Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

# OneStep hCG Combo Urine / Serum RapiCard™ InstaTest

REF 113029-1-20


| Sensitivity | 20 mIU/ml |
|---|---|

## INTENDED USE

Cortez Diagnostics, Inc. OneStep hCG Serum/Urine Combo RapiCard™ InstaTest A rapid one step test for the qualitative detection of human chorionic gonadotropin (hCG) in serum and urine.

## SUMMARY AND EXPLANATION

hCG is a glycoprotein hormone produced by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected as early as 7 to 10 days after conception. hCG levels continues to rise very rapidly, frequently exceeding 100mIU/mL by the first missed menstrual period, and peaking in the 100,000-200,000mIU/mL range about 10-12 weeks into pregnancy. The appearance of hCG in body fluid 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.

One Step hCG Serum/Urine Combo Pregnancy Test Cassette is a rapid test that qualitatively detects the presence of hCG in serum and urine specimens at the sensitivity of 20mIU/mL. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of hCG. At the level of claimed sensitivity, One Step hCG Pregnancy Combo Test shows no cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at high physiological levels.

## TEST PRINCIPLE

One Step hCG Serum/Urine Combo Pregnancy Test Cassette is a rapid chromatrographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in serum and urine to aid in the early detection of pregnancy. The test utilizes a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG. The assay is conducted by adding specimen to the specimen well of the test cassette and observing the formation of pink colored lines. The specimen migrates via capillary action along the membrane to react with the colored conjugate.

Positive specimens react with the specific antibody-hCG-colored conjugate and form a pink colored line at the test line region of the membrane. Absence of this pink colored line suggests a negative result. To serve as a procedural control, a pink colored line will always appear at the control line region if the test has been performed properly.

## REAGENTS

Coated Antibodies:
Control region: Goat anti-mouse (IgG) polyclonal antibody
Test region: Mouse monoclonal anti-hCG antibody A

Labeled Antibodies:
Colloidal gold conjugate of monoclonal anti-hCG antibody B

## SPECIMEN COLLECTION AND PREPARATION

**Serum Collection**
When collecting a blood sample, anticoagulant should not be used. Proper serum extraction techniques should be followed to avoid hemolysis. Only clear, non-hemolyzed serum specimens should be used.

**Urine Specimen Collection**
A fresh urine specimen should be used, no special pre-treatment is necessary. Specimens should be collected in a clean glass or plastic container.

**Specimen Storage**
Specimens may be refrigerated (2-8°C) and stored up to 2 days. For long term storage, freeze samples at -20°C or below. Refrigerated samples should be allowed to come to room temperature and mixed thoroughly before assaying. Frozen samples should be thawed completely and allowed to come to room temperature, and mixed thoroughly before assaying. Specimens should not be frozen and thawed repeatedly.

## MATERIALS AND COMPONENTS

### Materials provided with the test kits

1. One Step hCG Serum/Urine Combo Pregnancy Test RapiCard
2. Disposable pipette
3. Instructions for use

### Materials required but not provided

1. Clean glass or plastic container for specimens collection
2. Timer

## ASSAY PROCEDURE

Allow the test and the specimen to equilibrate to room temperature (15-30°C) prior to testing

1. To begin testing, open the sealed pouch by tearing along the notch. Remove the test kit from the pouch and use it as soon as possible.
2. Draw the urine or serum sample using the pipette provided, and dispense 3-4 drops (approx. 0.2 mL) onto the sample well of the cassette (see diagram).
3. Wait for the pink colored bands to appear. Depending on the concentration of hCG in the test specimen, positive results may be observed in as soon as 40 seconds. However, to confirm negative results, the complete reaction time of 5 minutes is required. It is important that the background is clear before the result is read.

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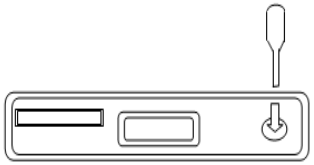
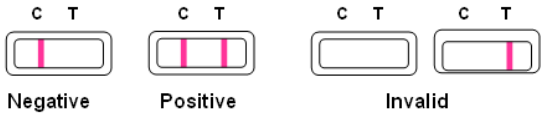


**Note: A low hCG concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 10 minutes.**

## RESULTS

- **Negative:** Only one pink colored band appears on the control region. No apparent band on the test region.
- **Positive:** Distinct pink color bands appear on the control and test regions. The color intensity of the test bands may vary since different stages of pregnancy have different concentrations of hCG hormone.
- **Invalid:** No line appears in the control zone "C", the test should be voided since an improper test procedure may have been performed or deterioration of reagents may have occurred. This is due to the internal control built in which a distinct control region (C) line should always appear. Repeat the test using a new cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.




## QUALITY CONTROL

A pink 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 controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing process.

Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

## PERFORMANCE CHARACTERISTICS

Normal urine and serum samples that were spiked with hCG concentrations of 62,500, 125,000, 250,000, 500,000, 1,000,000, and 2,000,000 mIU/mL were used to study the high dose hook effect on One Step hCG Serum/Urine Combo Pregnancy Test Cassette. It was noticed that both color bands at the test and the control region were visible. However, when hCG levels were over500,000 mIU/mL, the higher the hCG concentration became, the lighter the band at the test region became.

### Accuracy

An internal clinical evaluation was conducted comparing the results obtained using the One Step hCG Serum/Urine Combo Pregnancy Test Cassette to another commercially available One Step hCG Pregnancy Combo Test. Two hundred (200) female urine and serum samples collected and analyzed by trained technicians along with the commercially available test. The results demonstrated a 99.5% and 99% agreement for urine and serum samples respectively when trained technicians performed comparison testing on the tests. The results are shown in Tables 1 and 2.

**Table 1:** Comparison between Diagnostic Automation, Inc. vs. Predicate Urine cassette Format – Urine Samples

| | | Predicate | | Subtotal |
|---|---|---|---|---|
| | | + | - | |
| DAI | + | 123 | 0 | 123 |
| | - | 1 | 76 | 77 |
| Subtotal | | 124 | 76 | 200 |

Percent Accuracy = 99.5%
Discrepant Results = 0.5%

**Table 2**: Comparison between Diagnostic Automation, Inc. vs. Predicate Urine cassette Format – Serum Samples

| | | Predicate | | Subtotal |
|---|---|---|---|---|
| | | + | - | |
| DAI | + | 122 | 0 | 122 |
| | - | 2 | 76 | 78 |
| Subtotal | | 124 | 76 | 200 |

Percent Accuracy = 99%
Discrepant Results = 1%

### Sensitivity

One Step hCG Serum/Urine Combo Pregnancy RapidCard Test detects hCG concentrations greater than 20 mIU/ml as indicated by the appearance of a color band at the test region. Additionally, samples containing less than 20 mIU/mL hCG may also produce a positive result. To evaluate the sensitivity of One Step hCG Serum/Urine Combo Pregnancy test at low levels of hCG the following experiments were carried out. Serum and urine samples from 120 known non-pregnant subjects were spiked with hCG to the concentrations of 0, 10, 15, 20, 40, 100 mIU/ml. A total of twenty samples at each concentration were performed and blindly labeled and tested. The results are summarized in Table 3 and 4.

**Table 3:** Sensitivity of One Step hCG Serum/Urine Combo Pregnancy Test – Urine Samples

| hCG | 0 | 10 | 15 | 20 | 40 | 100 |
|---|---|---|---|---|---|---|
| # of Samples | 20 | 20 | 20 | 20 | 20 | 20 |
| Negative | 20 | 19 | 18 | 0 | 0 | 0 |
| Positive | 0 | 1 | 2 | 20 | 20 | 20 |

**Table 4:** Sensitivity of One Step hCG Serum/Urine Combo Pregnancy Test – Serum Samples

| hCG | 0 | 10 | 15 | 20 | 40 | 100 |
|---|---|---|---|---|---|---|
| # of Samples | 20 | 20 | 20 | 20 | 20 | 20 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Negative | 20 | 20 | 17 | 0 | 0 | 0 |
| Positive | 0 | 0 | 3 | 20 | 20 | 20 |

## Specificity

The cross reactivity of the One Step hCG Serum/Urine Combo Pregnancy RapiCard Test was evaluated using hCG homologous hormones. Homologous hormones FSH and TSH were added to serum and urine samples containing hCG at concentration of 0, 20 or 100 mIU/mL. No cross reactivity was observed. The results are shown in Table 5.

**Table 5:** Specificity of One Step hCG Cassette Pregnancy RapiCard Test

| hCG conc. in sample (mIU/mL) | Unspiked serum and urine samples | Serum and urine samples spiked with homologous hormones | |
|---|---|---|---|
| | | FSH | TSH |
| | | 1000 mIU/ml | 1000 µIU/ml |
| 0 | - | - | - |
| | - | - | - |
| | - | - | - |
| 20 | + | + | + |
| | + | + | + |
| | + | + | + |
| 100 | + | + | + |
| | + | + | + |
| | + | + | + |

## Interfering substances

The One Step hCG Serum/Urine Combo Pregnancy RapidCard Test was checked for possible interference from visibly hemolyzed, lipemic and icteric samples. Human hemoglobin, bilirubin or albumin was spiked into serum and urine samples with different concentration of hCG and tested using un-spiked samples as controls. No significant interference was observed in 20 sample testing results that were either positive or negative for hCG. The results, which have been pooled together due to little variance are shown in Table 6.

**Table 6: Non-Specific Interference on One Step hCG Serum/Urine Combo Pregnancy RapidCard Test**

| Sample No | Unspiked samples | Serum and urine samples spiked with (mg/mL) | | | |
|---|---|---|---|---|---|
| | | Hemoglobin | | Bilirubin | Albumin |
| | | 10 | 1 | 0.06 | 100 |
| 1 | - | - | - | - | - |
| 2 | - | - | - | - | - |
| 3 | - | - | - | - | - |
| 4 | - | - | - | - | - |
| 5 | - | - | - | - | - |
| 6 | - | - | - | - | - |
| 7 | - | - | - | - | - |
| 8 | - | - | - | - | - |
| 9 | - | - | - | - | - |
| 10 | - | - | - | - | - |
| 11 | + | + | + | + | + |
| 12 | + | + | + | + | + |
| 13 | + | + | + | + | + |
| 14 | + | + | + | + | + |
| 15 | + | + | + | + | + |
| 16 | + | + | + | + | + |
| 17 | + | + | + | + | + |
| 18 | + | + | + | + | + |
| 19 | + | + | + | + | + |
| 20 | + | + | + | + | + |

The following commonly used drugs and biological substances were added into hCG free and 50 mIU/mL hCG serum and urine samples. No interference was observed.

| | | | |
|---|---|---|---|
| Acetaminophen | 20 mg/mL | Caffeine | 20 mg/mL |
| Acetylsalicyclic Acid | 20 mg/mL | Gentisic Acid | 20 mg/mL |
| Ascorbic Acid | 20 mg/mL | Glucose | 2 g/dL |
| Atropine | 20 mg/mL | Hemoglobin | 1 mg/dL |

## LIMITATIONS OF PROCEDURE

1. False negative results may occur when the levels of hCG are below the sensitivity level of the test. When pregnancy is still suspected, a second test should be performed after 48 hours.
2. 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 or blood test should be performed after at least 48 hours.
3. Very low levels of hCG (less then 50mIU/mL) are present in specimen shortly after implantation. However, because a significant number of first trimester pregnancies terminate for natural reasons, a weakly positive result should be double confirmed after at least 48 hours.
4. 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. Therefore, the presence of hCG should not be used to diagnose pregnancy unless these conditions have been ruled out.
5. 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 of monoclonal antibodies for diagnosis or therapy may contain HAMA. Such specimens may cause false positive or false negative results.
6. 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 to be evaluated.

## PRECAUTIONS

1. In vitro diagnostic use for professional use only.
2. Do not use test kit beyond the expiry date.
3. The test cassette should not be reused.
4. Serum, urine, and other human-sourced specimens may be infectious; ensure proper handling and disposal of all used cassettes into a biohazard container.

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## REFERENCES


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# STORAGE

The test kit can be stored at temperatures between 2 to 30°C in the sealed pouch to the date of expiration. The test kit should be kept away from direct sunlight, moisture and heat. The expiration dating was established under these storage conditions.

| | |
|---|---|
| ISO 13485<br>ISO 9001<br>Diagnostic Automation/<br>Cortez Diagnostics, Inc.<br>21250 Califa St, Suite 102 and 116,<br>Woodland Hills, California 91367 USA | |
| **Date Adopted** | **2016-01-31** |
| REF 113029-1-20 | **CORTEZ- OneStep hCG Combo Urine/ Serum RapiCard ™ InstaTest** |
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| Revision Date: 2007-05-14 | |