Pregnancy Test
(Detecting Human chorionic gonadotropin in urine)
- Human chorionic gonadotropin:

• Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by a portion of the placenta following implantation.

• The qualitative hCG test can be used to see if a woman is pregnant or not.
- **hCG Role in pregnancy:**

  • Promotes the maintenance of the corpus luteum (which means yellow body in Latin) during the beginning of pregnancy in the ovary ➔ This allows the corpus luteum to secrete the progesterone during the first trimester. Progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can sustain the growing fetus.

  • Human chorionic gonadotropin also plays a role in cellular differentiation/proliferation.
- hCG levels:

• During the first trimester, hCG levels rise steadily and rapidly, peaking around 10 weeks’ gestation, and subsequently taper off to less than 10% of peak levels and remain constant for the duration of the pregnancy.
- hCG levels in pregnant and non pregnant women:

- In pregnant women, depressed urine hCG levels may indicate threatened abortion.

- In non pregnant women, elevated levels of hCG can lead to a cancer diagnosis since some cancerous tumors produce this hormone.
Practical Part
- **Objective:**
  - To detect and confirm pregnancy.

- **Principle:**
  - Urine pregnancy tests use the enzyme-linked immunosorbent assay (ELISA) technique, using a highly specific monoclonal antibody directed against the β-subunit of human chorionic gonadotropin (hCG).
- Principle cont’:

• Pregnancy test strip consist of:

1. The reaction zone (R zone): soluble anti-hCG antibody-enzyme conjugate. These are mouse monoclonal antibodies linked to an enzyme.

2. The test zone (T zone): contains immobilized polyclonal mixture of anti-hCG antibody + dye substrate.

3. The control zone (C zone): the dye substrates + anti-mouse antibodies can recognize epitopes on the mouse monoclonal. (control zone act as control sample)
- Different zones in pregnancy test strip:

• A nice animation explain the principle of pregnancy test:
  http://www.sumanasinc.com/webcontent/animations/content/pregtest.html
- Principle cont’:

**Sequence of events in pregnant women:**

1. A few drops of urine is transferred to the specimen well (loading well).
2. Urine will flow by capillary action from loading well towards R zone carrying along with it the HCG hormone.
3. At R zone, the HCG hormone will react and bind with the **soluble anti-HCG Ab-enzyme conjugates** forming a complex of **HCG hormone - HCG Ab - enzyme conjugate**. (excess Ab will not bind)
4. This complex will migrate towards T zone.
5. At T zone, this complex will react and bind with the **immobilized anti-HCG Ab**, once it binds with the immobilized Ab, this will **activate the enzyme** thus allowing to act on the dye substrate and produce a **color** that indicates a **positive pregnancy result**.
6. The **excess soluble HCG hormone - HCG Ab - enzyme conjugates complex** will pass from T zone to C zone.
7. At C zone, this complex will react and bind with the **immobilized anti-(anti-HCG Ab-Enzyme conjugates) Ab** there, once bound it will **activate the enzyme**, thus allowing to act on the dye substrate and produce the **color** detecting at C zone which is an indicator of the activity or reliability of the test.
Specimen Collection and Preparation:

- Collect at least 1 mL of urine in a clean, dry, plastic or glass container with no preservatives.
- Specimens may be collected at any time of the day, however the first morning sample generally has the highest concentration of hCG and is the specimen of choice.

- Procedure:

  - NOTE: Bring test components and specimens to room temperature prior to testing.
  1. Remove a Testing Device from the foil pouch by tearing at the "notch" and place it on a level surface.
  2. Holding a Sample Dropper vertically, add exactly three drops of the urine specimen to the sample well.
  3. Read results at time indicated in procedure.
-Results:

• Follow the instructions on the reagent package insert provided by the instructor to properly perform the test.

• Interpretation of Results:

• Based on the package insert correctly interpret the results of the pregnancy test on the patient sample.

• Record results as “Positive” or “Negative”

<table>
<thead>
<tr>
<th>SAMPLE TESTED</th>
<th>RESULT</th>
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-Discussion:

• Comment on the results and state whether the sample is pregnant or not.
- Urine test kit:
Osmolality in Serum and Urine
- Osmolality and osmolarity:

  • **Osmolality** is a measure of the moles (or osmoles) of solute per total weight of solvent in Kg. ➔ expressed as (mol/kg, molal, mOsm/kg).

  • **Osmolarity** is a measure of the moles (or osmoles) of solute per unite of total volume in L. ➔ expressed as (mol/L, M).

- osmolality test:

  • The osmolality test provides a snapshot of the number of solutes present in the blood (serum), urine, or stool.

  • **Osmometer** is a device for measuring the osmotic strength of a solution.
- Serum osmolality:

• Serum osmolality is primarily ordered to investigate hyponatremia (low sodium in serum).

• Higher than normal levels may be due to:
  ➔ High sodium level (hypernatremia).

• Lower than normal levels may be due to:
  ➔ Low sodium level (hyponatremia).
Urine osmolality:

- Urine osmolality is frequently ordered along with serum osmolality.
- This test helps check your body's water balance and urine concentration.
- Osmolality is a more exact measurement of urine concentration than the urine specific gravity test.
- Urine osmolality:

• Greater-than-normal measurements may indicate:
  ➔ Loss of body fluids (dehydration).

• Lower-than-normal measurements may indicate:
  ➔ Kidney failure.
  ➔ Diabetes insipidus.