INTRODUCTION

Vectra® pregnancy test is a rapid, visual, qualitative, enzyme immunoassay for the determination of human chorionic gonadotropin (hCG), a marker for pregnancy in urine/serum.

SUMMARY

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the synciotrophoblastic cells of the placenta, after fertilization. In normal pregnancy, hCG can be detected approximately 6-8 days after conception. Following conception approximately after 6-8 days, hCG is released and its concentration doubles approximately every 1.7 days. The level of hCG rise rapidly reaching peak levels after 60-80 days.

The appearance of hCG soon after conception and its subsequent rise in concentration makes it an ideal marker for early detection and confirmation of pregnancy. However elevated hCG levels are frequently associated with trophoblastic and non-trophoblastic neoplasms and hence these conditions should be considered before a diagnosis of pregnancy can be made.

Vectra® enzyme immunoassay for pregnancy detects the presence of hCG in urine/serum specimens, qualitatively, at concentrations of 25 mIU/ml of hCG and above in about 10 minutes.

PRINCIPLE

Vectra® pregnancy test utilizes the principle of sandwich enzyme immunoassay, with a unique mono-mono antibody combination specific against hCG present in urine/serum.

The patient's urine/serum specimen is allowed to react with the monoclonal antibody directed against hCG, coated on the microtiter wells and the monoclonal antibody - enzyme conjugate complex. If hCG is present in the test specimen, antibody-hCG-antibody enzyme complex will be formed on the surface of the microtiter well. Washing the well under running tap water will clear of the unbound complex and the unreacted conjugate. Incubating the well with substrate reagent results in the development of blue colour. The intensity of the blue colour is proportional to the concentration of hCG present in the urine/serum specimen. Visual comparison of the intensity of blue colour with test specimen well as against the positive control well indicates the concentration of hCG greater than or equal to 25 mIU/ml of hCG in the test specimen.

REAGENTS AND MATERIAL REQUIRED

Reagents for performing the test:
1. Sealed pouches containing breakable microtiter wells predispensed with monoclonal anti-hCG antiserum (8 wells/pouch).
2. Enzyme conjugate containing monoclonal anti-hCG antibody peroxidase conjugate.
3. Substrate reagent containing Tetra methyl benzidine in appropriate buffer.
4. Negative control serves to validate the test procedure.
5. Positive control provides colour equivalent to 25 mIU/ml of hCG.

Materials for performing the test:
1. Well holder.
2. Disposable sample droppers.

ADDITIONAL MATERIAL REQUIRED
1. Sample collection container.
2. Timer/stopwatch.
3. Running tap water or distilled water.
4. Absorbent paper/tissue.
STORAGE AND STABILITY
The kit must be stored at 2-8°C till the duration of shelf life as indicated on the carton label and the respective individual kit component labels.

NOTE
1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
2. Since the reagents from the same lot are optimized as a system, it is important that reagents of the same lot are used for performing the test.
3. Foil pouches may be cut open carefully from the sealed end. In case of less number of microtiter wells being used, replace back the unused wells into the foiled pouch and seal appropriately for future use. Protect the wells from direct exposure to moisture.
4. Do not interchange reagent bottle caps.
5. Once the test is performed, immediately replace the kit at 2-8°C.
6. Avoid exposure of the substrate solution to direct light during use or storage.
7. Do not use the kit beyond expiry date.
8. Addition sequence of samples and reagents must be observed meticulously for achieving accurate results.

SPECIMEN COLLECTION
Though random urine specimens can be used, first morning specimen is preferable as it contains the highest concentration of hCG. Specimens should be collected in clean glass or plastic containers. If testing is not immediate, the urine specimen may be stored at 2-8°C for upto 72 hours. Turbid specimens should be centrifuged or allowed to settle and only the clear supernatant should be used for testing.

TEST PROCEDURE
1. Bring all the reagents to room temperature before testing. Also refrigerated specimens must be brought to room temperature prior to testing.
2. Cut open the foiled pouch to retrieve the required number of microtiter wells.
3. Place the required number of microtiter wells on the well holder.
4. Tighten the vial cap of all the reagent bottles provided with the kit in clockwise direction to pierce the dropper bottle nozzle.
5. Dispense two drops of urine/ serum sample to be tested using the dropper provided with the kit to the appropriate microtiter wells.
6. Add two drops each of POSITIVE and NEGATIVE control into the appropriate wells.
7. Add one drop of ENZYME CONJUGATE to the test, positive and negative control wells.
8. Incubate for 5 min. at room temperature.
9. Empty the entire content of the wells into the sink by flicking off the liquids.
10. Wash the wells under running tap water (or distilled water) 6 times by filling and emptying the wells. Take care to avoid well to well contamination from water overflow during rinsing.
11. Blot dry after the last wash.
12. Add two drops of SUBSTRATE REAGENT into each well.
13. Incubate for 5 min. at room temperature.
14. Visually compare the development of blue colour in test well as against the positive control well against a white background.

INTERPRETATION OF RESULTS
POSITIVE RESULT:
Wells showing blue colour equal or more intense than the positive control well indicates a positive result.

NEGATIVE RESULT:
Wells showing no colour at the end of final incubation of 5 mins. indicates that the concentration of hCG is less than 25 mIU/ml or a negative result.

REMARKS
1. A number of conditions other than pregnancy including trophoblastic and non-trophoblastic neoplasms such as hydatidiform mole, choriocarcinoma etc. can cause elevated levels of hCG. Such clinical conditions must be ruled out before a diagnosis of pregnancy can be made.
2. Highly dilute urine specimens and specimens from early pregnancy may not contain representative levels of hCG. If pregnancy is still suspected, repeat the test with first morning urine after 48-72 hours.
3. As with all diagnostic tests, the results must be correlated with clinical findings.
4. Avoid air bubble formation during addition of reagents, positive and negative control, urine sample to be tested while performing the test.
5. A slight blue tinge in the test well with intensity lesser than the positive control well indicates insufficient washing and should be considered negative.
6. It is recommended to run a positive and negative control simultaneously with each assay run.

BIBLIOGRAPHY
3. Data on File: Qualpro Diagnostics (P) Ltd.