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One-Step Pregnancy Test Device Urine
Package Insert
Catalog Number: APT-121

A rapid and sensitive one-step test for the qualitative detection of human chorionic gonadotropin (hCG) in urine.

For Professional In Vitro Diagnostic Use Only

Intended Use

The *AZOG* hCG One-Step Pregnancy Test Device (Urine) is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in urine to aid in the early detection of pregnancy.

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 both urine and serum as early as 7 to 10 days after conception (1-4). hCG levels continue to rise rapidly, frequently exceeding 100 mIU/mL by the first missed menstrual period (2-4), and peaking in the 100,000-200,000 mIU/mL range about 10-12 weeks into pregnancy. The appearance of hCG in both urine and serum 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 *AZOG* One-Step Pregnancy Test Device (Urine) is a rapid test that qualitatively detects the presence of hCG in urine specimen at the sensitivity of 25 µIU/mL. 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 AZOG One-Step Pregnancy Test Device (Urine) shows no cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at high physiological levels.

Principle

The AZOG One-Step Pregnancy Test Device (Urine) 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 caprine polyclonal alpha hCG antibody and a monoclonal hCG antibody specific to the beta subunit of hCG to selectively detect elevated levels of hCG. The beta-hCG is conjugated to colloidal gold and the alpha-hCG is immobilized on the test region. The assay is conducted by adding a 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 dried conjugate of colloidal gold-monoclonal antibody. The urine reconstitutes the dried conjugate. If hCG is present in the sample, it will react with the monoclonal antibody to form a complex of colloidal gold-monoclonal antibody-hCG. This complex migrates up the membrane strip chromatographically and through the band of immobilized goat anti-hCG (alpha) antibody. Because the immobilized goat anti-hCG (alpha) is ale to bind to the hCG molecule of the migrating complex, a visible reddish band is formed along the exact location of the immobilized goat anti-hCG (alpha) antibody. If there is no hCG present in the urine sample, the colloidal gold-monoclonal antibody conjugate will pass through the immobilized hCG (alpha) band and no colored line will form – a negative result.

Further up the membrane, pass the anti-hCG test region, is a control region consisting of a band of immobilized goat anti-mouse IgG. This band of antibody will bind only conjugate and form a colored line, regardless of whether hCG is present in the urine or not. Appearance of the control line assures reagent integrity as well as correct testing procedure.

Reagents

The test device contains monoclonal (murine) anti-hCG coated particles and polyclonal (caprine) anti-hCG coated on the membrane.

Precautions

- For professional *in vitro* diagnostic use only. Do not use test after the expiration date.
- Do not remove test device from the sealed pouch until ready to perform test.
- All specimens should be considered potentially hazardous and handled in the same manner as infectious agents.
- The test should be discarded in proper biohazard container after testing.

Storage & Stability

Store as packaged in the sealed pouch at 4-30°C, out of direct sunlight. **DO NOT FREEZE.** The test device is stable until the expiration date printed on the sealed pouch. The test must remain in sealed pouch until use. Do not use beyond the expiration date.

Materials

Materials Provided

- Test device in desiccated pouch
- Disposable specimen dropper
- Package insert

Materials Required But Not Provided

- Specimen collection container
- Timer
- External controls

Specimen Collection & Preparation

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

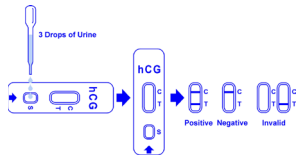
Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

Directions for Use:

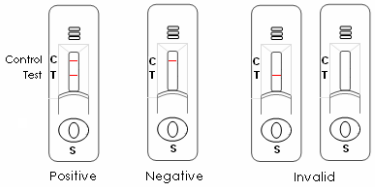
Allow the test device, urine specimen and/or controls to equilibrate to room temperature (4-30°C) before testing.

1. Bring the pouch to room temperature before opening it. Open pouch at notch. Remove the test device from pouch and use 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 (approx. 100µl) to the specimen well (S) of the test device, and then start the timer and wait for the red line(s) to appear. Avoid trapping air bubbles in the specimen well (S). See the illustration below.



3. Wait for the red line(s) to appear. The results should be read at 3 minutes. It is important that the background is clear before the result is read.



Note: A low hCG concentration may result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 10 minutes.

Interpretation of 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).

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 immediately and contact your local distributor.

NOTE: The intensity of the reddish 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.

Quality Control

A procedural control is 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 also required.

It is recommended that a positive hCG control (containing 25-250 mIU/mL hCG) and a negative control (containing '0" mIU/mL hCG) be evaluated to verify proper test performance when a new shipment of test devices is received.

Users should follow their federal, state or local and laboratory guidelines concerning frequency for running external controls.

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. If 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 fertilization. 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. A number of conditions other than normal pregnancy, including, trophoblastic disease and certain non-trophoblastic neoplasms including testicular tumors, prostate cancer, breast cancer and lung cancer can cause elevated hCG (6-7). Therefore, the presence of hCG in urine should not be used to diagnose pregnancy unless these conditions have been ruled out by a physician.
5. 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.

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 *AZOG* One-Step Pregnancy Test Device (Urine) has a sensitivity of 25 mIU/mL, and is capable of detecting pregnancy as early as 1 day after the first day of missed menses.

Performance Accuracy

Method Comparison

A clinical evaluation was conducted comparing the results obtained using the *AZOG* One-Step Pregnancy Test Device (Urine) to another commercially available urine membrane hCG test. The study included 150 urine specimens. Both assays identified 76 negative and 74 positive results. The results demonstrated 100% overall agreement (for a percent concordance of $\geq$ 99%) of the AZOG One-Step Pregnancy Test Device (Urine) when compared to the other urine membrane hCG test.

Reference hCG Method

		Positive	Negative
AZOG	**Positive**	**74**	**0**
Method	**Negative**	**0**	**76**

Sensitivity and Specificity

The *AZOG* One-Step Pregnancy Test Device (Urine) detects hCG at a concentration of 25 mIU/mL and greater. The test has been standardized to the W.H.O. Third International Standard. The addition of LH (1000 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.

Acetaminophen	20 mg/mL
Acetylsalicylic Acid	20 mg/mL
Albumin (human)	2 g/dL
Ascorbic Acid	20 mg/mL
Atropine	20 mg/mL
Bilirubin	2 mg/dL
Caffeine	20 mg/mL
Estriol 17-Beta	1.4 mg/dL
Ethanol	1%
EDTA	80 mg/dL
Ephedrine	20 mg/dL
Gentisic Acid	20 mg/mL
Glucose	2 g/dL
Hemoglobin	1 mg/dL
Pregnanediol	1.5 mg/dL
Salicylic Acid	20 mg/dL

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

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PI-00002

	CE		
IVD		4°C 30°C	
			EC REP
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