age 40-55;	27
C	Patients suspected to be pregnant from missing of expected period.	56
D	Patients in very early pregnancy (0-30 days),	27
E	Pregnant patients in the first trimester (31-100days).	27

The study coordinator tested each sample using both the Clarity Pregnancy One Step Rapid Test and the predicate test.

		Predicate device		
		Positive	Negative	Total
Test device	Positive	72	1	73
	Negative	3	91	94
	Total	75	92	167

Agreement= (163/167)*100%= 97.6% (95% C.I. = 95%-100%)

The possible reasons of discrepant results may lie in the following aspects:

a. The concentrations of some specimens were around the cutoff and the colors of the test lines were light and vague, which lead to different result interpretation from different technicians;

b. The concentrations of some specimens were around the cutoff and the volumes of the specimens added to the devices might be slightly different (from 2 to 3 drops), which lead to different color thickness;

c. Any difference between the predicate and our device, which may have influence on result reading in an unknown and complicated way (e. g. raw material, structure design and manufacturing method).

BIBLIOGRAPHY

L. A. Bastian, K. Nanda, V. Hasselblad, and D. L. Simel, Diagnostic Efficiency of Home Pregnancy Test Kits: A Meta-analysis. Arch Fam Med, September 1, 1998; 7(5): 465 - 469.

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CLIA '88 WAIVED CLIA '88

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LOG SHEET

Record Built-in Procedural Controls on the first patient tested each day.

	Date	Patient Name	Positive Procedural Control	Test Results Read at 5 minutes Do not read the test results after 10 (Ten) minutes	Lot Number and Exp. Date	Technician
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

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LOG SHEET

	Date	External Positive Control Result	External Negative Control Result	Lot Number and Exp. Date	Technician
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					