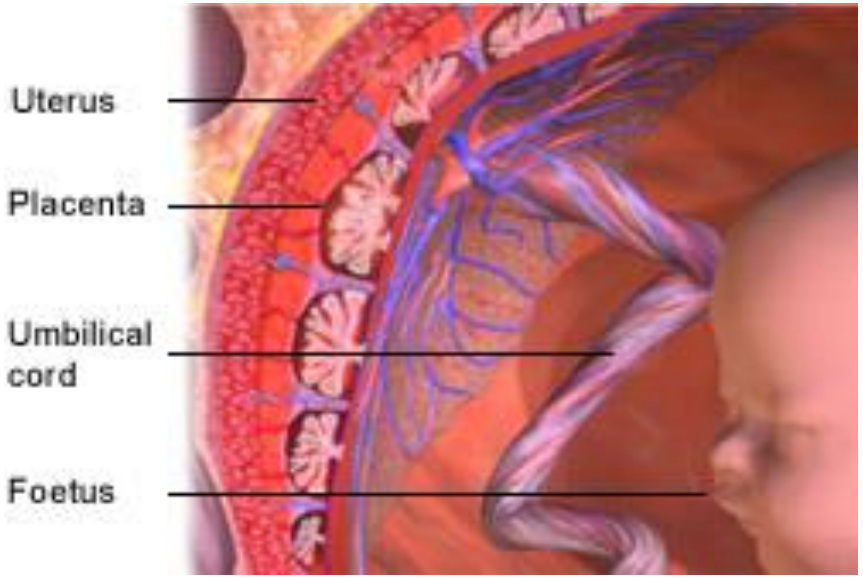
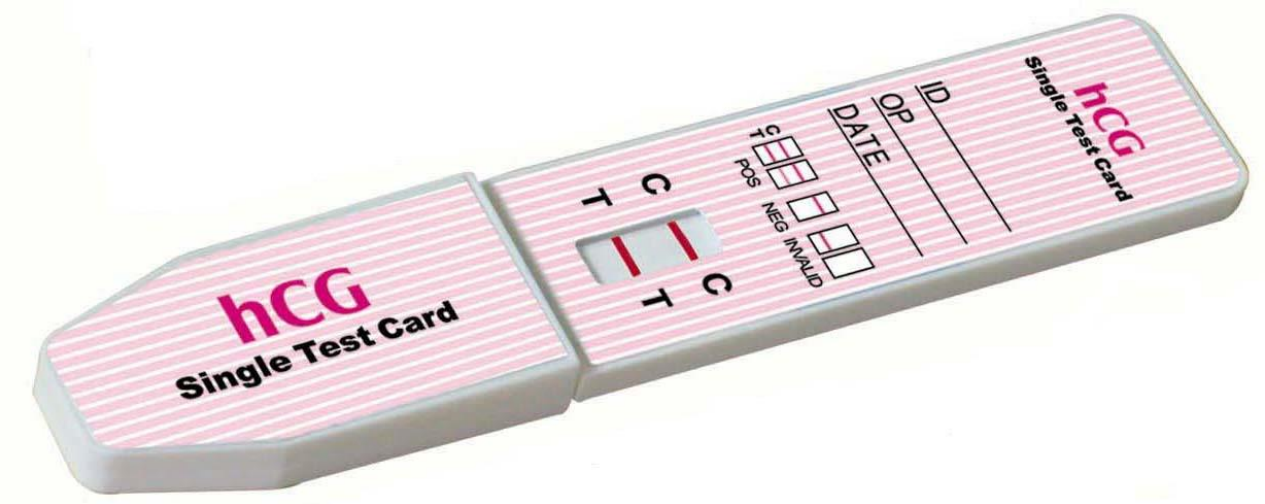
Pregnancy Test on a Urine Sample

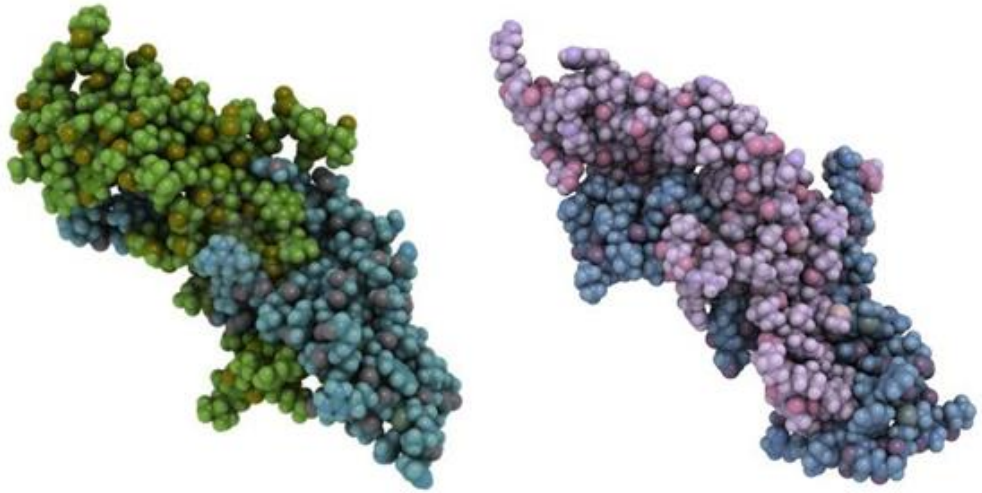




Molecular characteristics of HCG

- hCG is a glycoprotein composed of two subunits, αand β, held together by ionic and hydrophobic forces.
- The α - subunit is a glycopeptide of 92 aminoacids stabilized by five disulfide linkages. The aminoacid sequence of this subunit is *identical to that of the pituitary glycoprotein hormones, follicle stimulating and thyroid stimulating hormones.*
- The β-subunit is a glycopeptide of 145 amino acids stabilized by six disulfide linkages. The β-subunits of the glycoprotein hormones are *unique and give them their different biological characteristics*

Structurally, FSH, and hCG are all related. They are all di-meric glycoproteins (composed of two separate protein pieces, each with sugars attached). What differentiates them from one another is the other protein piece (the "beta subunit").

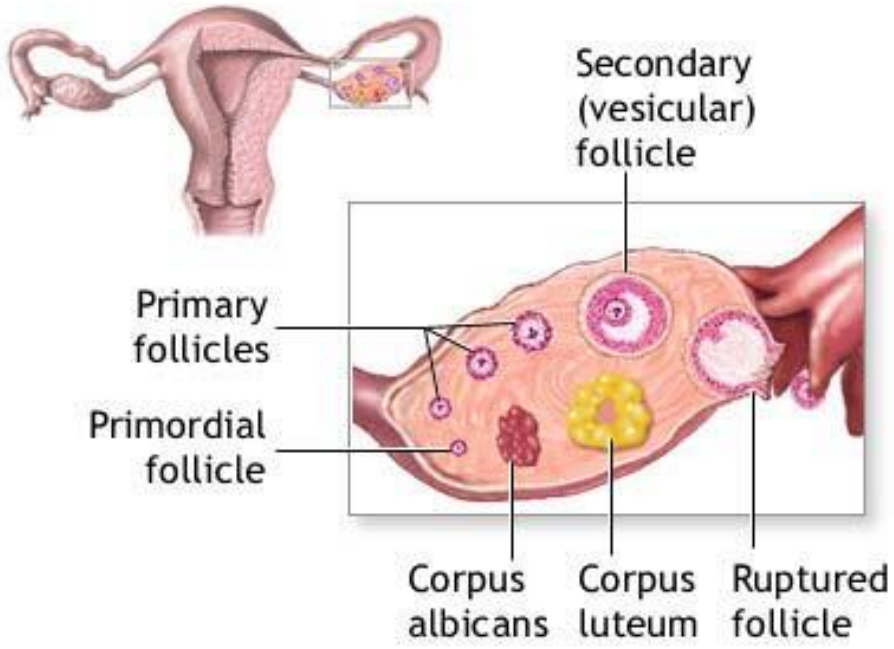


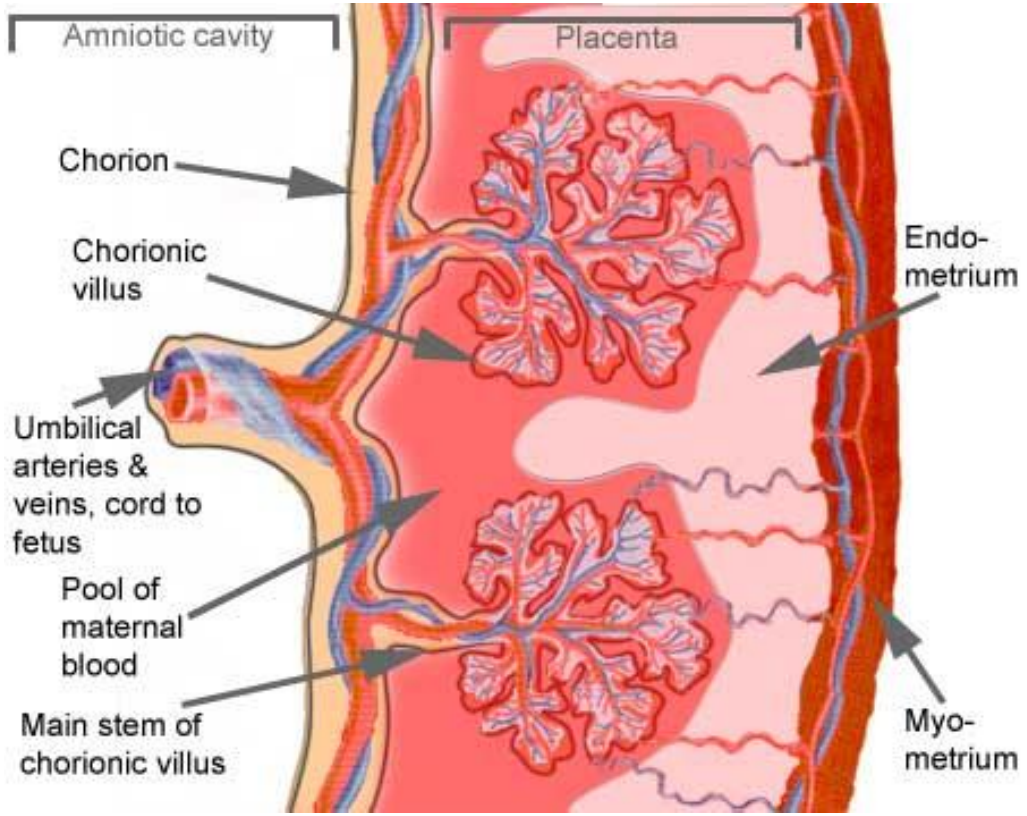
follicle-stimulating hormone (FSH)

human chorionic gonadotropin (hCG)

Function of the human chorionic gonadotropin:

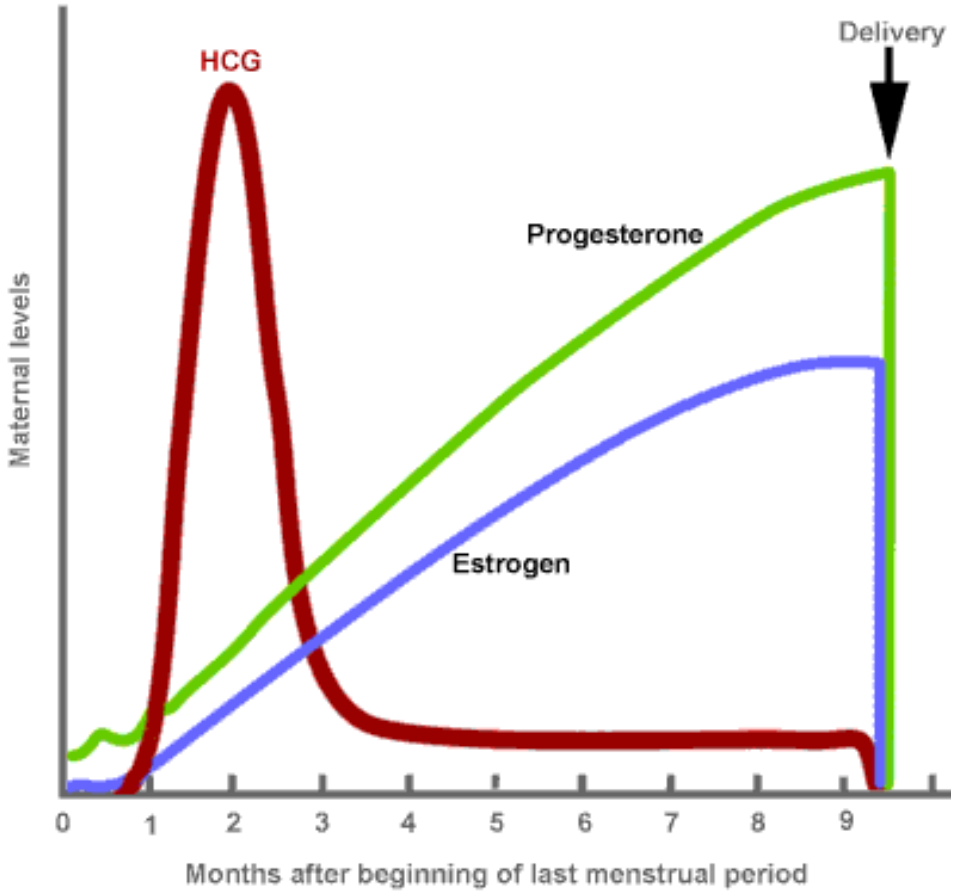
1. promotes the maintenance of the corpus luteum during the beginning of pregnancy in the ovary . This allows the corpus luteum to secrete the progesterone during the first trimester. Progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can sustain the growing fetus.
2. Human chorionic gonadotropin also plays a role in cellular differentiation/proliferation.





Hormonal changes during pregnancy

- During the first trimester, hCG levels rise steadily and rapidly, peaking around 10 weeks' gestation.
- The secretion of hCG reaches a peak 60 to 80 days after the last menstrual period, decreasing rapidly afterwards.
- By the end of the third month it has reached a low level which will remain constant for the duration of the pregnancy.
- With this decrease of hCG secretion, the placenta begins to secrete large quantities of estrogen and progesterone and the dependence on the corpus luteum for the maintenance of pregnancy disappears



Variation in secretion of hGC

The following is a list of serum hCG levels. (LMP is the last menstrual period.)

3 weeks since LMP: 5 - 50 mIU/ml

4 weeks since LMP: 5 - 426 mIU/ml

5 weeks since LMP: 18 - 7,340 mIU/ml

6 weeks since LMP: 1,080 - 56,500 mIU/ml

7 – 8 weeks since LMP: 7,650 - 229,000 mIU/ml

9 – 12 weeks since LMP: 25,700 - 288,000 mIU/ml

13 – 16 weeks since LMP: 13,300 - 254,000 mIU/ml

17 – 24 weeks since LMP: 4,060 - 165,400 mIU/ml

25 – 40 weeks since LMP: 3,640 - 117,000 mIU/ml

Non-pregnant females: <5.0 mIU/ml

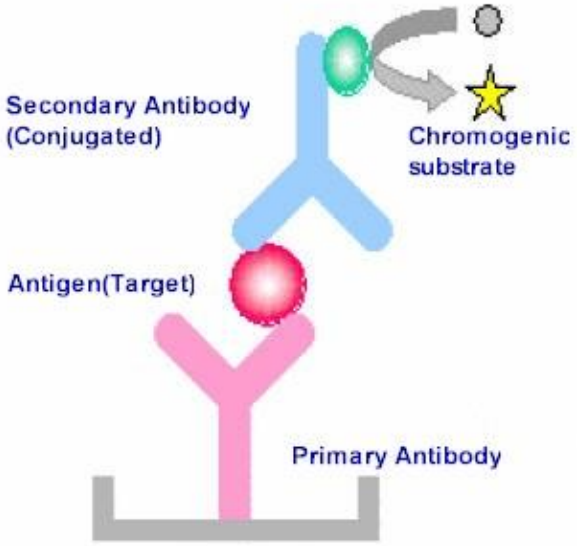
Postmenopausal females: <9.5 mIU/ml

Objective:

1. To detect and confirm pregnancy .

Principle of the test

- Many hCG immunoassays are based on the sandwich principle, which uses antibodies to hCG labeled with an enzyme, while pregnancy *urine dipstick* tests are based on the *lateral flow technique*.
- The urine test may be a *chromatographic immunoassay* or any of several other test formats, Published detection thresholds range from 20 to 100 mIU/ml.
- The serum test, using 2-4 mL of venous blood, is typically a fluorimetric immunoassay that can detect β-hCG levels as low as 5 mIU/ml and allows quantification of the β-hCG concentration.
- If HCG is not present in the sample, or present at very low levels, only the positive control will react.



sandwich enzyme-linked immunosorbent assay (ELISA)

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.*

Limitations of the Procedure

A "*false positive*" pregnancy test is one in which the pregnancy test is positive but the patient is not pregnant.

1. HCG has been found in patients with both **trophoblastic disease**.

Gestational trophoblastic disease *(GTD) is a term used for a group of pregnancy-related tumors . These tumors are rare, and they appear when cells in the womb start to proliferate uncontrollably. The cells that form gestational trophoblastic tumors are called trophoblasts and come from tissue that grows to form the placenta during pregnancy.*

Limitations of the Procedure

A "*false negative*" pregnancy test is one in which the patient is pregnant but the result of the pregnancy test

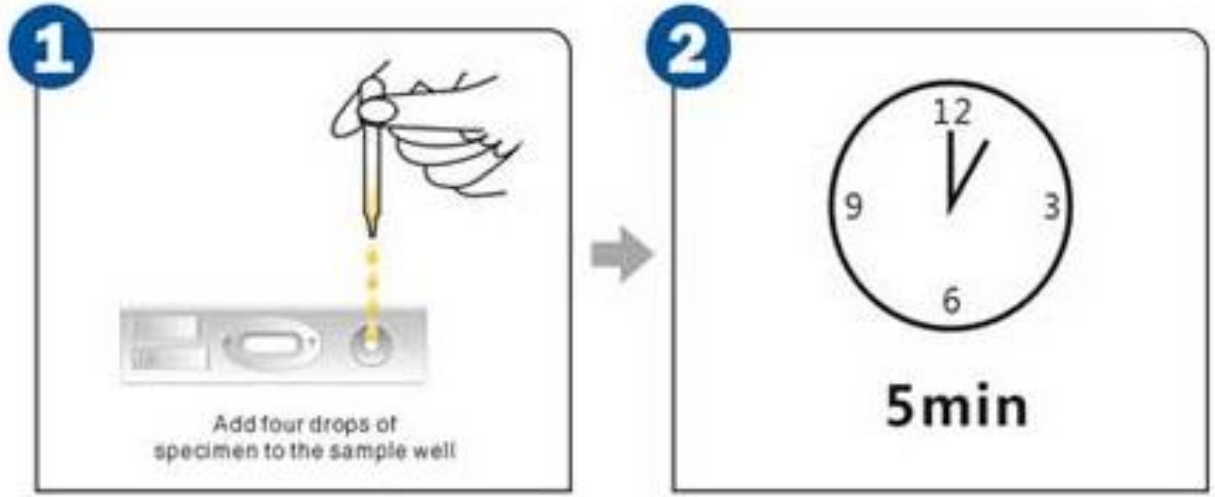
is negative.

False negative results are more common.

1. The primary cause of false negative pregnancy tests is **inaccuracies in timing of the test or in the procedure itself.**
2. A second cause is that the **sample was not a first morning** and the patient is **recently pregnant** (HCG levels are too low).

Test Procedure

Urine LH Cassette



Result Reading

PROCEDURE

- NOTE: Bring test components and specimens to room temperature prior to testing.
- Remove a Testing Device from the foil pouch by tearing at the "notch" and place it on a level surface.
- Holding a Sample Dropper vertically, add exactly four drops of the urine specimen to the sample well. NOTE: Picture shows incorrect orientation of dropper to test area, must be completely vertical to ensure adequate sample.
- Read results at time indicated in procedure.

Results

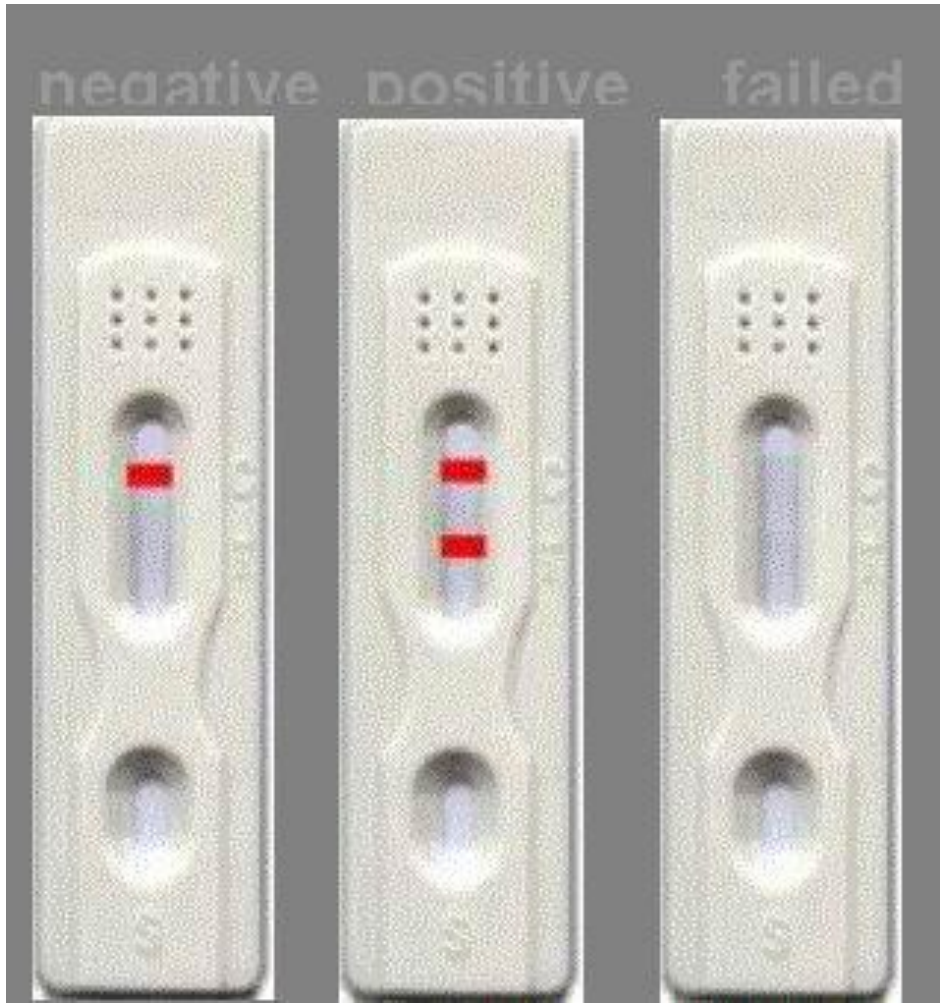
- Follow the instructions on the reagent package insert provided by the instructor to properly perform the test..

Interpretation of Results:

- Based on the package insert correctly interpret the results of the pregnancy test on the 2 patient samples
- Record results as "***Positive***" or "***Negative***"

SAMPLE TESTED	RESULT
CONTROL	
1	
2	

Urine test kit



Discussion

Comment on the results and state which sample is pregnant .

Questions

Can HCG hormone used as tumor marker? and if it is yes in which cases?

References:

- http://www.medicine.mcgill.ca/physio/vlab/other_exps/endo/reprod_horm.htm
- https://infertilechemist.wordpress.com/tag/hcg/