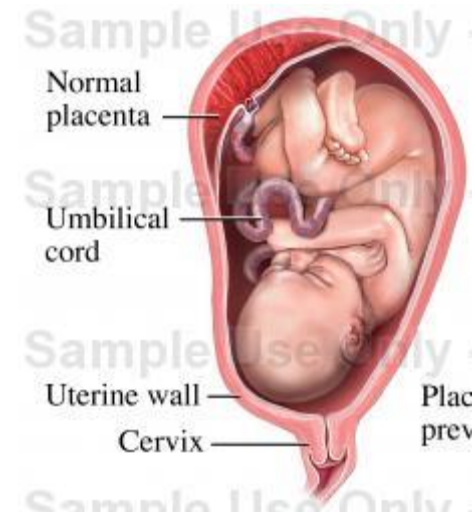


Pregnancy Test

(Detecting Human chorionic gonadotropin in urine)

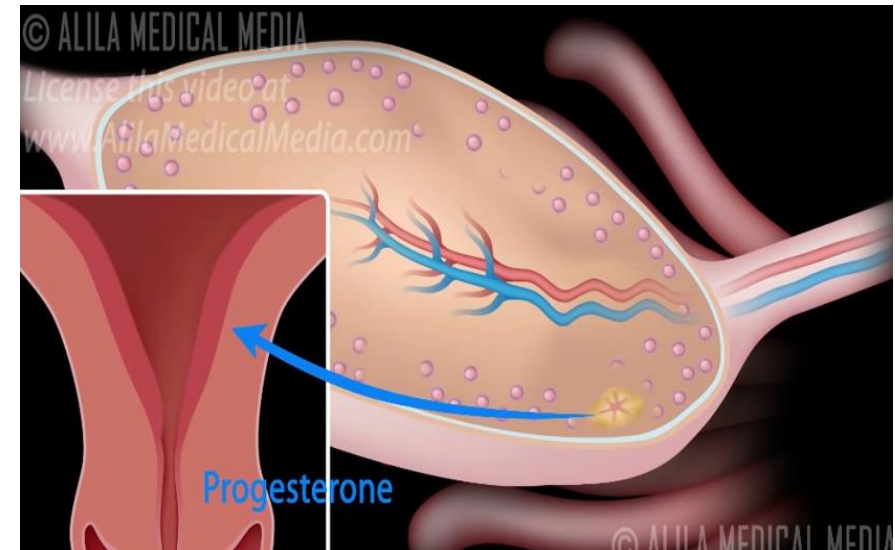
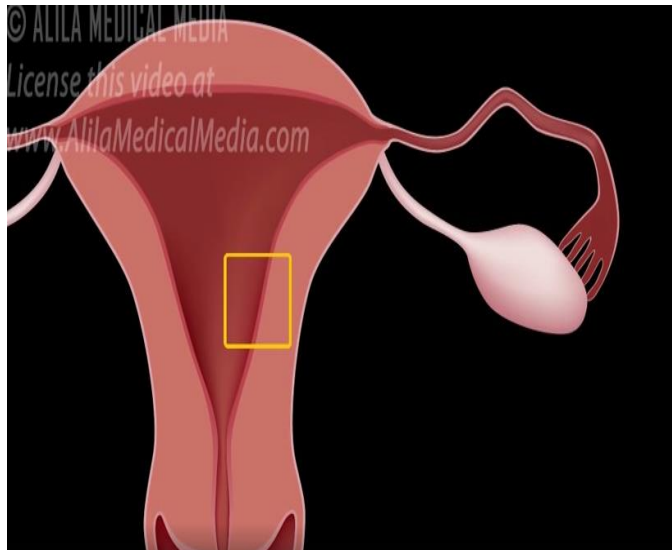
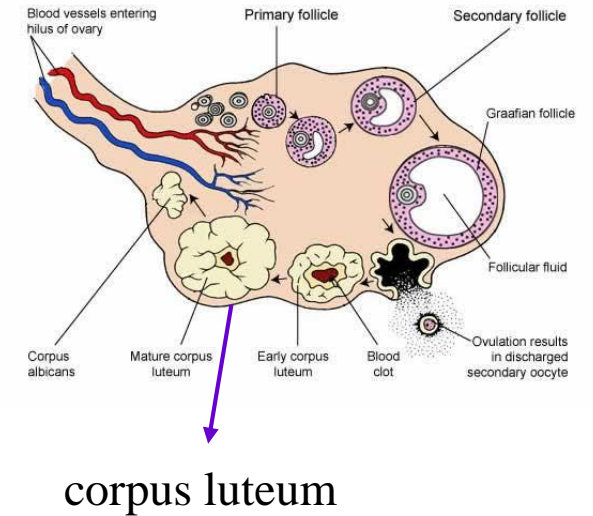
-Human chorionic gonadotropin:

- Human chorionic gonadotropin (hCG) is a glycoprotein hormone comprising 2 subunits, alpha and beta, which 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.



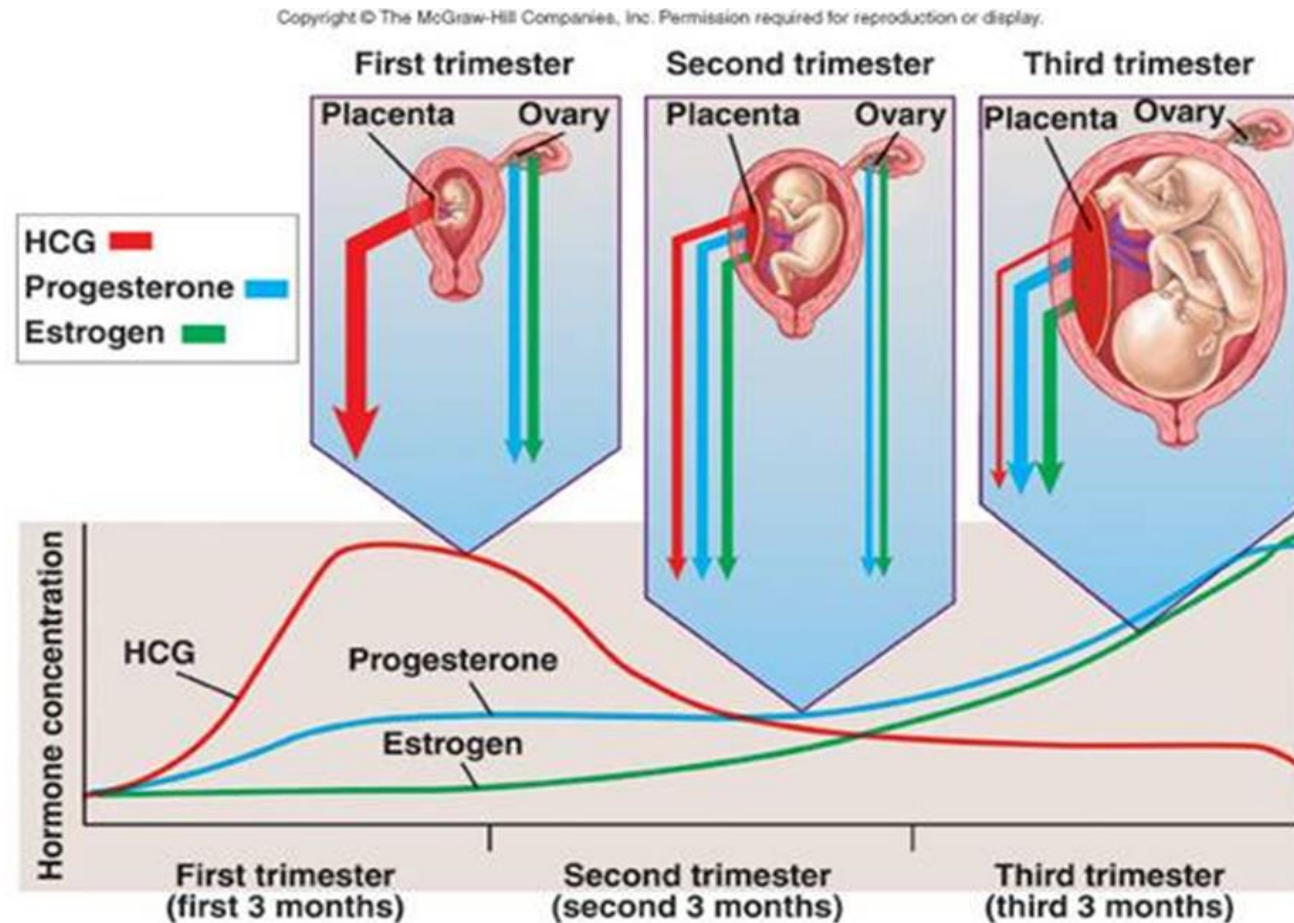
-hCG Role in pregnancy:

- **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 or men, elevated levels of hCG can lead to a cancer diagnosis since some cancerous tumors produce this hormone (tumor marker).

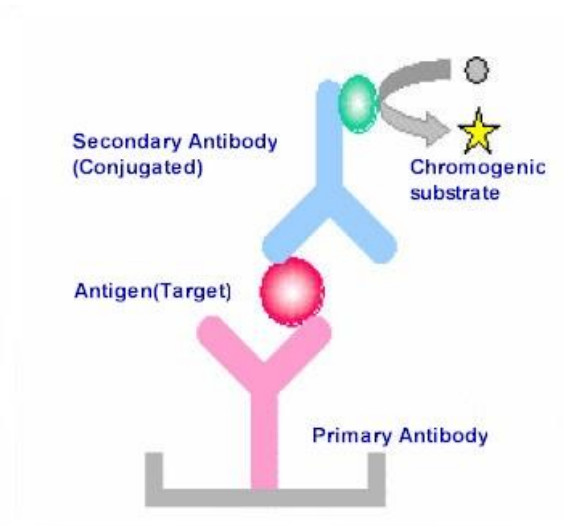
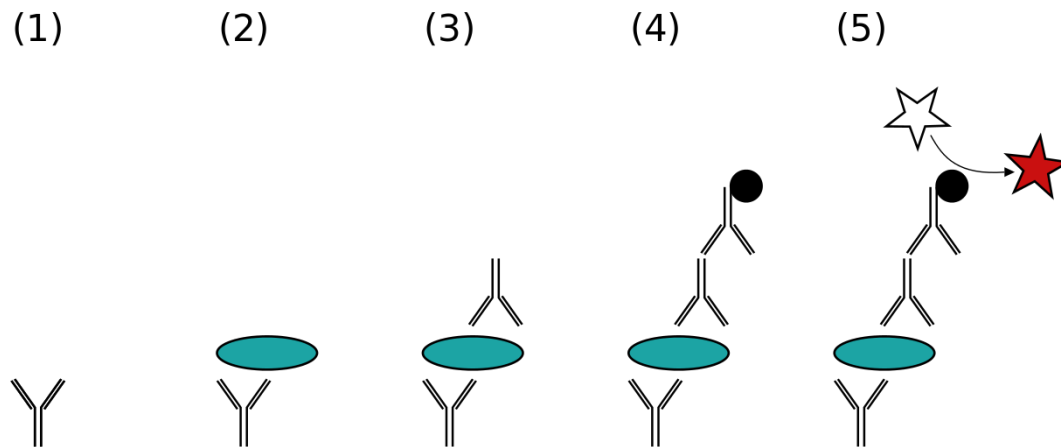
Practical Part

-Objective:

- To detect and confirm pregnancy using hCG test strip.

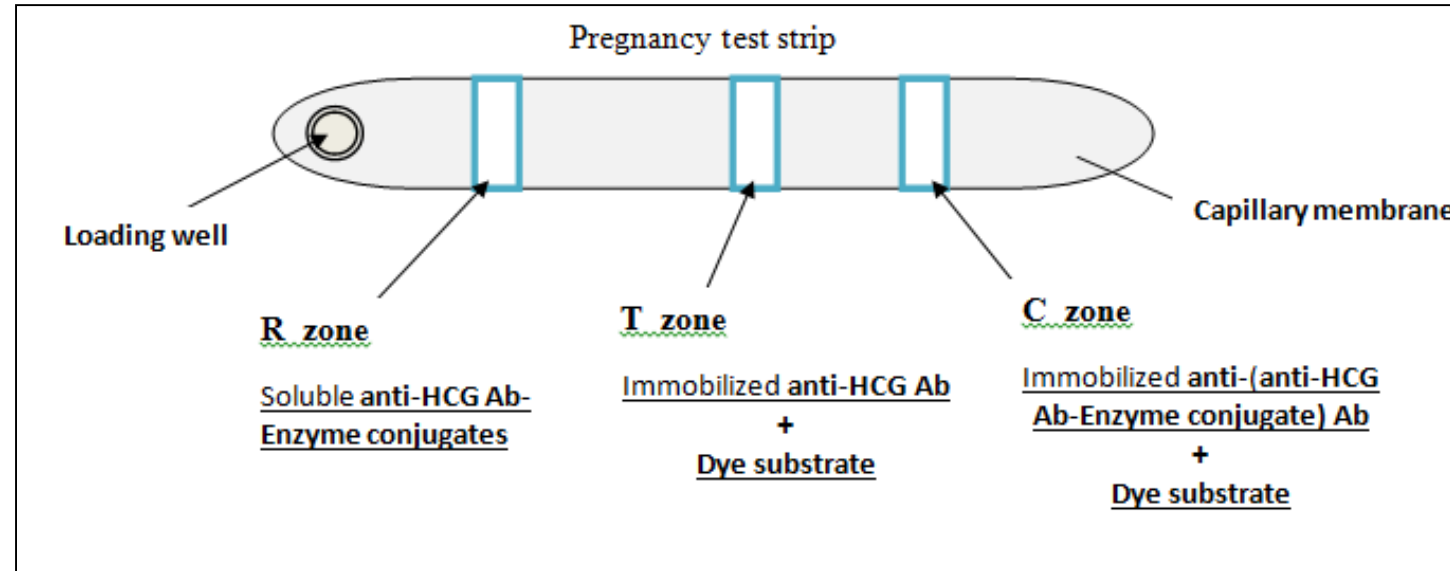
-Principle:

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



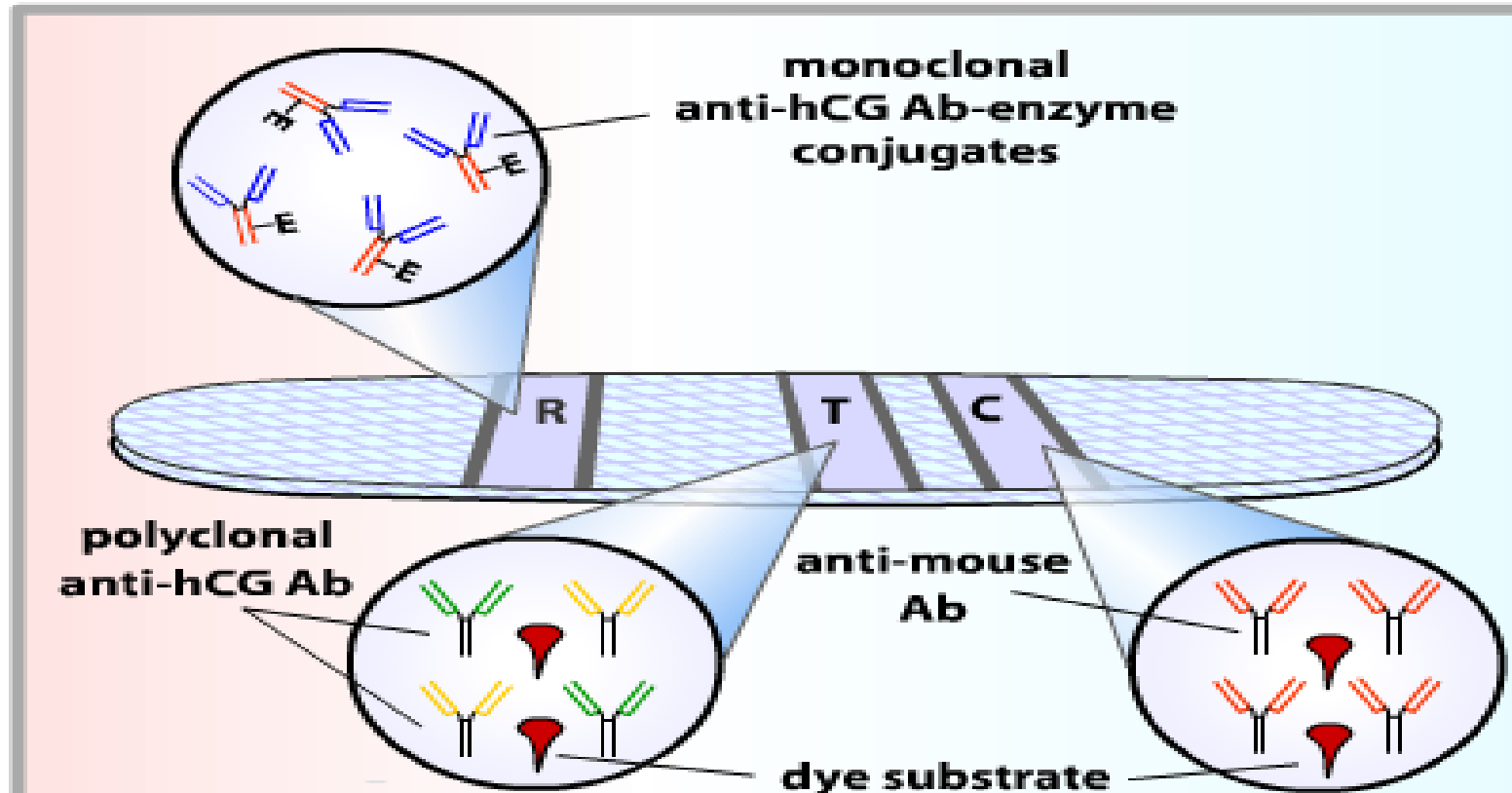
-Principle cont':

- 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 is like a control sample)

-Different zones in pregnancy test strip:

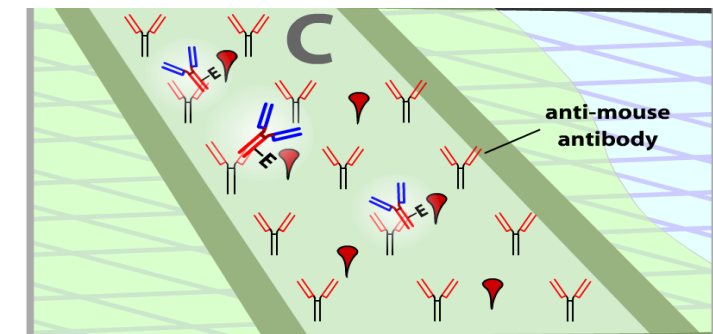
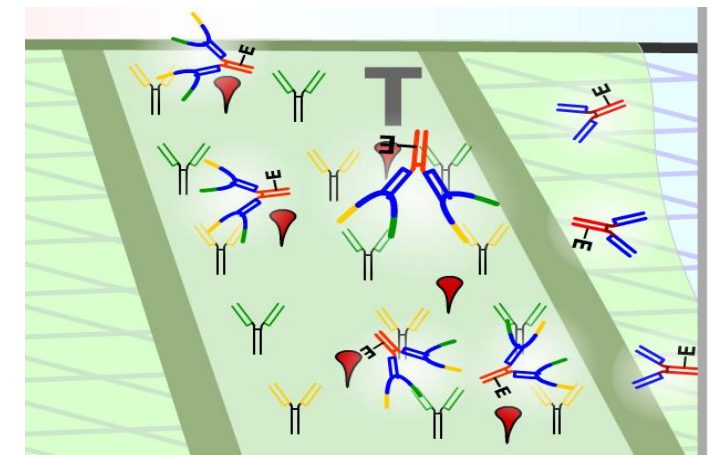
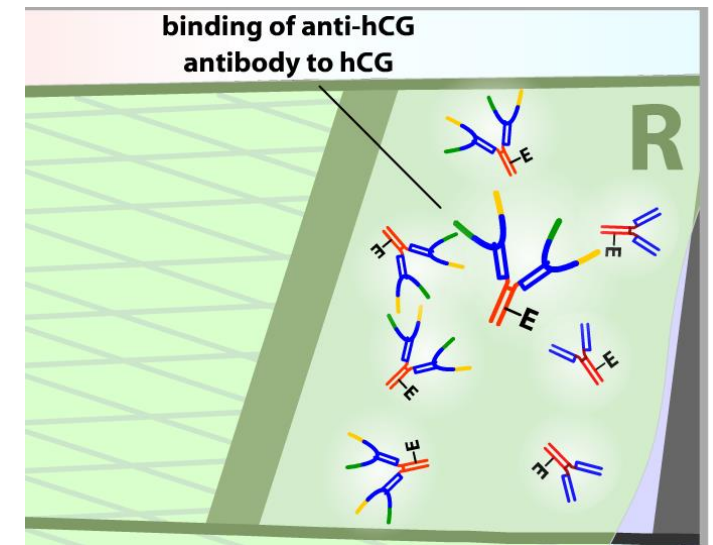


- A nice animation explain the principle of hCG test strip:
<http://www.sumanasinc.com/webcontent/animations/content/pregtest.html>

-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 Ab - enzyme conjugates 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.
- Follow the instructions on the reagent package insert provided by the instructor to properly perform the test.

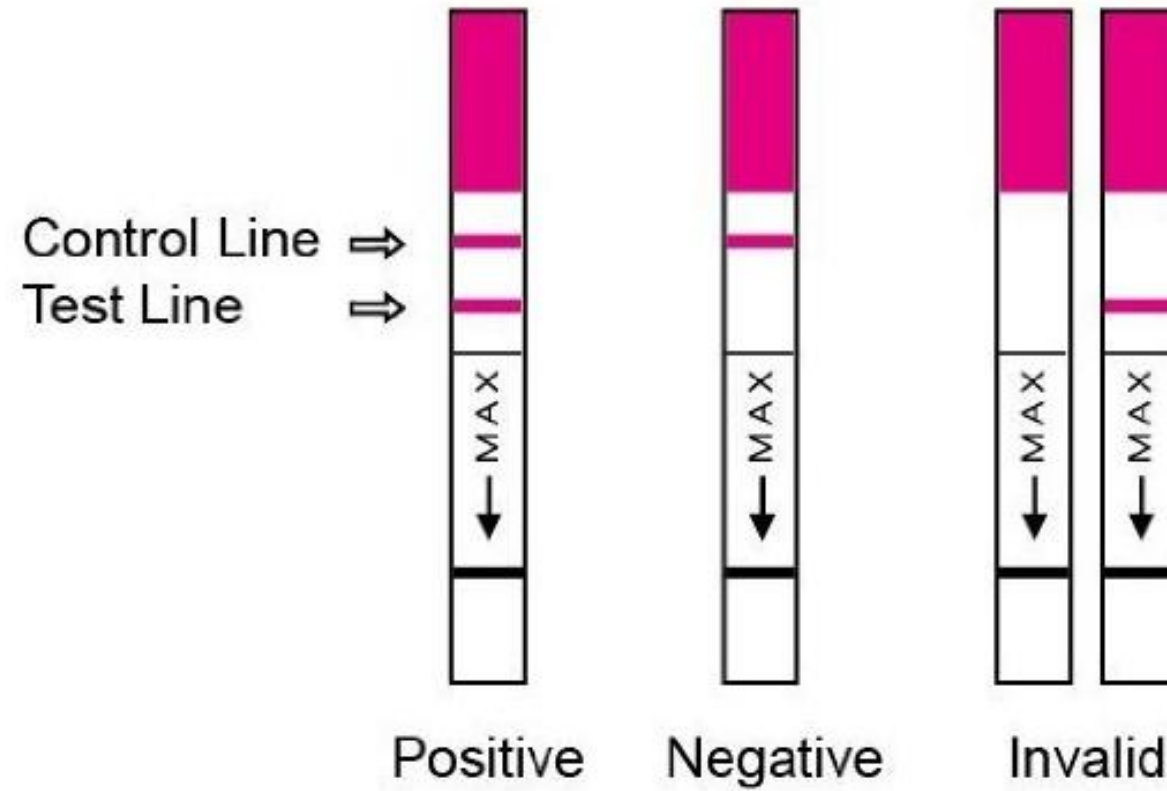
-Results:

- Record results as “**Positive**” if two lines appeared (in T and C zones) or “**Negative**” if only one line appeared in C zone.

SAMPLE TESTED	RESULT
1	

- Comment on the results and state whether the sample is pregnant or not .

-Urine test kit:



-Homework:

- Name one old method was used to test pregnancy , and explained it briefly.

-References:

- Russo IH, Koszalka M, Russo J. Effect of human chorionic gonadotropin on mammary gland differentiation and carcinogenesis. *Carcinogenesis* 1990;11:1849-1855.
- Fischbach FT, Dunning MB. *A Manual of Laboratory and Diagnostic Tests*. Lippincott Williams & Wilkins, 2009.
- Cole LA. New discoveries on the biology and detection of human chorionic gonadotropin. *Reprod. Biol. Endocrinol.* 2009.
- https://embryology.med.unsw.edu.au/embryology/index.php/Human_Chorionic_Gonadotropin#cite_note-PMID22455390-1