جامعة الملك سعود
King Saud University
1957

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.

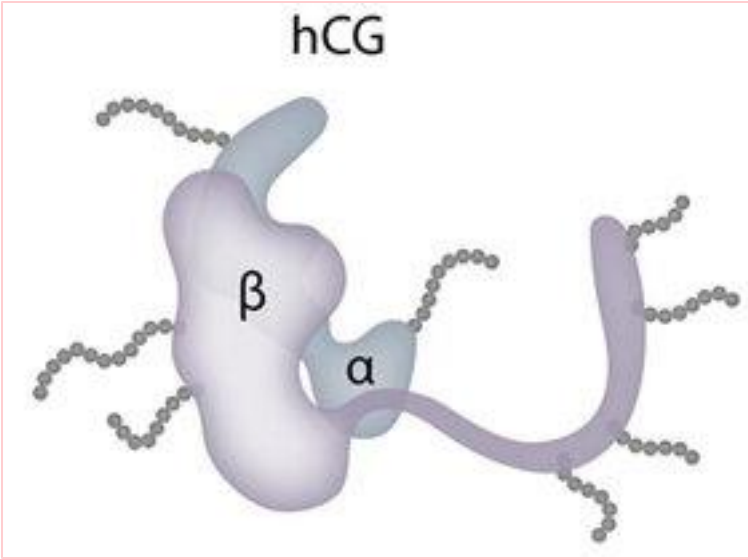
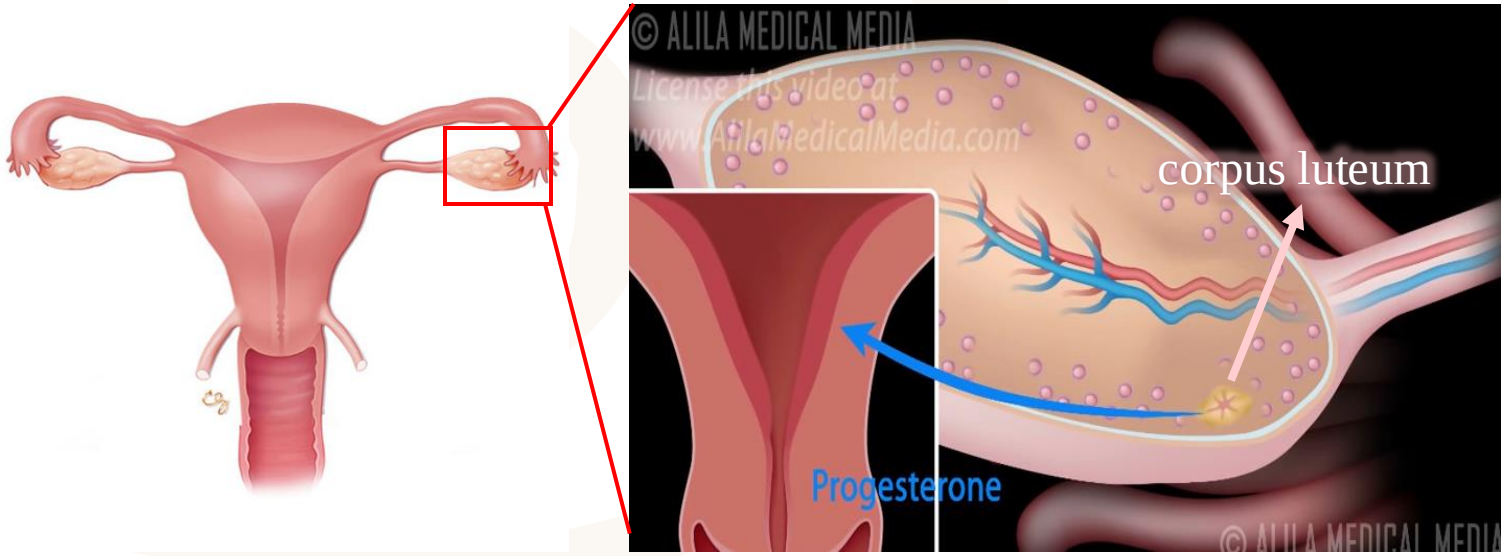


Figure 1. hCG structure, Source: researchgate.net

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.



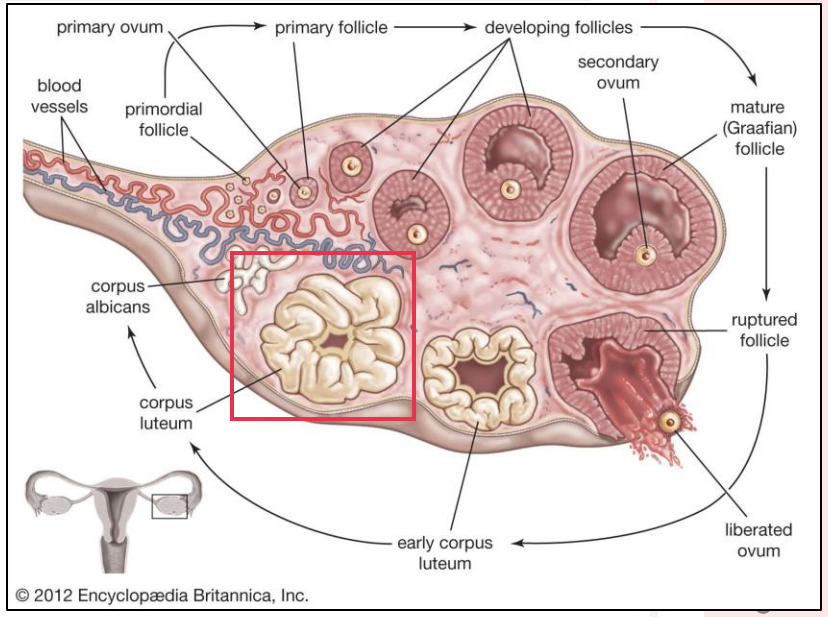


Figure 2. The steps of ovulation, Source: britannica.com

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.

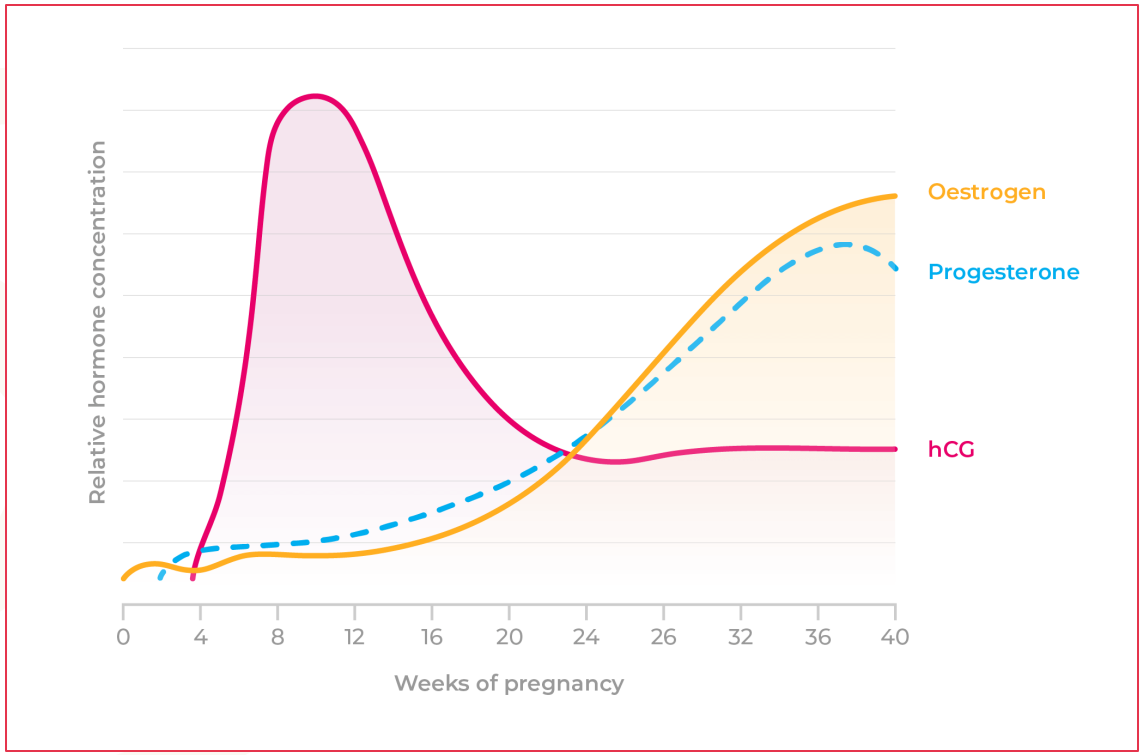


Figure 3. hormones levels during pregnancy, Source: medichecks.com

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.

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 **(stillbirth)**.

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**).

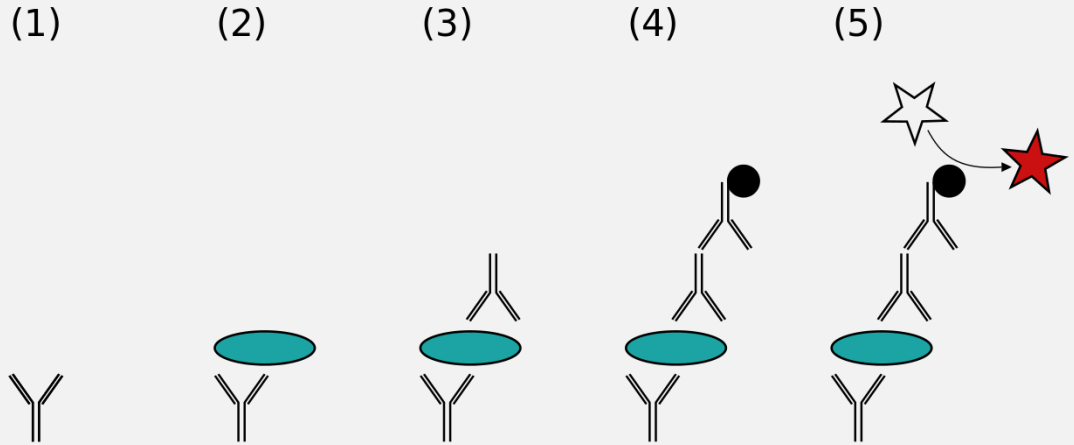
Practical Part

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).**



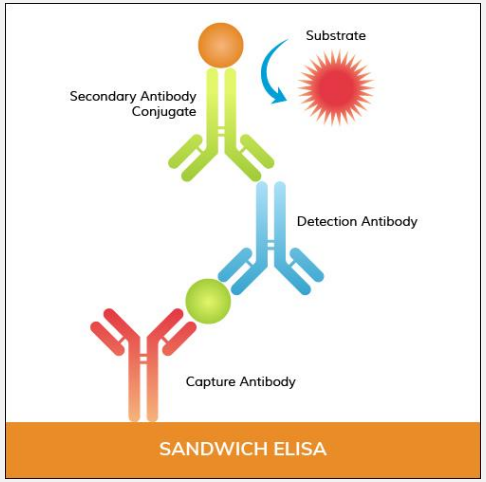


Figure 4. Sandwich ELISA principle, Source: bosterbio.com

Principle cont':

Pregnancy test strip consist of :

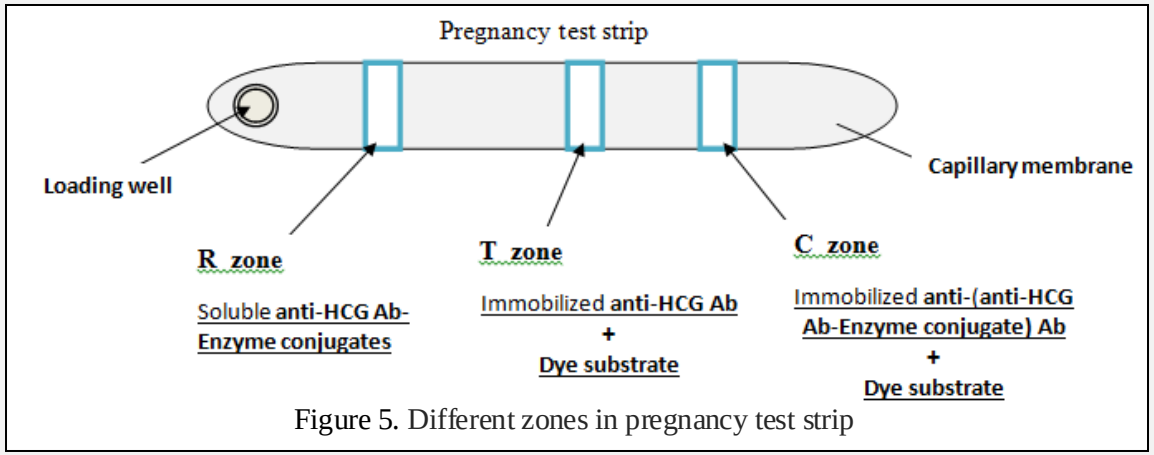


Figure 5. Different zones in pregnancy test strip

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)

Principle of hCG test strip

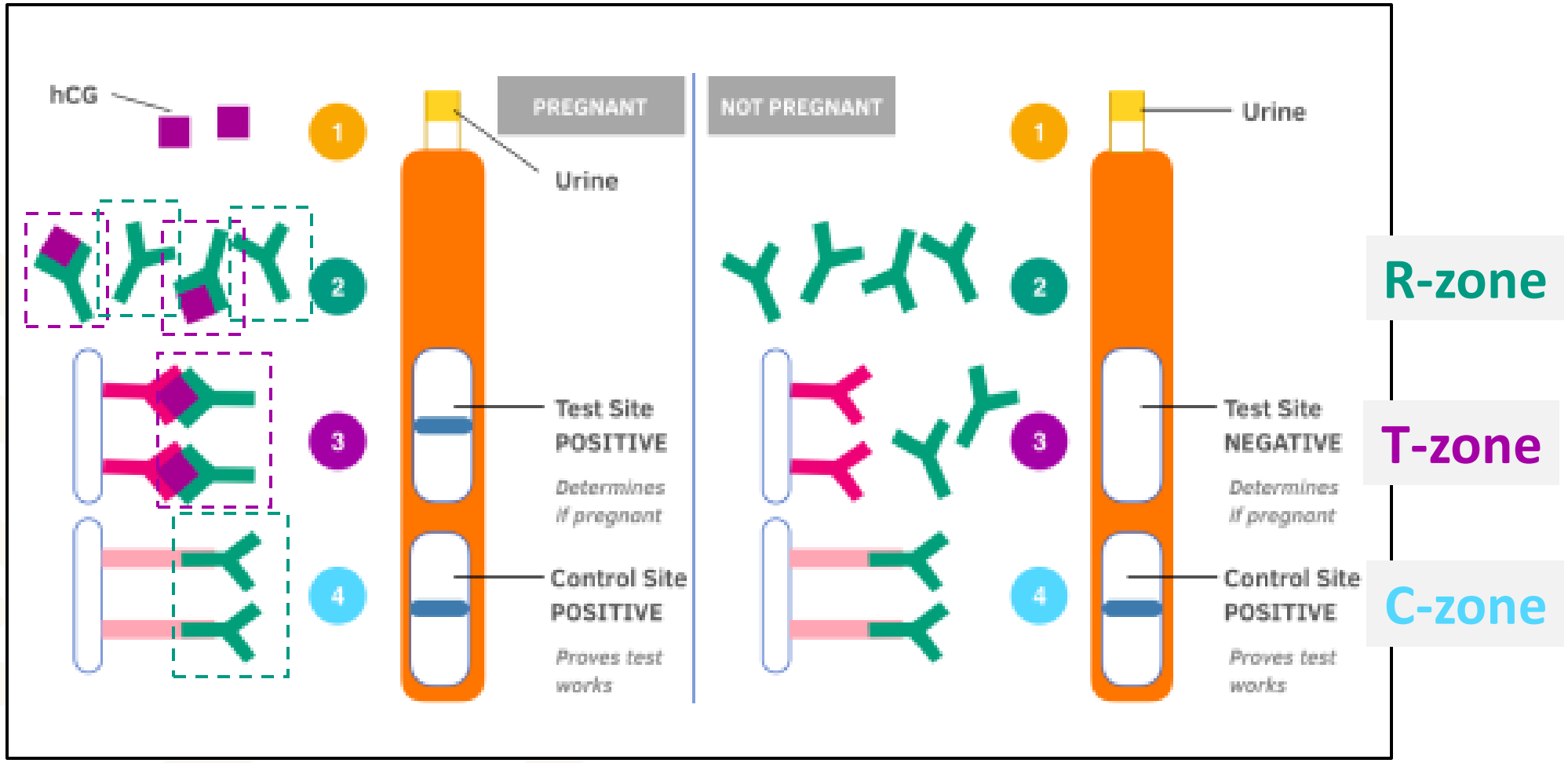


Figure 6. Principle of pregnancy test. Source: studymind.co.uk

- A nice animation explain the principle of hCG test strip: https://www.sumanasinc.com/webcontent/animations/content/pregnancytest.html

Sequence of events in pregnancy test:

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) This complex will migrate towards T zone.
4. 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. The excess soluble HCG Ab - enzyme conjugates will pass from T zone to C zone.
5. At C zone, excess soluble HCG Ab - enzyme conjugates 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:

1. Bring test components and specimens to room temperature prior to testing.
2. Follow the instructions on the reagent package insert provided by the instructor to properly perform the test.

Results:

1. 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	

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

Urine test kit:

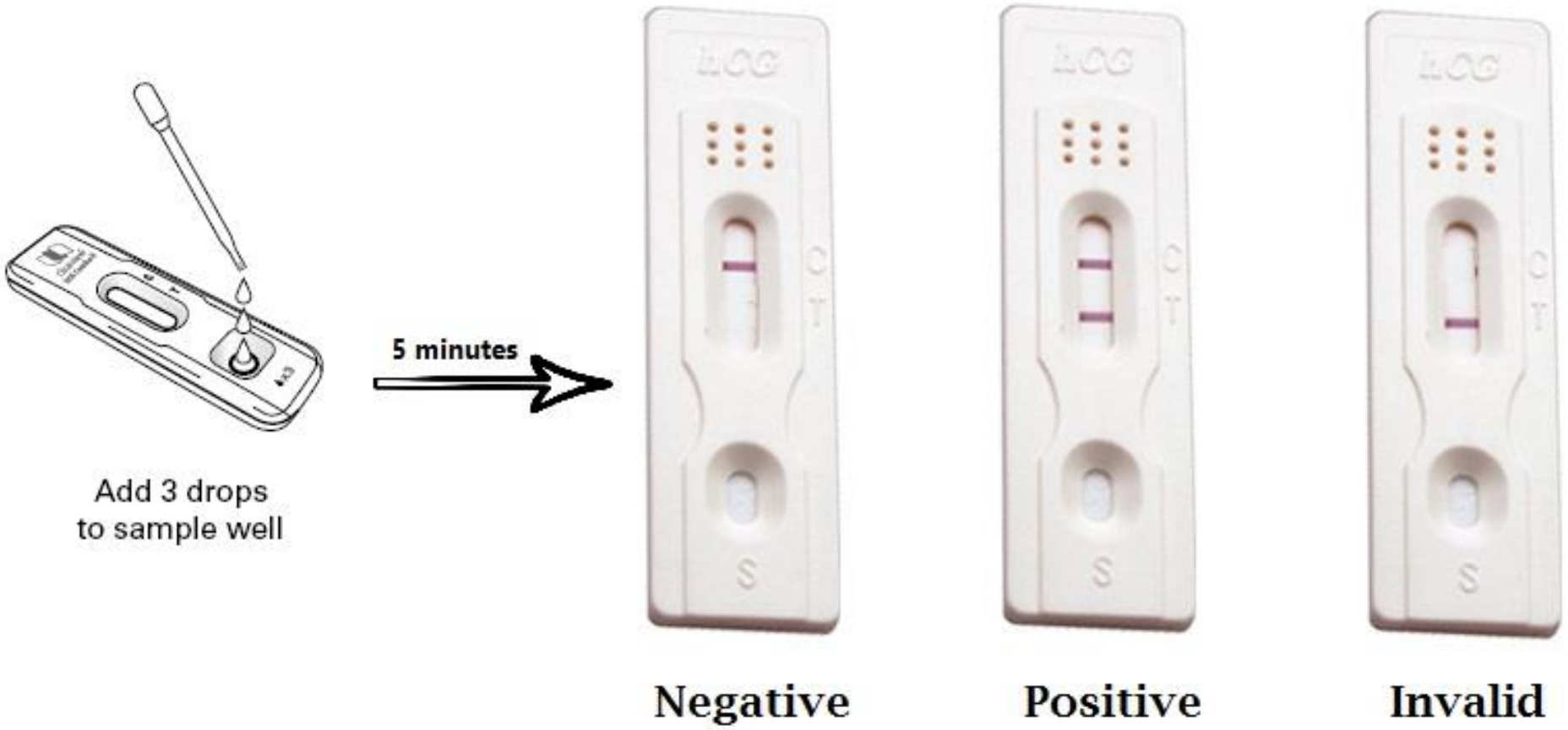


Figure 7. Pregnancy test results and interpretation. Source: laboratorytests.org

Homework:

Name one old method used to test pregnancy, and explain it briefly.

References

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