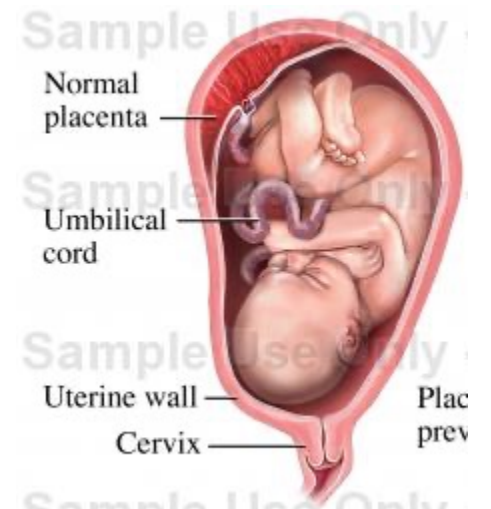


Pregnancy Test

(Detecting Human chorionic gonadotropin in urine)

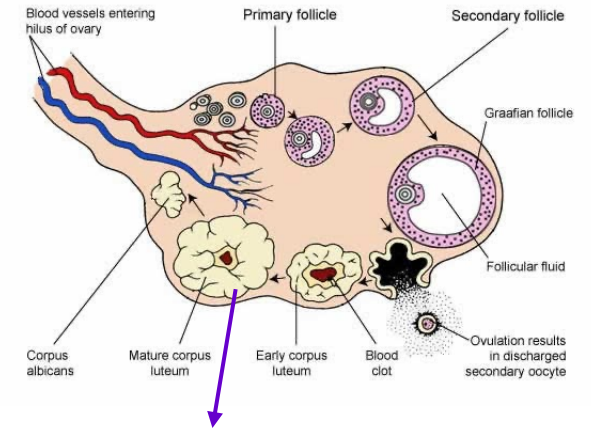
-Human chorionic gonadotropin (hCG):

- 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**.

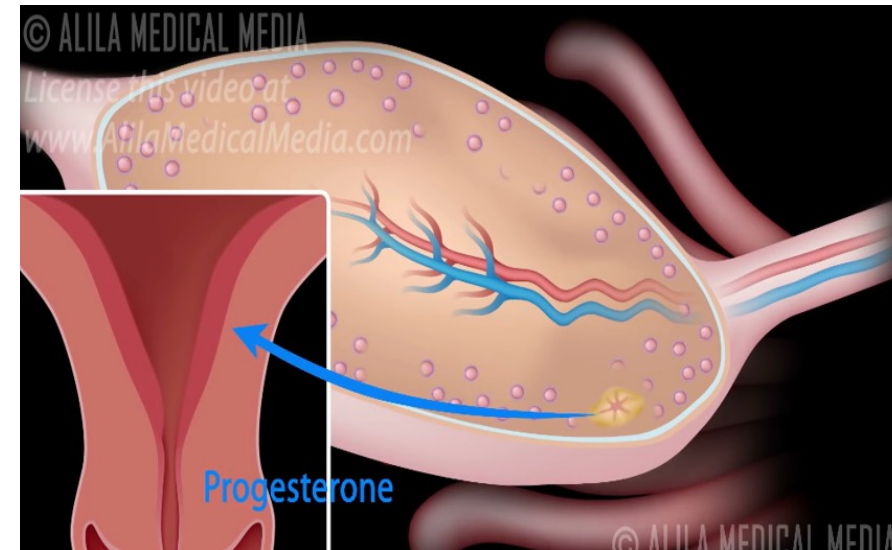
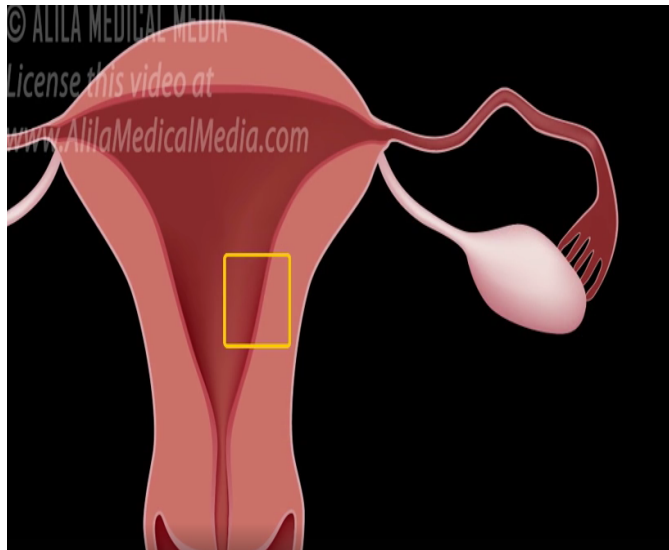


-hCG Role in pregnancy:

- 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.

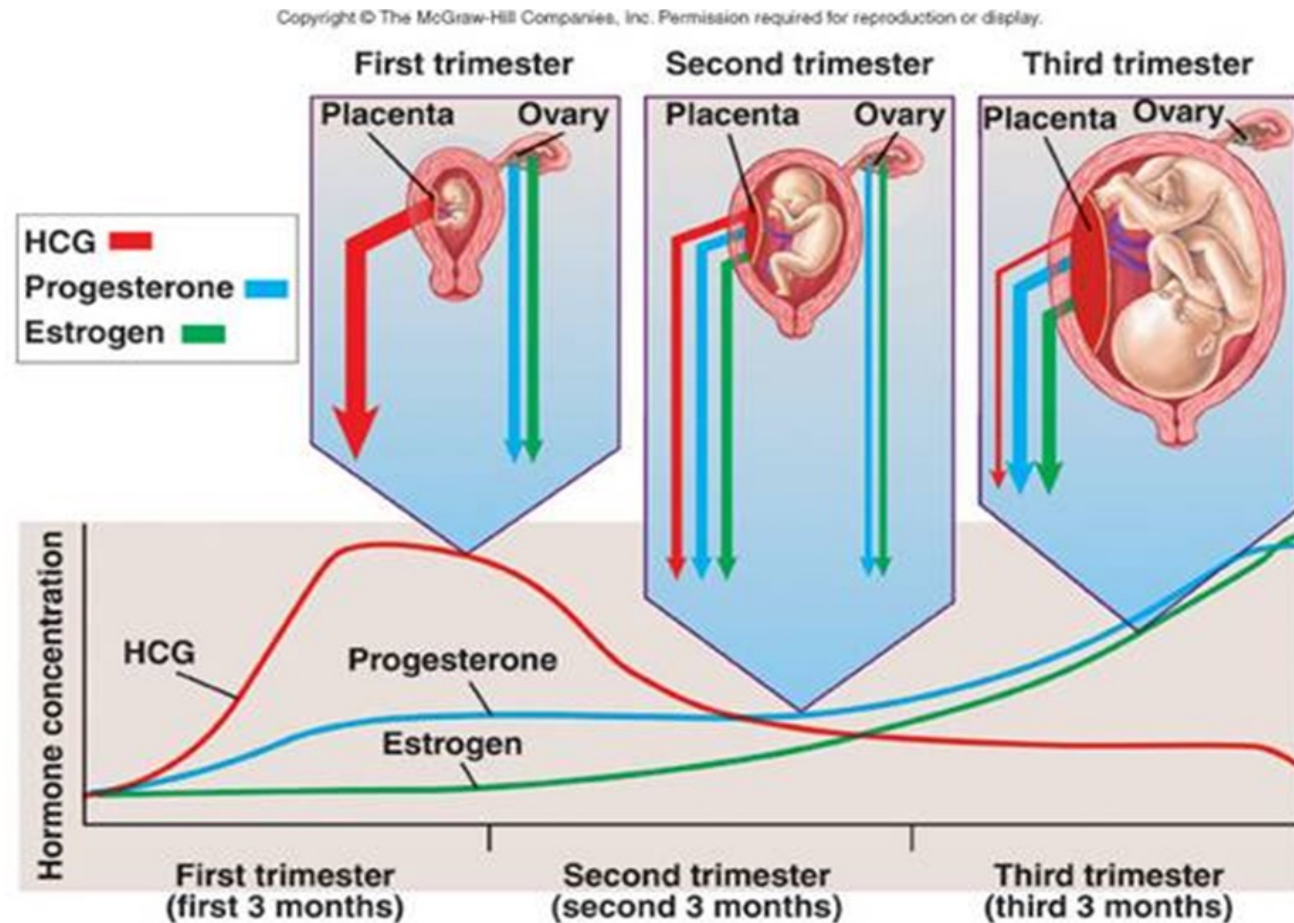


corpus luteum



-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.



-Quantitative hCG determination:

- **Quantitative** hCG measurement helps determine the exact age of the fetus. It can also assist in the diagnosis of abnormal pregnancies and possible miscarriages.
- **What Abnormal Results Mean:**
 - **Higher than normal level may indicate:**
 - A multiple pregnancy, such as twins or triplets.
 - A molar pregnancy, when an abnormal mass forms inside the uterus after fertilization instead of a normal embryo.
 - **Lower than normal level may indicate:**
 - Threatened spontaneous abortion (miscarriage).
 - Ectopic pregnancy.
 - Fetal death.

-hCG levels in men and nonpregnant women:

- 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).

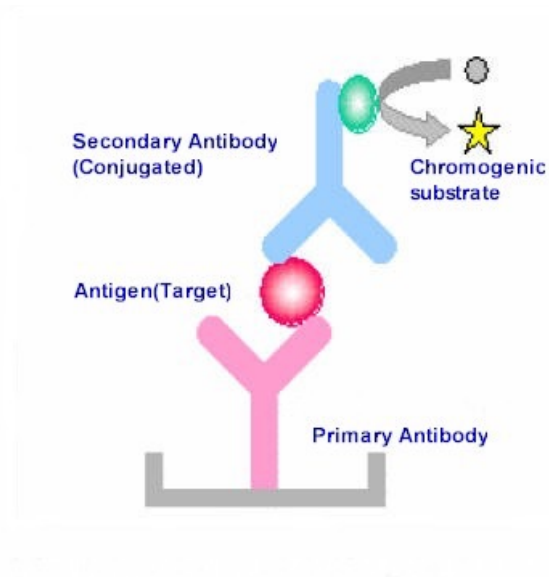
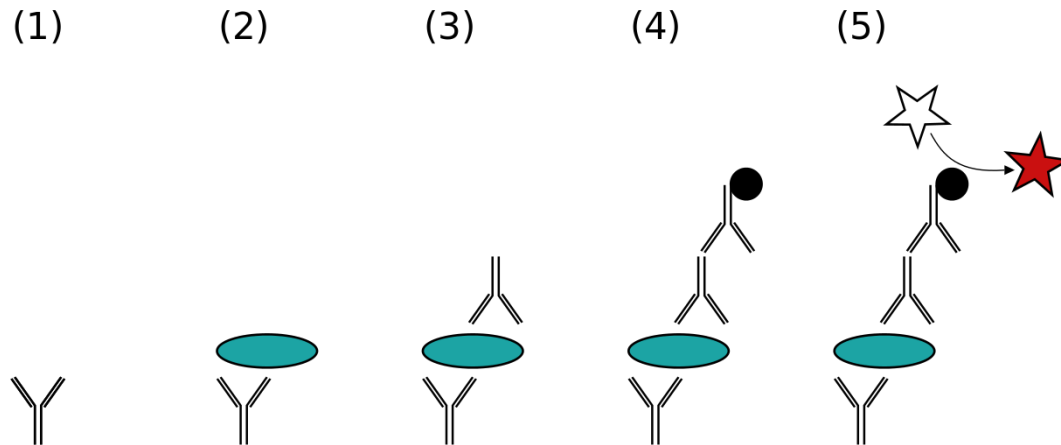
Practical Part

-Objective:

- To detect and confirm pregnancy using hCG test strip.

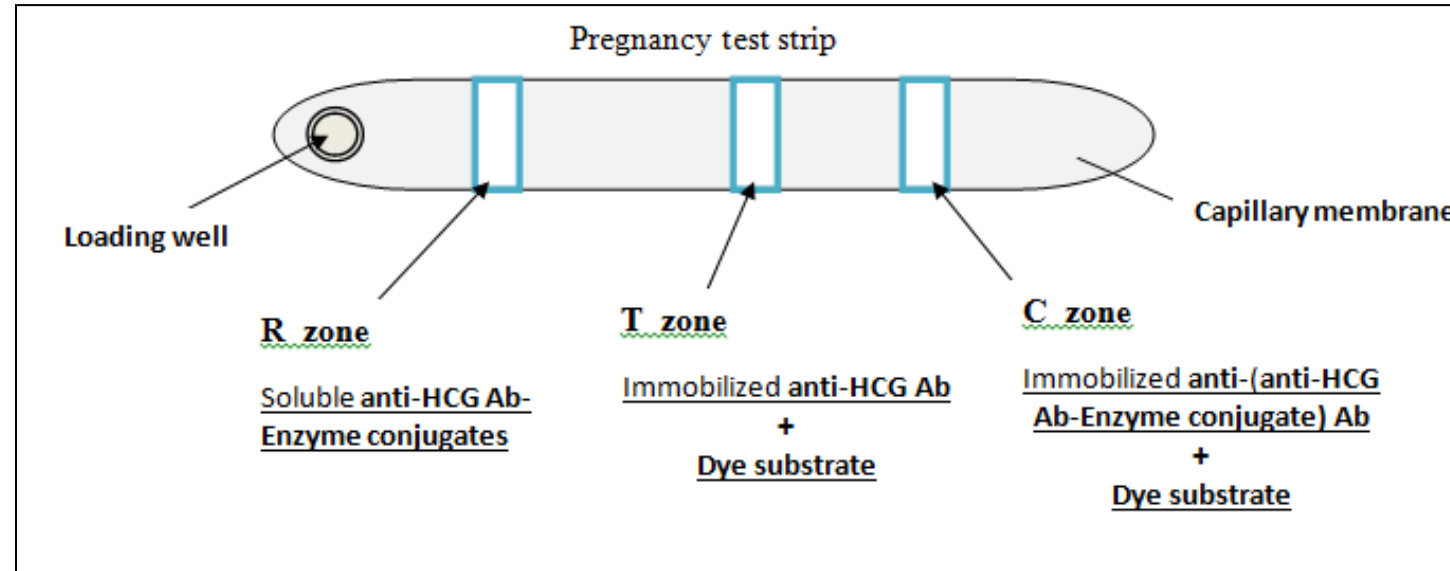
-Principle:

- 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)**.



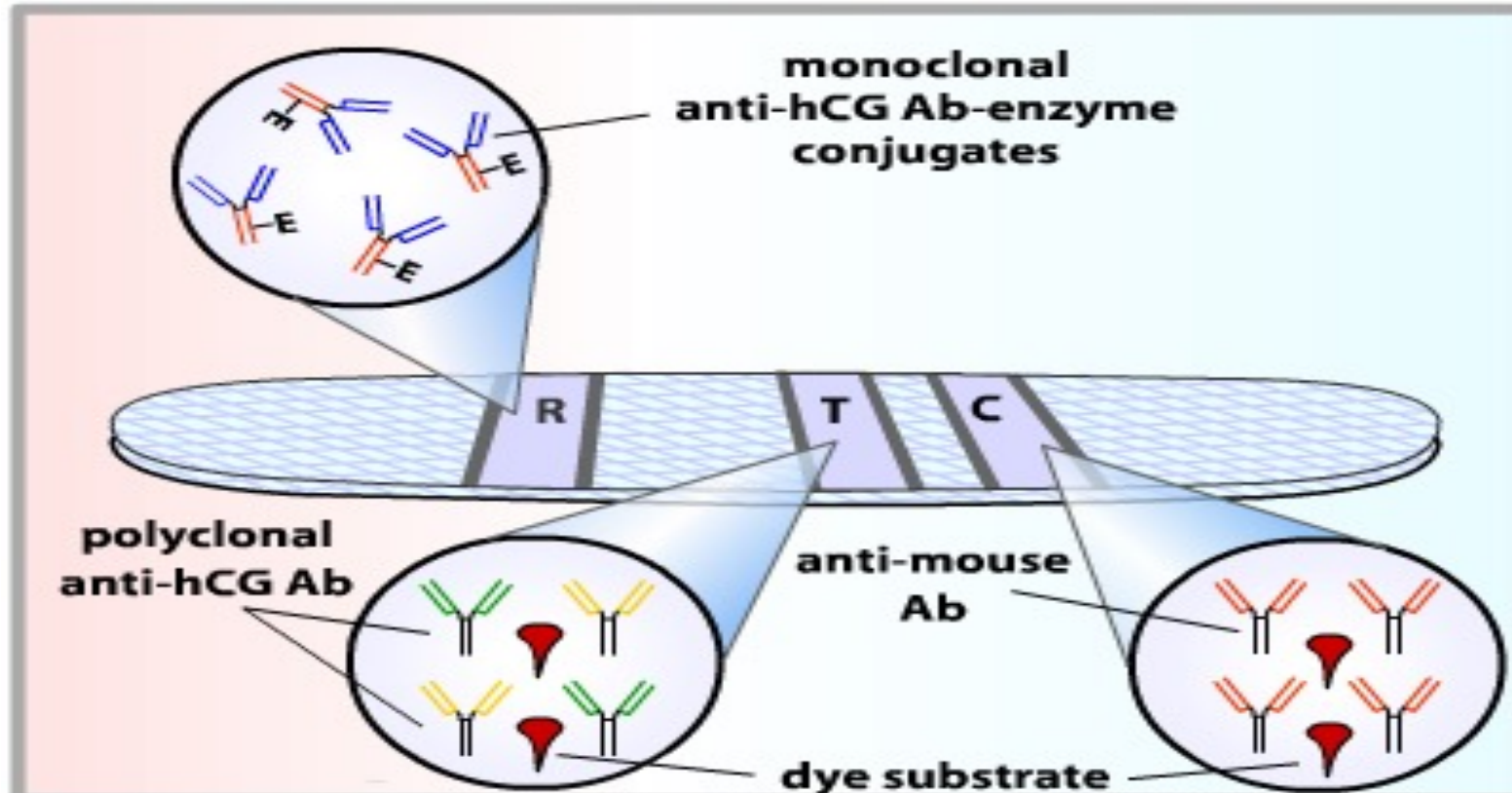
-Principle cont':

- Pregnancy test strip consist of :



1. **The reaction zone (R zone):** soluble anti-hCG antibody-enzyme conjugate. These are 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-antibodies can recognize epitopes on the monoclonal. (control zone is like a control sample)

-Different zones in pregnancy test strip:

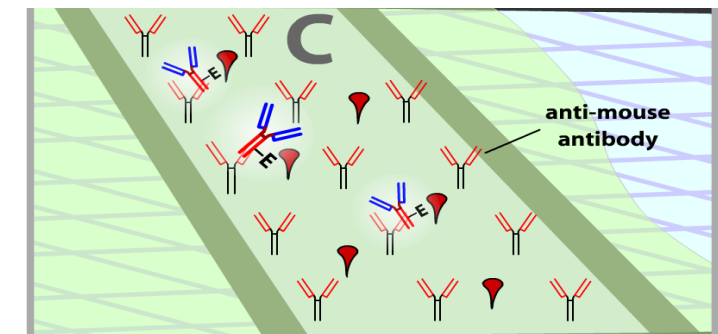
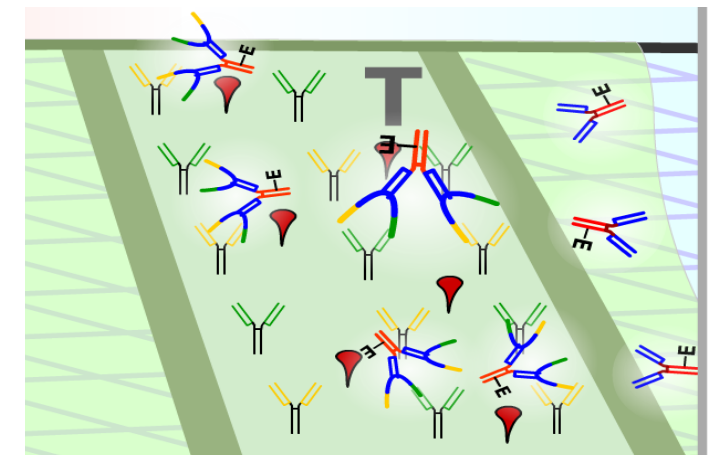
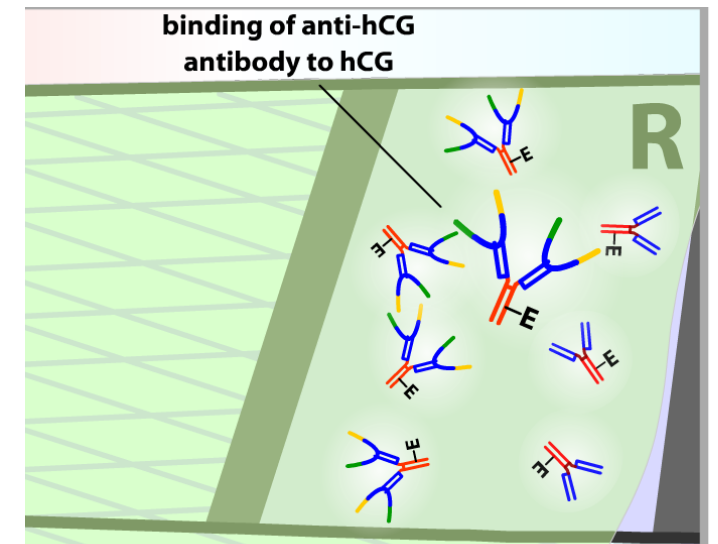


- A nice animation explain the principle of hCG test strip:
<http://www.sumanasinc.com/webcontent/animations/content/pregtest.html>

-Principle cont':

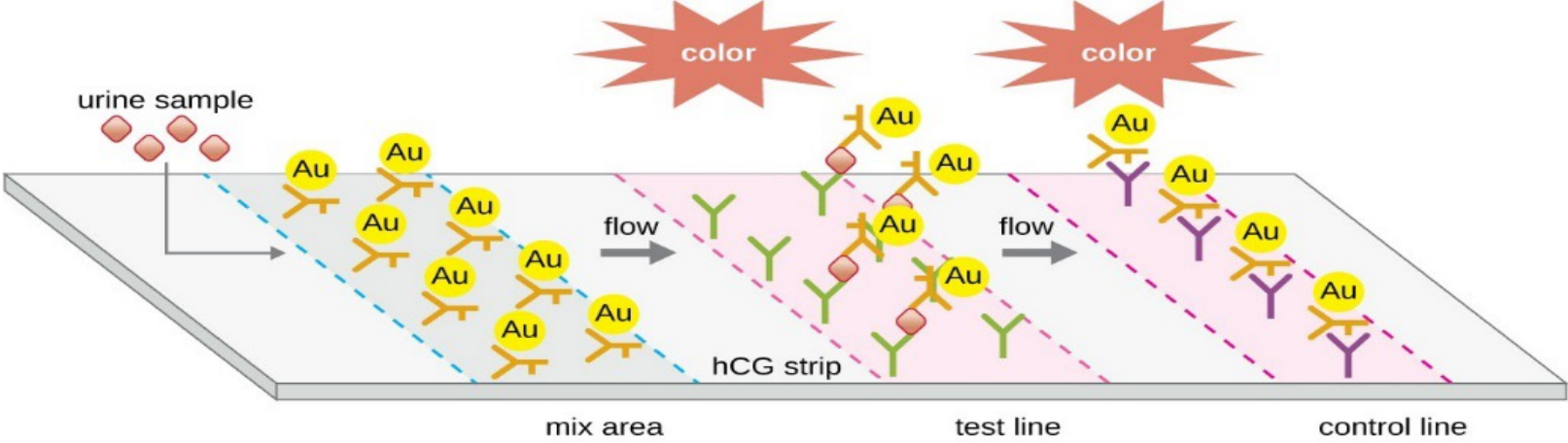
• Sequence of events in pregnant women:

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**.

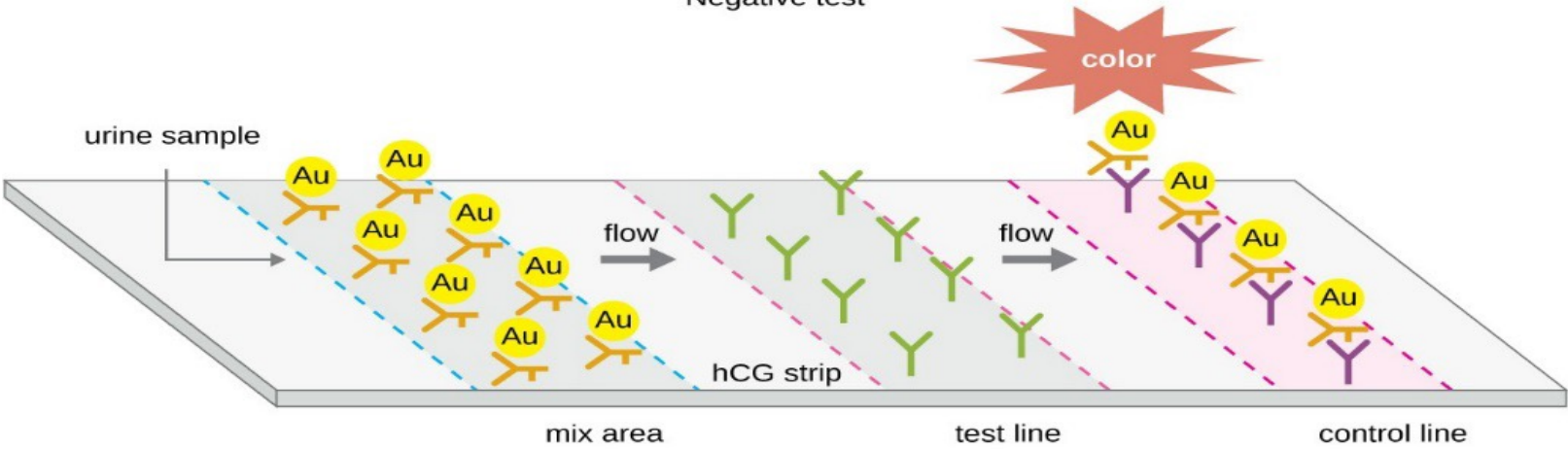


Positive test

- 1 Human chorionic gonadotropin (hCG) urine sample is applied to absorbent sample pad.
- 2 hCG antigen bonds with the anti-hCG antibody-colloidal gold conjugates.
- 3 hCG antigen bound to anti-hCG antibody-colloidal gold conjugate is captured by immobilized anti-hCG antibody.
- 4 Free hCG antibody-colloidal gold is captured by antibodies.



Negative test

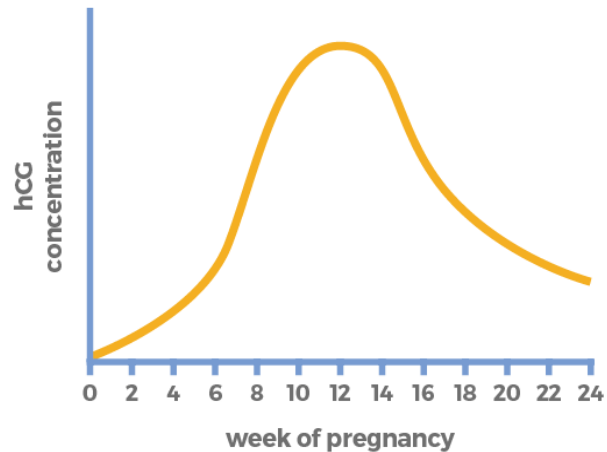


Y hCG-first antibody
 ● human chorionic gonadotropin (hCG)
 Au hCG-second antibody-AuNPs (hCG-GC)
 Y IgG

HOW DO PREGNANCY TESTS WORK?

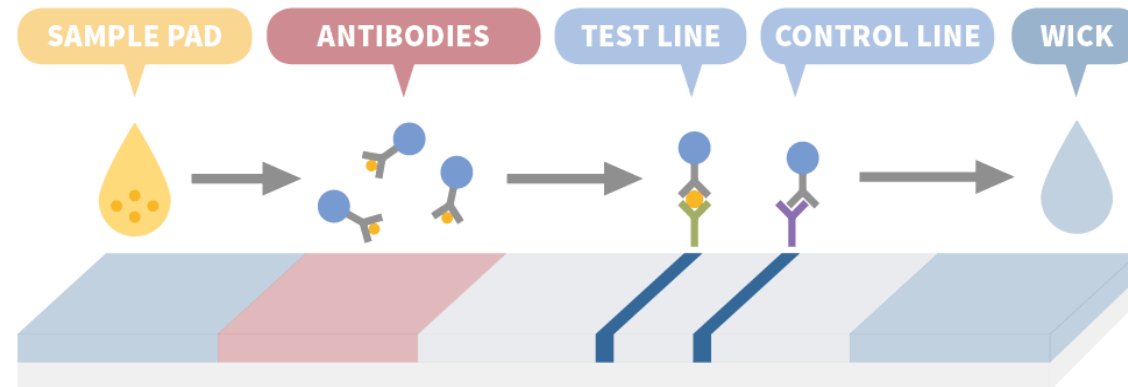
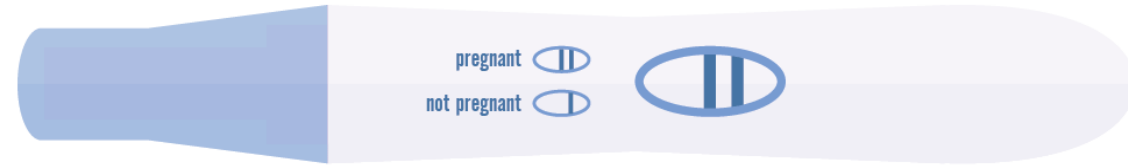
WHAT DO PREGNANCY TESTS DETECT?

Pregnancy tests detect a hormone called human chorionic gonadotropin (hCG). This hormone is produced by the placenta from the time at which the embryo attaches to the uterus.



hCG is essential for the function of the corpus luteum, a temporary structure in the ovaries that produces the hormones progesterone and estrogen. It has also been linked to early pregnancy symptoms such as nausea and vomiting. hCG is eliminated in urine and can be detected by pregnancy tests around 9 days after fertilisation.

HOW DO PREGNANCY TESTS WORK?



Urine applied to the sample pad. If a woman is pregnant, urine contains hCG.

hCG binds to mobile antibodies. These antibodies also have an enzyme attached to them.

Immobilised antibodies in the test zone bind to hCG. The enzyme on the first antibody changes the test line colour. Excess antibodies bind to immobilised antibodies in the control zone to show the test worked correctly.

-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:

- 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.

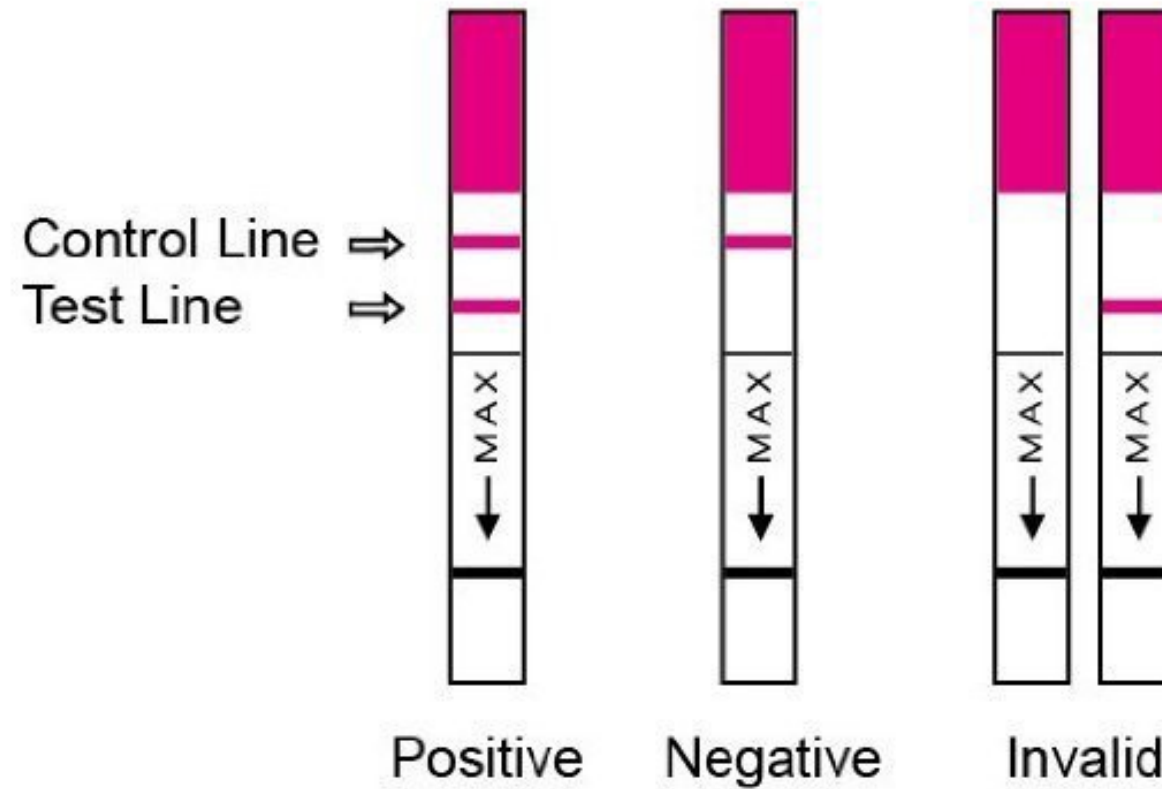
-Results:

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

	Result
Sample tested	

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

-Urine test kit:



-References:

- Russo IH, Koszalka M, Russo J. Effect of human chorionic gonadotropin on mammary gland differentiation and carcinogenesis. *Carcinogenesis* 1990;11:1849-1855.
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- https://embryology.med.unsw.edu.au/embryology/index.php/Human_Chorionic_Gonadotropin#cite_note-PMID22455390-1
- <https://www.ucsfhealth.org/medical-tests/hcg-blood-test---quantitative#:~:text=Quantitative%20HCG%20measurement%20helps%20determine,screening%20test%20for%20Down%20syndrome.>