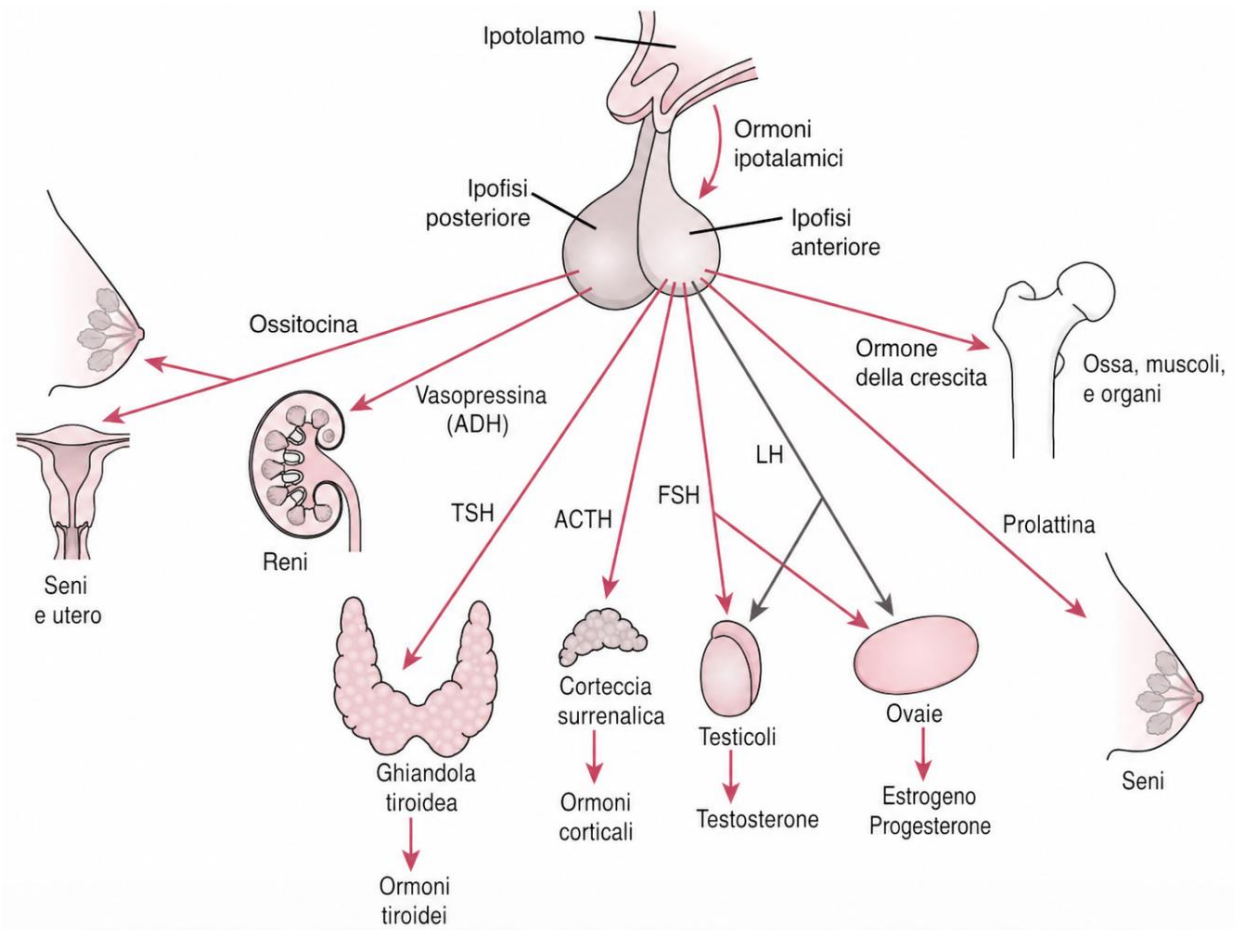


Lab session°1

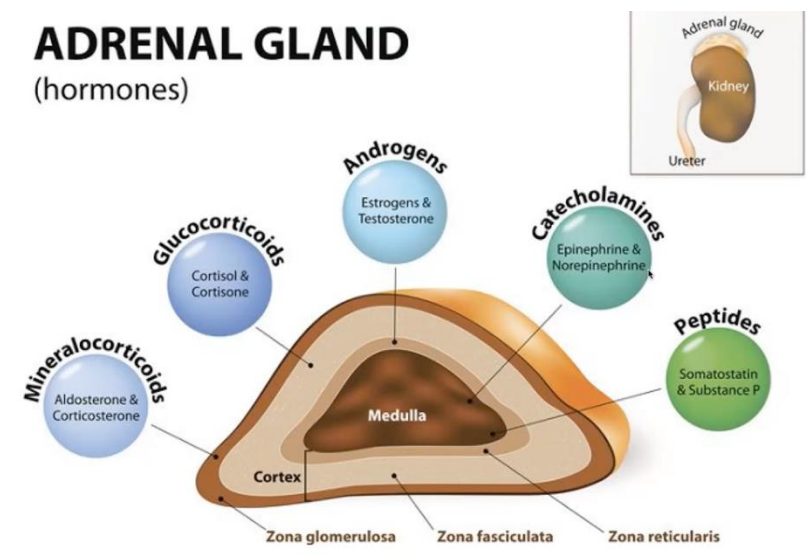
Quantification of cortisol in the saliva

ELISA Competitivo

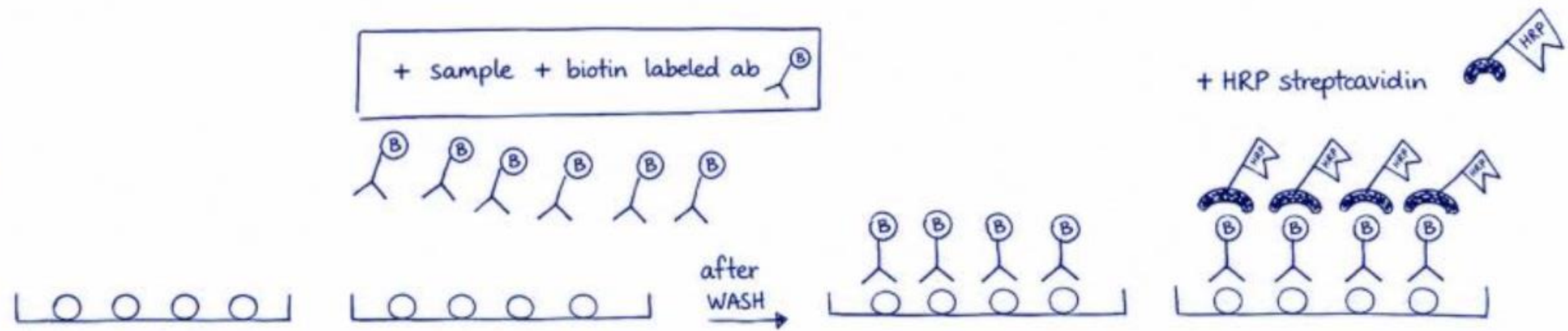
Enzyme-Linked Immunosorbent Assay.



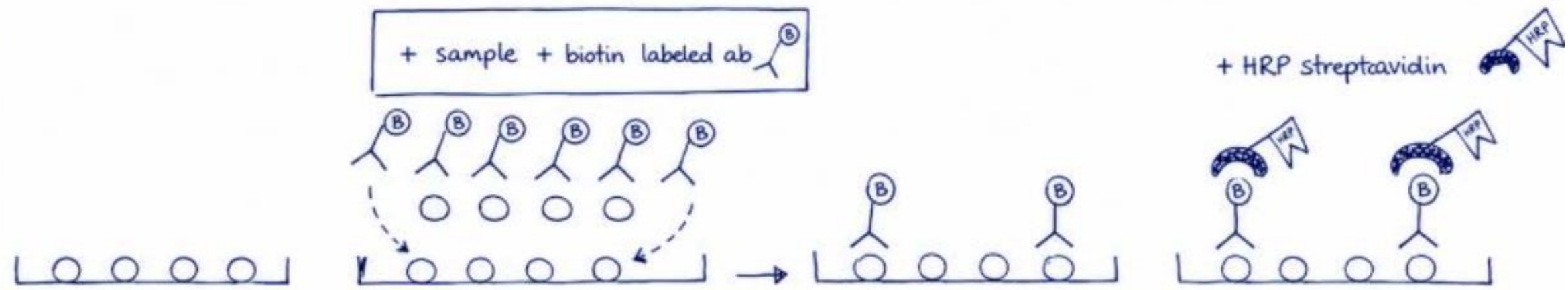
ADRENAL GLAND (hormones)



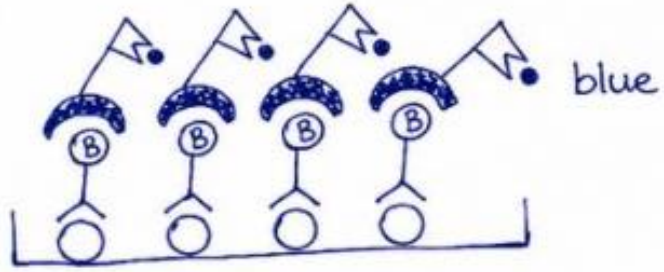
SAMPLE W/O CORTISOL



SAMPLE WITH CORTISOL



+ TETRAMETHYLBENZIDINE (TMB) ●



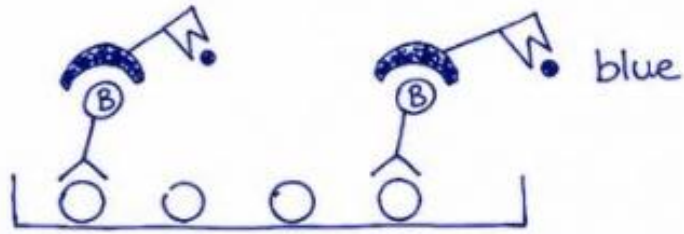
+ ACIDIC SOLUTION

blue → yellow

==

↓ ABSORBANCE AT
450nm
SIGNAL

+ TETRAMETHYLBENZIDINE (TMB) ●



+ ACIDIC SOLUTION

blue → yellow

==

↓ ABSORBANCE AT
450nm
SIGNAL

Electromagnetic spectrum



Radiation type



Wavelength (approximate)

30 mm

1 mm

10 nm

0.01 nm

Visible light



700 nm

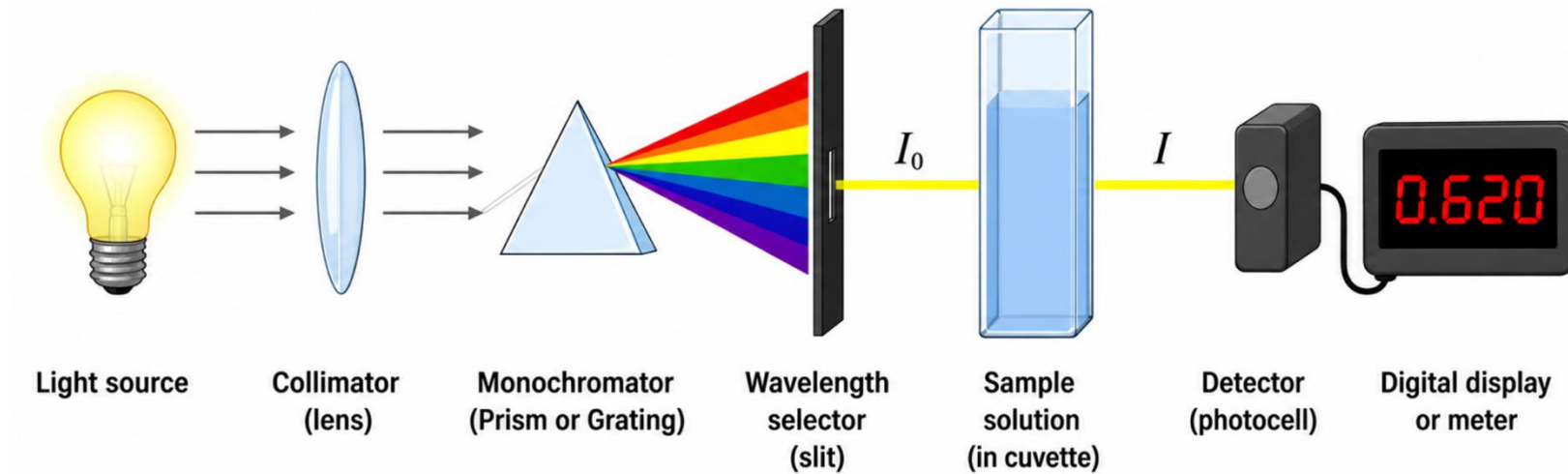
600 nm

500 nm

400 nm

Light spectrum

How does a spectrophotometer work?



FUNCTION

UV-Vis lamp
Es. Deuterium
or tungsten lamp

Organizes the
beam

Separates the
light beam into
different beams
of light at
different
wavelengths.

A slit that allows
only a selected
wavelength to
pass through.
 I_0

Test tube
containing the
sample. Part of
the light passes
through it, while
part is absorbed.

A sensor that
measures how
much light has
arrived.
 I

Display showing
the value


The spectrophotometer measures how much light passes through the sample and, using the Lambert–Beer law, provides the absorbance value A.

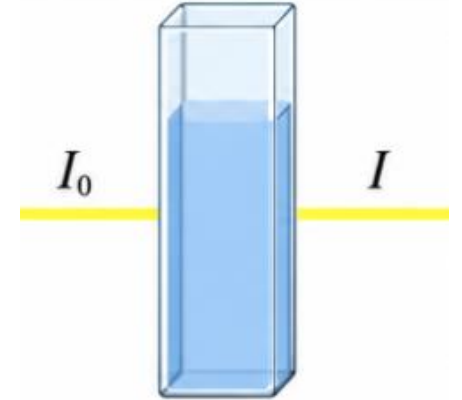
At the entrance there is light with intensity I_0 , while at the exit (after the cuvette) there is light with intensity I .

The instrument performs this calculation to obtain absorbance.

$$A = \log_{10}(I_0/I) = \boxed{\epsilon l c}$$

Definita in scala logaritmica





Thanks to the Lambert–Beer law, it is then possible to relate this value to concentration. This law states that absorbance and concentration are directly proportional.

NOTE:

Extinction: in optics, this indicates the decrease in the intensity of radiation passing through a medium.

Molar: because it refers to a concentration expressed in mol/L; it is related to the number of molecules present.

Equation of the 4-parameter logistic curve (4PL)

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

Inverted 4PL equation to calculate x given y.

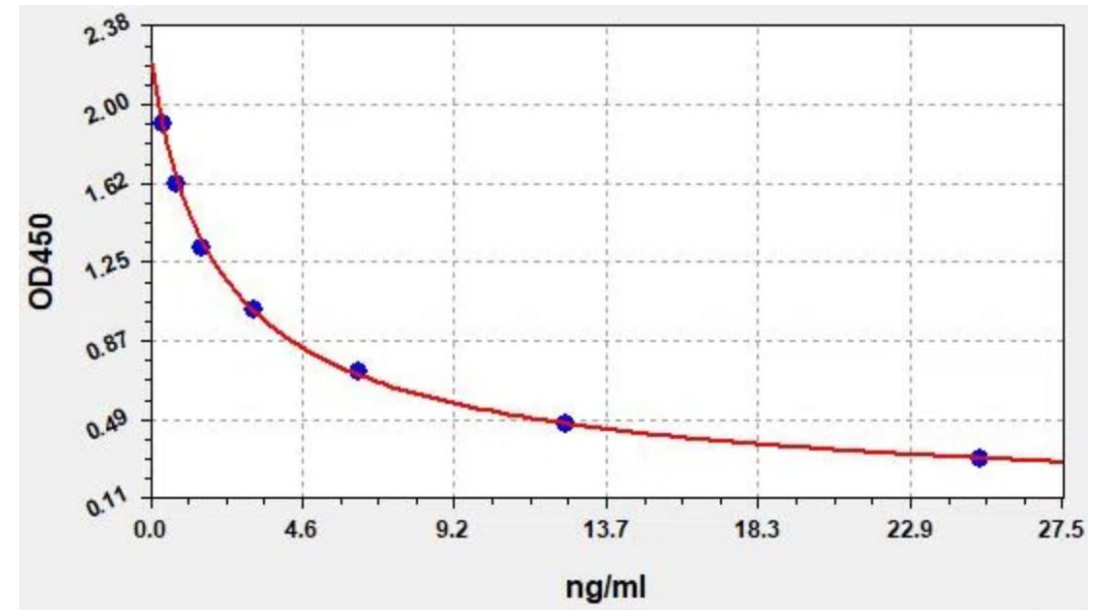
$$x(y) = C \left(\frac{A - y}{y - D} \right)^{\frac{1}{B}}$$

A = maximum absorbance

D = minimum absorbance

C = x value at the midpoint between A and D

B = curve slope



We substitute the obtained values into the equation:

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

$$R = 0,99$$

$$y = 2,263 + \frac{0,4674 + 2,263}{1 + \left(\frac{X}{2000}\right)^{-0,9604}}$$

$$y = D + \frac{4 - D}{1 + \left(\frac{X}{C}\right)^B}$$

REFERENCE EQ

$$x(y) = C \left(\frac{A - y}{y - D} \right)^{\frac{1}{B}}$$

$$X = 2,000 \left[\left(\frac{0,4674 + 2,263}{y - 2,263} \right)^{-1} \right]^{\frac{1}{-0,9604}}$$