

**Ref.** RDT-PRC.72P, 50 Test

## INTENDED USE

CARD test for detection of hCG in Urine.

1. Easy to use
2. High Specificity
3. Sensitivity better than 20 mIU/ml
4. 5 minutes test

## INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by trophoblastic cells and later by the placenta. Hence in a normal pregnancy hCG appears in urine soon after conception. Concentration of the hormone increases rapidly and therefore it serves as an indicator of pregnancy. The level of urinary hCG is about 100 mIU/ml at the time of the first missed menstrual period. The highest values (1,00,000 - 2,00,000 mIU/ml) can be demonstrated towards the end of the first trimester.

Elevated serum hCG levels are also associated with trophoblastic or nontrophoblastic neoplasms like hydatidiform mole, choriocarcinoma, therefore such diseases should be ruled out before a positive hCG result is considered diagnostic for pregnancy.

## PRINCIPLE

The hCG Card includes a sample (filter) pad, a conjugate pad, conjugate consisting of anti  $\beta$  hCG (monoclonal) antibody, a test line (zone) on the chromatographic membrane with coating of anti  $\alpha$  hCG (monoclonal) antibody and a control line (zone) consisting of Goat anti-mouse IgG and finally attached to another filter pad acting as sink for excess flow.

Testing is started by placing the test card. Put the sample in to the sample well. The sample flows through a filter into the chromatographic membrane where it comes into contact with different reagent zones. At first zone the sample rehydrates the pink-purple conjugate reactive with anti  $\beta$  hCG. The hormone in the sample attaches on the conjugate and the complex migrates further in the membrane towards the stationary antibody zone where the antibody conjugate hCG complex is capture by anti  $\alpha$  hCG antibody leading to the formation of a pink-purple line in the test window (test line). The test includes excess of the conjugate complex which migrates further in the membrane where it will be captured by the second stationary antibody reactive to the antibody conjugate leading to the formation of a second pink-purple line in the test window (control line), indicating the proper performance of the test. If the specimen does not contain hCG hormone or if the hormone level is very low, the antibody conjugate hCG complex will not be formed. The antibody conjugate reagent will freely pass over the first stationary antibody zone, will be captured by the second antibody in the membrane and leading to the formation of only one pink-purple line (control line).

The sensitivity of hCG Card has been adjusted so that it will detect the urinary hCG level equal or higher than 20 mIU/ml. (The World Health Organisation, Third International Standard.) This level is normally reached in approximately 10 days after conception.

## PRESENTATION

Pregnancy - hCG (Eva™ Cards)	50 Tests 50 Cards
------------------------------	----------------------

## PRECAUTION

- Do not cut the Pouch and leave the card exposed to air. Cut as many pouches as required and use immediately.

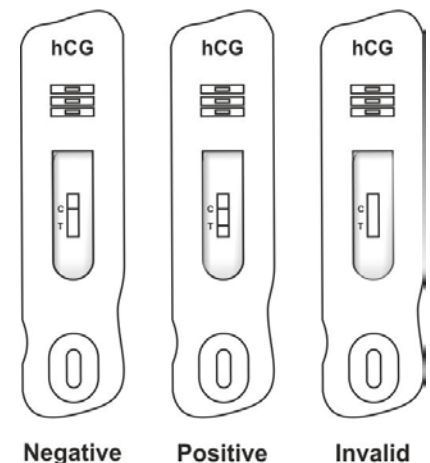
## STORAGE AND STABILITY

hCG test card should be stored at 4°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on the pouch as well as on the carton label. The test kit should be kept away from direct sunlight, moisture and heat.

## SPECIMEN COLLECTION AND STORAGE

The concentration of hCG is highest in the first urine of the morning. Therefore, it is recommended to perform the test using this specimen. However, if it is not possible any other urine sample can be used. The sample can be stored at room temperature (25 ± 5°C) for upto 8 hours and at 2°C-8°C for upto 4 days.

## TEST PROCEDURE



1. Open the pouch and remove the card.
2. Bring the specimen to room temperature.
3. Place the card on a flat surface, fill the urine dropper with specimen and holding it vertically, dispense 2-3 drops (50-75  $\mu$ l) without air bubbles into the sample well.
4. Read the result within 5 to 10 minutes.

## INTERPRETATION OF RESULTS

1. **NEGATIVE:** Only one pink-purple line appears on the control (C) region. The test result is negative.
2. **POSITIVE:** In addition to the control line a clearly distinct pink-purple line appears on the test region (T). The test result is positive.
3. **INVALID:** A total absence of pink-purple line in both (C) and (T) regions or no pink-purple line appears on the control region is an indication of procedure error and/or the test reagent has deteriorated. Repeat with a new test card.

## NOTE

1. Filtered urine gives a better test result.
2. Read the results within 5-10 minutes. Appearance of 2 lines is indicative of presence of hCG.

3. Observe the card up to 5 to 10 minutes. In many cases a faint test band may appear, for the following reasons.
  - a. A very early pregnancy. The card test is very very sensitive and reads well below the claimed (20 mIU/ml) value.
  - b. Improper storage of urine resulting in partial loss of hCG.
  - c. A non-specific binding due to urine contaminated with bacteria, and or presence of protein (albumin) etc.
  - d. Patients on drugs and drug addicts.
4. If a faint test band appears after 5 minutes, it is always advisable to repeat the test after 2 to 3 days before final interpretation.

## SPECIFICITY

No interference or false positive results observed from structurally related hormones such as Luteinizing Hormone (LH) Follicle Stimulating Hormone (FSH) and Thyroid Stimulating Hormone (TSH) at physiological possible levels.

## LIMITATIONS

1. Signs and symptoms of the patient must be clinically correlated for the interpretation of pregnancy test.
2. During certain conditions like trophoblastic diseases and nontrophoblastic neoplasms, hCG levels are elevated, comparable to normal pregnancy. The diagnosis should be based on appropriate clinical evidences.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. A false positive result may be seen in case of drug addicts.
5. A positive card result which shows negative on subsequent testing (after 1 to 2 days) indicates an early abortion due to uterine pathology.
6. The card may show positive for hCG due to the tumors of ovaries and testicles like Dysgerminoma, Embryonal carcinoma with STGC, yolk sac tumor with STGC, Seminoma, Seminoma with STGC, Primary epithelioma of ovaries etc. (STGC = Syncytiotrophoblastic Giant Cells)

## HIGH DOSE EFFECT

Normal Urine that were spiked with hCG concentration of 62,500, 1,25,000, 2,50,000, 1,000,000 and 2,000,000 mIU/mL were used to study the high dose hook effect on one step hCG Pregnancy Test. It was noticed that both color bands at the test band region and the control region were visible. However, when hCG levels were over 500,000 mIU/mL, the higher the hCG concentration became, the lighter the band at the test region became.

## REFERENCES

1. Catt. K. J., Dufan, M.L., and Varitukaitis, J.L., J. Clin. Endocrinol Metab. Vol. 40,537
2. Lenton, E. A., Neel, L.M., Sulaiman, R., Fertility & Sterility Vol. 37,773 (1982)
3. Batzer F. R., Fertility & Sterility, Vol. 34,1 (1980)
4. Jacques Wallace, Interpretation of Diagnostic Tests Fifth edition Pg. (538-539).

