



Rapid Visual ELISA Test for the Qualitative Determination of Human Chorionic Gonadotropin (hCG) in Urine or Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Visual hCG Enzyme Immunoassay

INTENDED USE

For the qualitative determination of Human Chorionic Gonadotropin (hCG) in human urine or serum.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected generally as early as 7 days following conception (1-4), 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 100,000-200,000 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. (5) Elevated hCG levels comparable to those observed in early pregnancy may also be associated with trophoblastic or non-trophoblastic neoplasm (6-7) such as hydatidiform mole, choriocarcinoma; therefore, the possibility of such disease should be

ruled out before a positive hCG result is considered diagnostic for pregnancy.

PRINCIPLE OF THE TEST

The Visual hCG ELISA Test is a sandwich enzyme immunoassay (8-9) for the determination of human chorionic gonadotropin in urine or serum. The method employs two monoclonal antibodies to selectively identify hCG in urine/serum with a high degree of sensitivity. In less than 10 minutes, elevated levels of hCG as little as 20 mIU/ml can be detected.

The patient's specimen is allowed to react with the antibody enzyme conjugate and the antibodies on the solid phase simultaneously. In the presence of hCG, a specific antibody-hCG-antibody-enzyme complex will form on the surface of microtiter well. After unbound enzyme conjugate is removed by rinsing under a stream of distilled water, the well is incubated with TMB Reagent. The development of blue color in the well indicated the presence of hCG.

Comparing the color intensity of patient samples with that of the provided known reference, the amount of hCG can be visually estimated to be greater or less than 20 mIU/ml.

REAGENTS

Materials provided with the kit:

1. Microtiter Wells: mouse monoclonal anti- α -hCG coated wells, 96 wells.
2. Enzyme Conjugate: containing mouse monoclonal anti- β -hCG peroxidase conjugate in protein stabilizer, 7 ml (Red cap).
3. HCG Standard: containing 0 mIU/ml hCG, 1 ml (White cap).
4. HCG Standard: containing 20 mIU/ml hCG, 1 ml (Yellow cap).
5. HCG Standard: containing 150 mIU/ml hCG, 1 ml (Black cap).
6. TMB Reagent (One-Step), 7 ml (Amber cap).
7. Stop Solution: 1N HCl, 7 ml (Natural cap).

Materials required but not provided:

- Specimen collection containers
- Timer

- Distilled or deionized water
- Absorbent paper towels

SPECIMEN COLLECTION AND PREPARATION

The specimen must be collected in a clean, dry container, either plastic or glass, without preservative. Specimen collected at anytime may be used. However, the first morning urine generally contains the highest concentration of hCG.

All specimens may be refrigerated (2-8°C) and stored up to 72 hours prior to testing. If specimens are refrigerated, they must be equilibrated to room temperature before testing. Urine specimens exhibiting visible precipitates should be filtered, centrifuged or allowed to settle.

No special preparation of the patient specimen is required. Additives such as sodium azide should be avoided. Limited sample studies indicated that plasma sample prepared from EDTA can be used in lieu of serum. Serum not to be assayed immediately must be stored in a refrigerator or a freezer. Bring these specimens to room temperature prior to testing. Do not freeze and thaw repeatedly.

STORAGE INSTRUCTIONS

Store reagents at refrigerator temperature (2-8°C) when not in use. Do not freeze.

Bring reagents and specimens to room temperature (18-25°C) before testing.

PRECAUTIONS

Do not add sodium azide to the specimens as preservative since it inhibits the enzymatic activity. Do not mix reagents from different lots and do not use kit components beyond expiration date. Do not interchange bottle caps.

TEST PROCEDURE

All reagents and specimens must be brought to room temperature and mixed thoroughly before beginning the test.

A. Qualitative ELISA Testing

1. Place Microtiter Wells for your test on the holder.
2. Dispense 1 drop (50 µl) of hCG of patient sample and/or 1 drop (50 µl) of hCG Standards and Negative Reference, if desired, into the appropriately labeled Microtiter Wells. Use a separate disposable pipette for each specimen.
3. Add 1 drop (50 µl) of Enzyme Conjugate into each well. Mix gently for 10 seconds.
4. Incubate at room temperature for 5 minutes.
5. Remove content by flicking the microtiter well holder into sink, followed by rinsing the wells 5 times with distilled or deionized water.
Note: Avoid well to well contamination from water over flow during the first rinse. Separating wells on the well holder would help.
6. Add 1 drop of TMB Reagent into each well.
7. Mix gently for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Compare the color developed in specimen wells to that of the positive reference well (20 mIU/ml).

INTERPRETATION OF RRESULTS

Positive: Wells showing blue color stronger than the 20 mIU/ml Reference Standard indicate the presence of hCG, or positive results.

Negative: Wells showing no color or faint blue color weaker than the 20 mIU/ml Reference Standard indicate non-detectable amount or less than 20 mIU/ml of hCG in the specimen.

A slight bluish tinge, much lighter than the positive reference well, may result from insufficient washing and should be considered negative. If the patient sample shows a negative result but pregnancy is suspected, the test should be repeated using a fresh specimen obtained 2-3 days later.

Note:

Depending on the concentration of hCG in the specimen, the color may develop instantaneously. Incubation of the TMB Reagent beyond 5 minutes may result in a slight shade of blue much less intense than that of the positive reference (20 mIU/ml). This should still be considered as negative.

B. Quantitative Reader Procedure

In order to run a standard curve, test hCG standards included in the kit by the same method as the test specimens.

Following step 7 in Procedure A, if a microtiter reader is available for quantitative reading at 450 nm, proceed immediately after five minutes incubation:

1. Rapidly add 1 drop (50 µl) of Stop Solution (1N HCl) into each well including your test specimen, all hCG Standards and Negative Reference.
2. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.
3. Calculate the hCG concentration from the standard curve.

LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

PERFORMANCE CHARACTERISTICS

Healthy men and healthy non-pregnant women do not have detectable hCG by the Visual ELISA Test. The hCG level of 20 mIU/ml can be reached in 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 within days after parturition.

Sensitivity and Specificity

The sensitivity of the Visual ELISA Test is set at 20 mIU/ml. The 20 mIU/ml Positive reference (calibrated to the 2nd international Standard) was designed as the cut off for the test because hCG concentrations in this range are usually achieved during the 2nd week after conception.

Specificity of the Visual ELISA Test was determined from cross reaction studies with known amounts of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and Thyroid Stimulating Hormone (TSH). 500 mIU/ml LH, 1000 mIU/ml FSH and 1000 µIU/ml TSH all give negative results to the test.

Accuracy

Visual ELISA Test shows 99.4% agreement with results obtained by the use of other qualified immunological pregnancy tests under actual clinical conditions.

Urine samples from five known non-pregnant subjects were spiked with hCG to concentrations of 0, 40, 100 mIU/ml. A total of 75 of these samples were blind labeled and tested with Visual ELISA Test. Results are summarized in Table 1.

Table 1

HCG (mIU/ml)	0	40	100
Number of sample	25	25	25
Negative	25	0	0
Positive	0	25	25

Interference Testing

The following substances were added in 20 mIU/ml hCG spiked negative urine specimens. None of the substances at concentration tested 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
Gentisic Acid	20 mg/dl
Glucose	2 g/dl
Hemoglobin	1 mg/dl

The Bureau of Biologics has requested all manufactures of pregnancy test products to include the following statement, for your information.

“False positive and false negative biological and immunological pregnancy tests have been reported in tests of specimens from individuals taking a variety of drugs. The false reactions may be related to the donor and/or the drug. Whenever possible, it is best to test the specimens from donors who are not taking drugs.” (10)

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