NCCLS

Accutest®
Rapid Urine/Serum Pregnancy Test
(Catalog No. PF-58A240)

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 Serum or Urine
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Accutest® Rapid Urine/Serum Pregnancy Test
Catalog No. PF-58A240

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.

Accutest® Rapid Urine/Serum Pregnancy Test
One-Step Cassette 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
INTENDED USE

For the rapid detection of human chorionic gonadotropin (hCG) in serum and 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 \(^{(2-4)}\) and peaking in the 30-200,000 mIU/ml range by 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 Accutest® Rapid Urine/Serum Pregnancy Test is a rapid test to detect the presence of hCG in a qualitative format sensitive to 20 mIU/ml. The test utilizes monoclonal antibodies to selectively detect elevated levels of hCG in serum or urine specimens. 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 Accutest® Rapid Urine/Serum Pregnancy Test is a chromatographic immunoassay (CIA) for the rapid qualitative determination of hCG in serum and urine. The membrane is pre-coated with anti-alpha hCG capture antibody on the test band region and goat anti-mouse on the control band region. During testing, the urine or serum specimen is allowed to react with the colloidal gold particles that have been coated with anti-beta hCG monoclonal antibody. The mixture then moves along the membrane chromatographically by capillary action. For a positive result, a pink-colored band with a specific antibody-hCG-antibody-colloidal gold particle complex will form on the membrane in the test band region. Absence of a pink-colored band in the test band region indicates a negative result. To serve as a procedural control, a pink-colored band at the control region will always appear.
SPECIMEN COLLECTION

URINE SPECIMEN

Method of collection
The urine specimen must be collected in a clean, dry container.

Volume of specimen required
5 pipette drops (included) or approx. 0.2 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.

SERUM SPECIMEN

Method of collection
A whole blood specimen should be collected by acceptable medical technique. Process to separate the serum using aseptic methods.

Volume of specimen required
5 pipette drops (included) or approx. 0.2 ml

Special patient preparation requirements
No special preparation of the patient’s specimen is required.

Handling Conditions of specimen
Serum not assayed immediately must be stored in a refrigerator (2-8°C, up to 72 hours) or frozen (-20°C, for up to 3 months). Do not freeze and thaw sample repeatedly.

Criteria
Grossly hemolyzed samples should not be used.
MATERIALS

Materials Provided

- 40 individually wrapped test devices. Each device contains an anti-alpha hCG capture antibody coated membrane and colloidal gold particles coated with mouse anti-beta hCG monoclonal antibody.
- 40 disposable dropper pipettes
- One instruction insert

Materials Required but Not Provided

- Specimen collection container
- Timer

Storage and Stability

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

TEST PROCEDURE

Review “Specimen Collection” instructions. Test device, 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 device from its protective pouch (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identifications.

2. Draw sample to the line marked on the pipette (approximately 0.2 ml). Dispense entire contents into the sample well. For each sample or control, use a separate pipette and device.

3. Wait for pink-colored bands to appear. Depending on the concentration of hCG, positive results may be observed as soon as 40 seconds. However, to confirm negative results with urine samples the complete reaction time of 4 minutes is required. To confirm a negative result with serum samples the complete reaction time of five minutes is required.
INTERPRETATION OF RESULTS

POSITIVE: In addition to a pink colored control band (C), a distinct pink colored band will appear in the patient test region (T). The shade of pink on the (T) test band region 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.

NEGATIVE: Only one pink-colored band appears on the control region (C). No distinct pink-colored band appears in the patient test region (T).

INVALID: A total absence of pink-colored bands in both regions is an indication of procedural error or that test reagent deterioration has occurred.

Notes on the interpretation of results

• Negative test results in patients suspected to be pregnant should be re-tested 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 should contain the highest concentration of hCG.

• Borderline samples (those showing a very faint line in the test region) that later test negative may be attributed to falling hCG levels following a spontaneous or induced abortion. Natural termination occurs in 22% of clinically unrecognized pregnancies (5).

LIMITATIONS

1. A number of conditions other than pregnancy including trophoblastic disease and certain nontrophoblastic neoplasms 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 (≈ 200,000 mIU/ml) may yield a test line 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.
6. The presence of heterophile antibodies or non-specific protein binding may cause false-positive results in sensitive immunoassays. If a qualitative interpretation is inconsistent with the clinical evidence, results should be confirmed by an alternative hCG method.

QUALITY CONTROL

A procedural control is included in the test. A colored band appearing on the control region (C) is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the results window 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, however, each laboratory should follow their state and local requirements. For this purpose, we recommend the use of Accutest® hCG (Positive and Negative) controls.

COMMENTS AND TECHNICAL ASSISTANCE

Expected Values

Healthy men and healthy non-pregnant women do not have detectable hCG by the Accutest® Rapid Urine/Serum Pregnancy Test. HCG levels of 100 mIU/ml 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 Accutest® Rapid Urine/Serum Pregnancy Test has been standardized to the World Health Organization First International Reference Preparation (IRP 75/537).

Technical Service Representative

For technical assistance contact:

Jant Pharmacal Corporation
16255 Ventura Blvd., Suite 505
Encino, CA 91436
Phone: (818) 986-8630
Fax: (818) 986-0235
PERFORMANCE CHARACTERISTICS

Sensitivity

The Accutest® Rapid Urine/Serum Pregnancy Test detects urinary and serum hCG concentrations equal to or greater than 20 mIU/ml (Calibrated according to the 1st IRP) as indicated by the development of a pink-colored band in the patient test region. Additionally, samples containing less than 20 mIU/ml hCG may also produce a positive result. To evaluate the sensitivity of the Accutest® Rapid Urine/Serum Pregnancy Test at low levels of hCG, the following experiments were carried out:

A. Urine Samples: Urine samples from five known non-pregnant subjects were spiked with hCG to the concentrations of 0, 10, 20, 40, and 100 mIU/ml. A total of 100 samples were blind-labeled and tested with the Accutest® Rapid Urine/Serum Pregnancy Tests. Results are summarized in Table 1.

<table>
<thead>
<tr>
<th>hCG Concentration (mIU/ml)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Number of Negatives</td>
<td>20</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of Positives</td>
<td>0</td>
<td>8</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

B. Serum Samples: Pooled serum samples from five known non-pregnant subjects were spiked with hCG to the concentrations of 0, 10, 20, 40, and 100 mIU/ml. A total of 100 samples were blind-labeled and tested with the Accutest® Rapid Urine/Serum Pregnancy Tests. Results are summarized in Table 2.

<table>
<thead>
<tr>
<th>hCG Concentration (mIU/ml)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>100</th>
</tr>
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<tbody>
<tr>
<td>Number of Samples</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Number of Negatives</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of Positives</td>
<td>0</td>
<td>4</td>
<td>20</td>
<td>20</td>
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</tr>
</tbody>
</table>

Specificity

The specificity of the Accutest® Rapid Urine/Serum 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 Study Results

A. Urine Samples: One hundred randomly selected urine samples were analyzed using the Accutest® Rapid Urine/Serum Combo Pregnancy Test procedure in parallel with a commercially available one step hCG test. The results (Table 3) indicated a complete agreement (60 positive samples and 40 negative samples).

<table>
<thead>
<tr>
<th></th>
<th>Commercial hCG Test (Positive)</th>
<th>Commercial hCG Test (Negative)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accutest® Rapid Urine/Serum (Pos.)</td>
<td>60</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Accutest® Rapid Urine/Serum (Neg.)</td>
<td>0</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>40</td>
<td>100</td>
</tr>
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</table>

B. Serum Samples: One hundred randomly selected serum samples were analyzed using the Accutest® Rapid Urine/Serum Pregnancy Test procedure in parallel with a commercially available quantitative hCG immunoenzym-metric assay. As seen in Table 4, the results indicated 100% agreement.

<table>
<thead>
<tr>
<th></th>
<th>Commercial hCG Test (Positive)</th>
<th>Commercial hCG Test (Negative)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accutest® Rapid Urine/Serum (Pos.)</td>
<td>66</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Accutest® Rapid Urine/Serum (Neg.)</td>
<td>0</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>34</td>
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</tr>
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Interference Testing

The following substances were added to hCG-free and 20 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
REFERENCES


