INTRODUCTION

Insight® is a semi-quantitative immunoconcentration assay for the detection of human chorionic gonadotropin (hCG), a marker for pregnancy in urine specimen.

SUMMARY

Human chorionic gonadotropin (hCG), a glycoprotein hormone is secreted by synciotrophoblastic cells of the placenta, after fertilization. Following conception approximately after 6-8 days, hCG is released and its concentration doubles every 1.7 days. The levels of hCG rise rapidly reaching peak levels after 60-80 days. The appearance of hCG in urine soon after conception makes it an ideal marker for the early detection and confirmation of pregnancy. However elevated hCG levels are frequently associated with trophoblastic and non-trophoblastic neoplasms, hence these conditions should be considered before a diagnosis of pregnancy can be made.

Insight® hCG pregnancy test detects the presence of hCG in urine specimens, at a concentration of 25 mIU/ml and above.

PRINCIPLE

Insight® hCG test utilizes the principle of immunoconcentration and enables semi-quantitative comparison of colour developed at the test band with inbuilt reference band having colour intensity of 50 mIU/ml of hCG. The membrane assembly is predispensed with anti-hCG monoclonal antibodies at the test region and antimouse antibodies at the reference region and control region.

The membrane assembly is hydrated with the wash buffer. Urine specimen under test is mixed with anti-β hCG colloidal gold sol solution and transferred to the flow through membrane assembly. In case of negative result only the control and reference region bands appear. In case of a positive result in addition to the control and reference region bands appear. In case of a positive result in addition to the control and reference band, the test band also appears as the hCG-gold sol complex is captured and immobilized at the test region. Finally the excess unreacted gold sol conjugate is washed off and the results are visualized.

REAGENTS AND MATERIALS SUPPLIED

Each kit contains:

A. Individual pouches each containing a:
   1. Flow through membrane device. Each flow through assembly contains pre-dispensed monoclonal anti hCG antiserum at the test region and antimouse antiserum at the reference and control region.
   B. Reagent 1- Wash buffer solution.
   C. Reagent 2- anti β hCG colloidal gold conjugate.
   D. 25 disposable sample preparation cuvettes.
   E. Disposable sample droppers.
   F. Package Insert.

STORAGE AND STABILITY

1. Reagent 1 and Reagent 2 should be stored at 2-8°C, till the duration of shelf life as indicated on the vial.
2. The sealed pouches in the kit may be stored between 4-30°C, till the duration of the shelf life as indicated on the pouch. DO NOT FREEZE.

NOTE

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use.
2. Do not use beyond expiry date.
3. Flow through device assembly, Reagent 1 and Reagent 2 of the same lot are optimized as a system. It is important that kit components from the same lot are used for achieving accurate and reproducible results. Do not intermix reagents from different lots.
4. The sequence of addition of reagents should be followed meticulously for achieving accurate results.
SPECIMEN COLLECTION AND PREPARATION
Though random urine specimen can be used, first morning urine specimen is preferable as it contains the highest concentration of hCG. Specimen should be collected in a clean glass or plastic container. If testing is not immediate, the urine specimen may be stored at 2-8°C for up to 72 hours. Turbid specimen preferably should be centrifuged or allowed to settle and only the clear supernatant should be used for testing.

TEST PROCEDURE AND INTERPRETATION OF RESULTS
1. Bring the kit components to room temperature before testing. Also refrigerated specimen must be brought to room temperature before testing.
2. Retrieve the required number of sealed pouches as per the number of samples to be run. Open the pouch and remove the device. Appropriately label the device with the sample identity. Once opened the device must be used immediately.
3. Tighten the vial cap of the Reagent 1 and Reagent 2 in clockwise direction to pierce the dropper bottle nozzle.
4. Add two drops of Reagent 1 (Wash Buffer) to hydrate the flow through assembly. During addition of wash buffer ensure that there are no air bubbles formed and the membrane assembly has been completely soaked up.
5. In the meanwhile retrieve the required number of cuvettes as per the number of samples to be run. Label the cuvette(s) with the sample identity. Dispense two drops of Reagent 2 (Anti-β hCG colloidal gold sol solution) to the cuvette(s).
6. Add two drops of urine sample to be tested using the sample dropper provided with the kit to the appropriately labelled cuvette, mix by gentle shaking.
7. Dispense the entire content of the cuvette (sample + gold sol reagent mixture) using the sample dropper to the appropriately labelled and hydrated ‘Insight’ membrane device. Ensure that the mixture is to be added to the centre of the window on the flow through device to avoid formation of air bubbles during addition. Wait for a few seconds for the reaction mixture to soak completely through the membrane.
8. Add two drops of Reagent 1 (Wash Buffer) to the flow through assembly. During addition of wash buffer ensure that there are no air bubbles formed and the membrane assembly has been completely soaked up so that the unreacted conjugate and the unbound complex are cleared for clear visualization of results.
9. At the end of above mentioned steps read the results immediately as follows:

   **Negative**- Appearance of only two pink bands at reference and control region. A dark pink coloured band appears in the control region 'C'. A light pink coloured band appears in the reference region 'R'.

   **Positive**- Appearance of three pink bands at test, reference and control region.

10. If the intensity of pink test band is less than reference band, then the concentration of hCG in the sample is approximately in the range of 25-50 mIU/ml.
    If the intensity of the pink test band is same as the reference band, the concentration of hCG in the sample is approximately equal to 50 mIU/ml.
    If the intensity of the pink band at the test region is more than reference region, the concentration of hCG in the sample is greater than 50 mIU/ml.

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 the 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 before arriving at the final diagnosis.
4. Usage of contaminated / turbid samples should be avoided as they lead to high background colour and difficulty in test visualization and interpretation of results.
5. Avoid air bubble formation during addition of wash buffer and sample-gold sol conjugate mixture solution.
6. After addition of each solution to the membrane assembly during test procedure, ensure that the solution has soaked up completely before adding the next solution.
7. In case a sample-gold sol mixture does not flow through easily, verify if the sample is contaminated or contains suspended particles. Centrifuge such samples and use supernatant for subsequent testing.
BIBLIOGRAPHY
3. Data on File: Qualpro Diagnostics (P) Ltd.