

NCCLS

**Accustrip®
hCG Pregnancy Test**

(Catalog No. PF-51A150)

One Step Pregnancy Test

For Professional and In Vitro Diagnostic Use Only

**A Rapid One Step, Visual Test for the
Qualitative Detection of
Human Chorionic Gonadotropin
in Human Urine**

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This procedural bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *In Vitro* DIAGNOSTICS USE to provide a complete package insert in accordance with the format and specification recommended by the National Committee for Clinical Laboratory Standards, Villanova, PA 19085; this bulletin is intended for use in conjunction with the Physicians Office Laboratory Procedural Manual, NCCLS Document POL2-T.

Accustrip® hCG Pregnancy Test
One-Step Test

For Professional and In Vitro Diagnostic Use Only

A Rapid One Step, Visual Test for the
Qualitative Detection of
Human Chorionic Gonadotropin
in Human Serum or Urine

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INTENDED USE

For the rapid detection of human chorionic gonadotropin (hCG) in urine specimens. This test kit is used to obtain a visual, qualitative result and is intended for professional and laboratory use only.

BACKGROUND

Summary

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in serum as early as 7 days following conception.⁽¹⁻⁴⁾ The concentration of hCG continues to rise rapidly, frequently exceeding 100 mIU/ml by the first missed menstrual period.⁽¹⁻⁴⁾ and peaking in the 30-200,000 mIU/ml range about 10-12 weeks into pregnancy. The appearance of hCG soon after conception, and its subsequent rise in concentration during early gestational growth, make it an excellent marker for the early detection of pregnancy.

The Jant Pharmacal Accustrip® hCG Pregnancy Test is a rapid test to detect the presence of hCG in urine specimens in a qualitative format sensitive to 20 mIU/ml. The test utilizes monoclonal antibody reagents to selectively detect elevated levels of hCG in urine. The immunological specificity of the test kit virtually eliminates cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at physiological levels.

Principle of the Test Procedures

The Accustrip® hCG Pregnancy Test is a qualitative, two-site sandwich immunoassay (ref.5-6) for the determination of human chorionic gonadotropin (hCG) in urine. The membrane is pre-coated with anti-hCG capture antibody on the test band region and goat anti-mouse on the control band region. During testing, the urine specimen is allowed to react with the colored conjugate (mouse anti-hCG monoclonal antibody-colloidal gold conjugate) that has been pre-dried on the test strip. The mixture then moves upward on the membrane chromatographically by capillary action. For a positive result, a pink-colored band with the specific antibody-hCG-colored conjugate complex will form in the test region of the membrane. Absence of this pink-colored band in the test region suggests a negative result. Regardless of the presence of hCG, as the mixture continues to move across the membrane to the immobilized goat anti-mouse, a pink-colored band at the control region will always appear. The presence of this pink-colored band serves as: 1) verification that sample volume is sufficient, and 2) that proper flow is obtained.

SPECIMEN COLLECTION

URINE SPECIMEN

Method of collection	The urine specimen must be collected in a clean, dry container.
Volume of specimen required	Approximately 2 ml
Special patient preparation requirements	Specimens collected at any time of day may be used; however, the first morning urine generally contains the highest concentration of hormone. Fresh urine does not require any special pretreatment.
Handling Conditions of specimen	Urine specimens may be refrigerated (2-8°C) and stored up to 72 hours prior to testing. If samples are refrigerated, they must be equilibrated to room temperature before testing.
Criteria	Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle, and clear aliquots are obtained for testing.

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MATERIALS

Materials Provided

- 50 individually wrapped test strips. Each test strip contains an anti-alpha hCG capture antibody coated membrane and colloidal gold particles coated with mouse anti-beta hCG monoclonal antibody.
- One instruction insert

Materials Required but Not Provided

- Specimen collection container
- Timer

Storage and Stability

The test kit is to be stored at refrigerated (2-8°C) or room temperature (up to 30°C) in the sealed pouch for the duration of the shelf life.

TEST PROCEDURE

Review “Specimen Collection” instructions. Test strip, patient’s samples and controls should be brought to room temperature (20-30°C) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the test strip from its protective pouch (bring the test to room temperature before opening the pouch to avoid condensation of moisture on the membrane).
2. Immerse the test strip in the urine sample with the arrow end pointing toward the urine. Do not immerse the strip above the printed MAX (maximum) line. After a minimum of 15 seconds, remove the test strip from the urine and lay flat on a non-absorptive clean surface. Alternatively, the test strip may be left in the test sample, as long as the strip is not immersed above the MAX line. A separate test strip must be used for each sample or control.
3. Wait for pink-colored bands to appear. Depending on the concentration of hCG, positive results may be observed as soon as 60 seconds. However, to confirm negative results, the complete reaction time of 5 minutes is required. Do not interpret results after 10 minutes.

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INTERPRETATION

1. **Positive:** Two distinct pink-colored bands will appear, one in the test region and one in the control region.
2. **Negative:** Only one pink-colored band appears in the control region. No apparent pink band appears in the test region.
3. **Invalid:** A total absence of pink-colored bands in both regions. If neither a test band nor a control band appears on the membrane, the test should be considered void. Improper testing procedures or deterioration of reagents probably occurred.

Notes on the interpretation of results

Negative test results in patients suspected to be pregnant should be retested with a sample obtained 48 to 72 hours later, or by performing a quantitative assay. When testing with a urine specimen, the first morning specimen generally contains the highest concentration of hCG.

- The shade of pink in the test region (T) will vary depending on the concentration of hCG present. However, neither the quantitative value nor the rate of increase in hCG can be determined by a qualitative test.

LIMITATIONS

1. A number of conditions other than pregnancy, including trophoblastic disease and certain nontrophoblastic neoplasms may cause elevated levels of hCG. These diagnoses should be considered if appropriate to the clinical evidence.
2. If a urine specimen is too dilute (i.e., low specific gravity) it may not contain representative levels of hCG. If pregnancy is still suspected, a first morning urine should be obtained from the patient 48-72 hours later and tested.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. Immunologically interfering substances such as those used in antibody therapy treatments may invalidate the test result.
5. Some samples containing very high levels of hCG (~ 600,000 mIU/ml) may yield a test band with color intensity lighter than what is expected. When high dose "hook effect" is suspected a 1:10 dilution of the specimen with DI H₂O is recommended. The test should then be repeated with the diluted specimen.

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QUALITY CONTROL

A procedural control is included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the result area is considered an internal negative procedural control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result.

It is recommended that two levels of control specimens be used with each new kit. For this purpose, we recommend the use of the Accustrip® hCG (Positive and Negative) controls. However, each laboratory should follow their state and local requirements.

COMMENTS AND TECHNICAL ASSISTANCE

Expected Values

Healthy men and healthy non-pregnant women do not have detectable levels of hCG by the Accustrip® hCG Pregnancy Test. HCG levels of 100 mIU/ml⁽⁴⁾ can be reached on the day of the first missed menstrual period. HCG levels peak about 8-10 weeks after the last menstrual period and then decline to lower values for the remainder of the pregnancy. Following delivery, hCG levels rapidly decrease and usually return to normal within days after parturition.

Standardization

The Accustrip® hCG Pregnancy Test has been standardized to the World Health Organization First International Reference Preparation/3rd International Standard (Code# 75/537).

Technical Service Representative

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PERFORMANCE CHARACTERISTICS

Sensitivity

The Accustrip® hCG Pregnancy Test detects hCG concentrations in urine samples equal to or greater than 20 mIU/ml as indicated by the development of a pink-colored band in the test region. Additionally, samples containing less than 20 mIU/ml may also produce a faint positive result. To evaluate the sensitivity of the Accustrip® hCG Pregnancy Test at low levels of hCG, the following experiments were carried out:

Urine samples from 100 known non-pregnant subjects were spiked with hCG to the concentrations of 0, 10, 20, 40, 100 mIU/ml. The samples were blind labeled and tested with the Accustrip® hCG Pregnancy Test. Results are summarized in Table 1.

Table 1

hCG Concentration (mIU/ml)	0	10	20	40	100
Number of Samples	20	20	20	20	20
Number of Positives	0	15	20	20	20
Number of Negatives	20	5	0	0	0

Specificity

The specificity of the Accustrip® hCG Pregnancy Test was determined from cross-reactivity studies with known amounts of Luteinizing Hormone (hLH), Follicle Stimulating Hormone (hFSH), and Thyroid Stimulating Hormone (hTSH). Negative results were obtained from all tests conducted with 300 mIU/ml hLH, 1000 mIU/ml hFSH and 1000 µIU/ml hTSH.

Accuracy

Correlation with Qualitative Visual Tests Urine: 102 randomly selected urine samples were analyzed with the Accustrip® hCG Pregnancy Test procedure in parallel with a commercially available qualitative visual hCG test. The results indicated complete agreement (57 positive samples and 45 negative samples).

Additionally, 40 urine samples at the hCG concentrations of 0, 20, 40, 80, 100 mIU/ml were blind labeled and tested with the Accustrip® hCG Pregnancy Test at three doctor's offices and one medical laboratory. The results from this study gave 100% agreement with the expected results.

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Interference Testing

The following substances were added to hCG-free and 50 mIU/ml hCG-spiked urine samples. At the concentrations tested, none of the substances interfered in the assay.

Acetaminophen	20 mg/dl
Acetylsalicylic Acid	20 mg/dl
Ascorbic Acid	20 mg/dl
Atropine	20 mg/dl
Caffeine	20 mg/dl
Gentesic Acid	20 mg/dl
Glucose	2 g/dl
Hemoglobin	1 mg/dl

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