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QuickCard™ Pro HCG Test

Catalog # 9008-25 / Test Instructions

Intended Use

The Phamatech QuickCard™ Pro HCG Test is an in vitro diagnostic test for the qualitative detection of human Chorionic Gonadotropin (hCG) in urine. The test is a two-site immunoassay employing monoclonal and polyclonal antibodies and is designed to reliably produce a visually detectable pink to purple test line in the presence of hCG at a concentration of 25 mIU/ml or greater (WHO 1st IRP, 75/537) within five minutes of sample application.

Summary & Explanation

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In a normal pregnancy, hCG can be detected in serum as early as 7 days following conception1-4, doubling every 1.3 to 2 days. At the time of the first missed menstrual period, hCG concentration is about 100 mIU/ml, and peak levels of 100,000 to 200,000 mIU/ml are seen at the end of the first trimester. 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.

Elevated serum hCG levels comparable to those observed in early pregnancy may also be associated with trophoblastic or non trophoblastic neoplasms6-7 such as hydatidiform mole and choriocarcinoma, therefor, the possibility of such diseases should be ruled out before a positive hCG result is considered diagnostic for pregnancy.

The QuickCard™ Pro HCG Test is a rapid, visual, and easily read test to qualitatively detect the presence of hCG in urine specimens at concentrations as low as 25 mIU/ml. 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.

Principles of the Procedure

The QuickCard™ Pro HCG Test employs the basic immunochemical sandwich assay principle, relying on the recognition and formation of specific antibody / hCG / antibody complexes. The membrane is coated with goat anti-hCG on the Test Band region (T) and goat anti-mouse on the Control Band region (C). During the test, urine is allowed to react with colored conjugate (mouse anti-hCG monoclonal antibody - colloidal gold conjugate) which is pre-dried on the test strip. As urine migrates across the membrane, it carries the colored conjugate with it. If hCG is present, a specific antibody / hCG / colored antibody complex is formed on the membrane. The absence of this colored band suggests a negative result. To serve as a procedural control, a colored band will always appear in the Control region regardless of the presence of hCG in the test specimen.

To perform the test, urine is added to the device with the aid of a plastic transfer pipette. The sample migrates by capillary action from the sample pad through the label pad to the membrane test area. As stated previously, if hCG is present in the urine sample, a specific antibody / hCG / colored antibody complex

is formed. The test result is read in 5 minutes. The presence of two color bands within the test region of the device indicates a positive result.

Reagents & Materials Provided

1. 25 individually pouched Test Devices
2. Test Instructions

Materials Required, But Not Provided

1. Clock or timer
2. Sample collection containers

Warnings & Precautions

1. For in vitro diagnostic use only.
2. For testing human urine only.
3. Urine specimens may be potentially infectious; properly handle and dispose of all specimens and used reaction devices according to Universal Precautions for biohazardous materials.
4. Do not use kit beyond the expiration date.
5. Do not reuse the test device.

Storage

Store the test kit between 15 and 30 °C until the expiration date on the pouch or kit box.

Sample Collection and Preparation

Collect a urine sample in clean, dry container, either plastic or glass, without any preservatives. Samples may be refrigerated (2 - 8 °C) and stored up to 48 hours. For longer storage, freeze samples at ≤ –20 °C .

Procedural Notes

The following procedural notes should be read carefully to ensure the best results with QuickCard™ Pro.

Transfer of Specimens. A clean pipette should be used to transfer the specimen to a test tube or sample cup.

Incubation Time and Temperature. The QuickCard™ Pro assay performs with the stated sensitivity and specificity at room temperature (15 to 30 °C, 59 to 86 °F). Temperatures lower than this will result in less color development at the end of the 5-minute incubation.

Specimens with high hCG levels (>1000 mIU/ml) may give positive results in less than 5 minutes. For such specimens it is not necessary to wait the full 5 minutes to obtain a valid result.

Batch Processing. If several specimens are to be analyzed at one time, perform each test step for all specimens at timed intervals before proceeding to the next test step. It is recommended that no more than five (5) devices be run in any one batch.

Turbid Specimens. Turbidity and particulates slow the rate of phoresis, the speed which liquid passes across the membrane. In certain cases, an extremely turbid specimen may cause the liquid transfer to stop. In such cases, centrifuge the sample and retest using a fresh device.

Assay Procedure

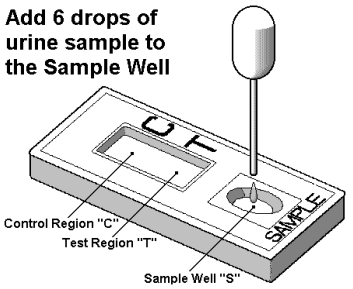
The test procedure for QuickCard™ Pro is performed at room temperature. Before performing the test, read the section entitled "Procedural Notes." The assay procedure is as follows:

Preparation

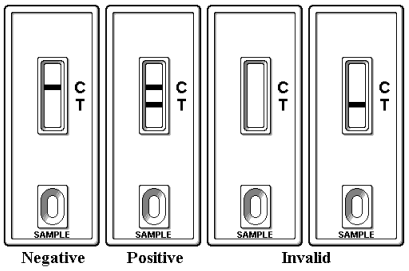
1. Bring the test device and sample to room temperature (15 - 28 °C) before testing.
2. Do not break the seal on the foil pouch until ready to perform the test.

Testing

1. Remove a QuickCard™ Pro HCG Test from its protective pouch. Verify that the test device is within its expiration dating.
2. Lay the test device on a clean, level surface. Using the sample pipette provided with the test, add six (6) drops of urine to the Sample Well. Allow each drop to be absorbed before adding the next.
3. Within 5 minutes a colored band will appear at the top of the test area to show that the test is complete.
4. Read test results immediately at 5 minutes. Results read after 10 minutes have elapsed should be considered invalid.



Interpretation of Results



Negative. If one (**1**) colored band appears in the Control Reaction Zone (**C**) but not in the Test Reaction Zone (**T**), this is a negative result and indicates the hCG level is below the detection sensitivity of 25 mIU/ml.

Positive. If two (**2**) color bands appear, one in the Control Reaction Zone (**C**) and one in the Test Reaction Zone (**T**), this is a positive result and indicates the hCG level is above the detection sensitivity of 25 mIU/ml.

Invalid. If no bands appear, or a Test Band appears without a Control Band, the device reagents may have deteriorated or the test may not have been performed properly. The presence of a Control Band is necessary to validate test performance. Retest the sample with a new test device.

Quality Control

An internal procedural control has been incorporated into the test to ensure proper kit performance and reliability. The development of the rose-pink Control Band demonstrates antibody recognition, verifies that the reagents are chemically active, and confirms that the test was performed correctly.

The use of external controls is recommended to verify proper kit performance. Quality control samples should be tested according to the quality control requirements of the testing facility. Assay controls in same method as urine specimens.

Limitations of the Procedure

1. This method has been tested using urine only. Other fluids have not been evaluated.
2. Positive results, as evidenced by the appearance of rose-pink Test and Control bands, may be apparent in as little as 1 minute. However, to confirm negative results the full 5 minute assay time should be allowed to elapse.
3. 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 specimen should be obtained from the patient and tested.
4. Occasionally, samples containing hCG levels below the cutoff sensitivity for the test may produce positive results.
5. A number of conditions other than pregnancy, including trophoblastic disease and certain non trophoblastic neoplasms, cause elevated levels of hCG. These diagnoses should be considered if appropriate to the clinical evidence.
6. The test is a qualitative screening assay and is not for determining quantitative concentration levels.
7. 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 the physician after all clinical and laboratory findings have been evaluated.

Expected Values

Healthy males and healthy non pregnant females do not have detectable hCG with the Phamatech QuickCard™ HCG Urine Test. HCG levels of 25 mIU/ml can be reached by the day of the first missed menstrual period. HCG levels peak about 8 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 soon after parturition.

Performance Characteristics

Sensitivity: A normal male urine pool was spiked with hCG to 0, 25, and 600 mIU/ml, then aliquoted, randomized, and coded. Technicians tested each coded aliquot with the QuickCard™ Pro HCG in a random order for a total of 10 replicates of each hCG level.

The coded results were visually interpreted as positive or negative by 4 Phamatech technicians (10 replicates of each level x 4 reads = 40 test results per hCG level) upon assay completion. Visual interpretation of the QuickCard™ Pro HCG Test resulted in the correct identification of 100% of all samples ≥ 25 mIU/ml hCG . At 25 mIU/ml, the assay sensitivity, 100% of the samples were correctly identified as positive.

Accuracy: A total of 118 female urine samples were collected from clinical sites from normal females who suspected they were pregnant. Testing was performed at Phamatech. Immediately upon completion of the assays, results were visually scored as "negative" (absence of hCG) or "positive" (presence of hCG) by the technician. Quality Assurance personnel then verified all results.

Overall, the QuickCard™ Pro HCG Test obtained an overall accuracy compared to the *RapidVue*™, the *Be Sure*™ Pregnancy Test, and the Quickstick™ One Step hCG Pregnancy Test (9010J) of 100% (354/354). The QuickCard test correctly detected 90 of 90 (100%) positive samples and 28 of 28 (100%) negative samples. These results are presented below.

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QuickCard™ Pro™ vs 3 Commercially Available hCG Tests

Characteristic	Observed vs Expected Results	% Correct
Sensitivity	**270 / 270**	**100%**
Specificity	**84 / 84**	**100%**
Accuracy	**354 / 354**	**100%**

Specificity. Various concentrations of hLH, hTSH and hFSH were spiked into a normal male urine pool which was previously spiked with 0, 25 or 100 mIU/ml hCG. Each sample was tested in triplicate using the Quick-Card™ test in accordance with the package insert procedure. The results were demonstrated that the Quick-Card™ Pro HCG Test has no cross-reactivity with hLH, hTSH or hFSH.

Interference. The following substances were added to hCG-free and 25 mIU/ml hCG-spiked urine samples and tested with the QuickCard™ Pro HCG Test. No interference with the expected results was observed from any of the substances at the indicated concentrations.

Chemical Analytes	Concentration
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	20 mg/dL
Benzoylecgonine	10 mg/dL
Cannabinol	10 mg/dL
EDTA	80 mg/dL
Ethanol	1.0 %
β-Hydroxybutyrate	2000 mg/dL
Methanol	10.0 %
Phenylpropanolamine	20 mg/dL
Uric Acid	20 mg/dL

Urine Analytes	Concentration
Albumin (serum)	2000 mg/dL
Estriol 17-beta	1400 µg/mL
Hemoglobin	1000 µg/dL
Pregnanediol	1500 µg/mL

Hormone	Concentration
LH	300 mIU/mL
FSH	1000 mIU/mL
TSH	1000 µIU/mL

Chemical Analytes	Concentration
Acetoacetic Acid	2000 mg/dL
Ascorbic Acid	20 mg/dL
Caffeine	20 mg/dL
DMSO	5.0 %
Ephedrine	20 mg/dL
Gentisic Acid	20 mg/dL
Methadone	10 mg/dL
Phenothiazine	20 mg/dL
Salicylic Acid	20 mg/dL

Urine Analytes	Concentration
Bilirubin	1000 µg/dL
Glucose	2000 mg/dL
pH	5 to 9
Specific Gravity	1.005 to 1.040

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