RADIOIMMUNOASSAY

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WE'LL FIND OUT ABOUT....

- The principles of the methods most commonly used in laboratories.
- The basic principle of the reactions we are working in our laboratory with ("*in vitro*").
- How to assure accuracy, trueness and reproducibility of results.

IMMUNOCHEMICAL REACTIONS

...form the basis for sensitive and specific clinical assays known as *immunoassays (IA)*.

In a typical IA, an antibody is used as a reagent to detect the analyte (antigen) of interest.

The specificity and high affinity of Ab for specific Ag, coupled with the unique ability of Ab to cross-link Ag, allows for identification and quantification of specific substances by a variety of methods.

IMUNOCHEMICAL REACTIONS

$$Ag + Ab \leftrightarrow AgAb$$

Ag..... ANTIGEN

Ab.....ANTIBODY

ANTIGEN AND ANTIBODY CHARACTERISTICS

- The strength of binding of an Ab and Ag is determined and described by *affinity* and *avidity*.
- AFFINITY: energy of interaction of a single Ab-combining site and its corresponding epitope on the Ag.
- AVIDITY: overall strength of binding of Ab and Ag; include the sum of the bindig affinities of all individual combining sites on the antibody.
- AFINNITY is an Ag characteristic, while AVIDITY is connected with Ab.

IMUNOCHEMICAL METHODS

- Because of the specificity of the reaction Ab-Ag, these methods are sensitive and reproducible.
- Mostly, they are used for hormones quantification.
- Significant and important benefits in physiology and endocrinology.

IMUNOCHEMICAL TECHNIQUES

According to the determination of the reaction between Ab-Ag, IC techniques are divided:

1. Non-labeled techniques

2. Labeled techniques.

Ag + Ab ↔ AgAb

$$K = \frac{[AgAb]}{[Ag][Ab]}$$

LABELED Ab EXCESS Ab	Ag i Ab IN RELATIVE EQILIBRIUM	LABELED Ag LIMITED Ab CONCETRATION
IMUNOMETRIC ASSAY "TWO SITES" or "SANDWICH" ASSAY	ELECTROIMMUNOASSAY IMMUNOELECTROPHORESIS NEPHELOMETRIC ASSAY TURBIDIMETRIC ASSAY	IMMUNOASSAY COMPETITION

TYPES OF LABELS

- RADIONUCLIDES
- ENZYMES
- FLUORESCENT MOLECULES
- CHEMILUMINISCENT MOLECULES

RADIONUCLIDES

- Iodine; ^{125}I (t ½ = 60 days)

  ^{131}I
- Carbon, ^{14}C
- Tritium, ^{3}H

ENZYMES

- HORSERADISH PEROXIDASE, HRP
- ALKALINE PHOSPHATASE, ALP
- GALACTOSIDASE
- GLUCOSE-6-DEHYDROGENASE, G6D

FLUORESCENT MOLECULES

FLUOROPHORE

- FLUORESCEIN ISOTHIOCYANATE
- EUROPIUM
- RHODAMINE B ISOTHIOCYANATE

CHEMILUMINISCENT MOLECULES

- ISOLUMINOL
- FLUORESCEIN
- ACRIDINIUM ESTERS
- RUTHENIUM (electrochemilum. assays)

WHAT IS MEASURED BY INSTRUMENTS ?

TYPE OF LABELS	*MEASURED CHARACTERISTIC*
RADIONUCLIDES	RADIOACTIVITY
ENZYMES	ENZYME ACTIVITY
FLUORESCENT MOLECULES	FLUORESCENCE
CHEMILUMINISCENT MOLEC.	CHEMINILUMINISCENCE

RADIOACTIVITY MEASURMENT

GAMA AND BETA COUNTERS

THE BASIC PARTS OF COUNTERS:

- Scintillation crystal
- Photomultiplier tubes
- Detector
- Printer

RADIOIMMUNOASSAY HISTORY

- RIA was developed in the 1960s by S. Berson and R. Yalow from New York Universitiy who found out how to determine insuline antibody using insuline labeled by iodine.
- Patient's insuline competes with the labeled insuline for the binding sites of an Ab.
- The method is named *radioimmunoassay* (RIA).

RADIOIMMUNOASSAY HISTORY

- In 1970s, Miles and Hales modified RIA, labeling Ab instead of Ag. A new, improved technique had better sensitivity and precision.
- The method is named as immunoradiometric assay (IRMA).

RADIOIMUNNOASSAY

There are two types of immunoassay according to the labeled reagent:

1. LABELED ANTIGEN (RIA)

2. LABELED ANTIBODY (IRMA)

$$Ag + Ab \leftrightarrow AgAb$$

$$K = \frac{[AgAb]}{[Ag][Ab]}$$

IRMA

LABELED Ab
EXCESS Ab

IMUNORADIOMETRIC ASSAY
"TWO SITES" OR "SANDWICH"

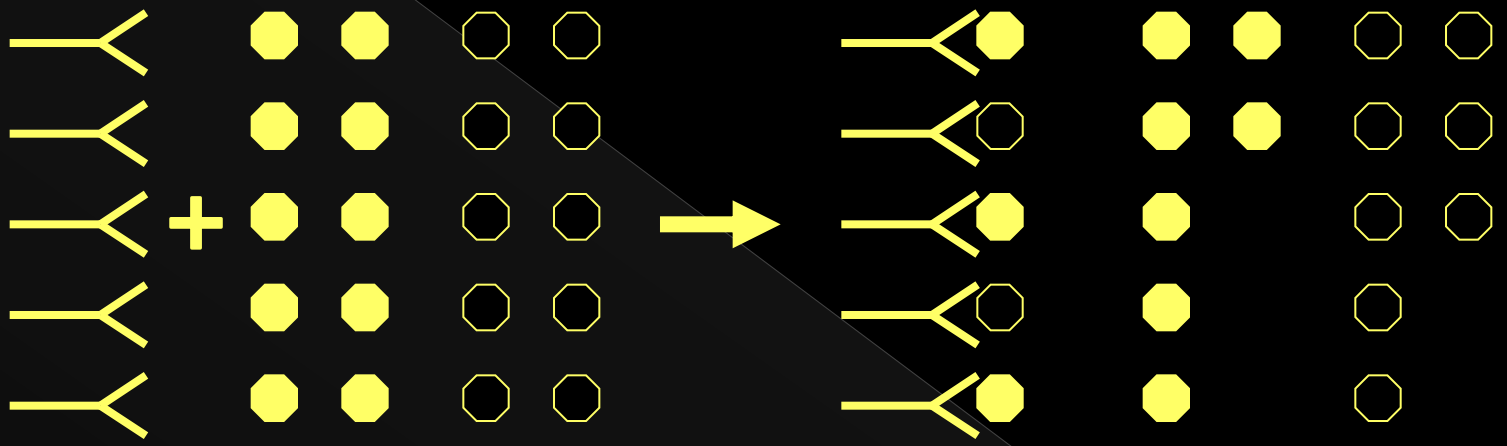
RIA

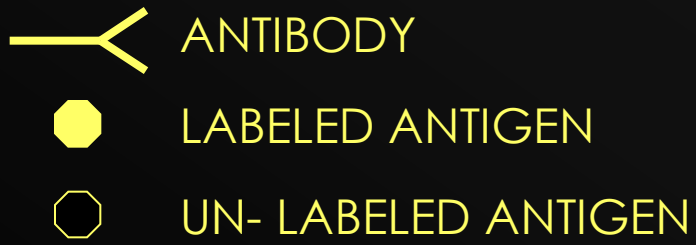
LABELED Ag
LIMITED Ab
CONCENTRATION

RADIOIMMUNOASSAY

COMPETITION

RIA (COMPETITION)





RIA (COMPETITION)

- Ag* and Ab are in comercial kits for hormone analysis.
- Ag is antigen (hormone) which concentration we want to determine (to quantify).
- Ag* and Ag have the same chemical and immunochemical characteristics which MUSTN'T be changed by labeling.
- The labeling is complex procedure of ^{125}I incorporation into antigen or antibody molecules (polypeptides, proteins, glycoproteins, steroids...)

- Radionuclide (isotope) labeling should be:
  - Strong enough to assure reliable and reproducible measurement
  - Strong enough to be in physiologicaly important interval for desirable antigen/antibody
  - But not too strong, to avoid molecules desintegration.

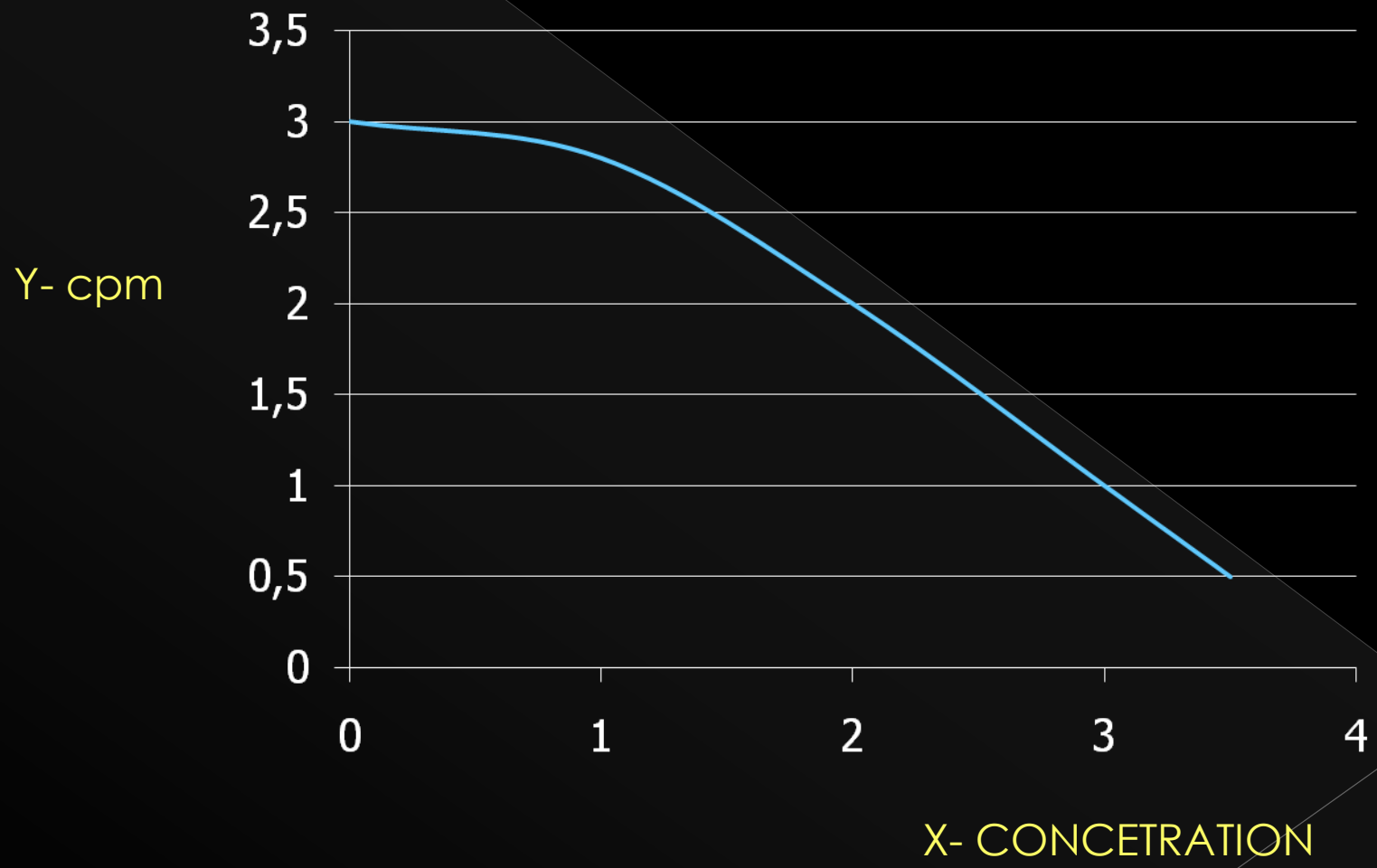
RIA (COMPETITION)

Ag^{*} + Ab + Ag → Ag^{*} Ab + Ag^{*} + Ag Ab

Volume of added Ab is limited to assure competiton!

*Ag** *LABELED ANTIGEN*
Ag *NON-LABELED ANTIGEN*
Ab *ANTIBODY*

RIA



IRMA (SANDWICH)

$$Ag + Ab \rightarrow Ag\,Ab + Ab^{*} \rightarrow Ab^{*}Ag\,Ab$$

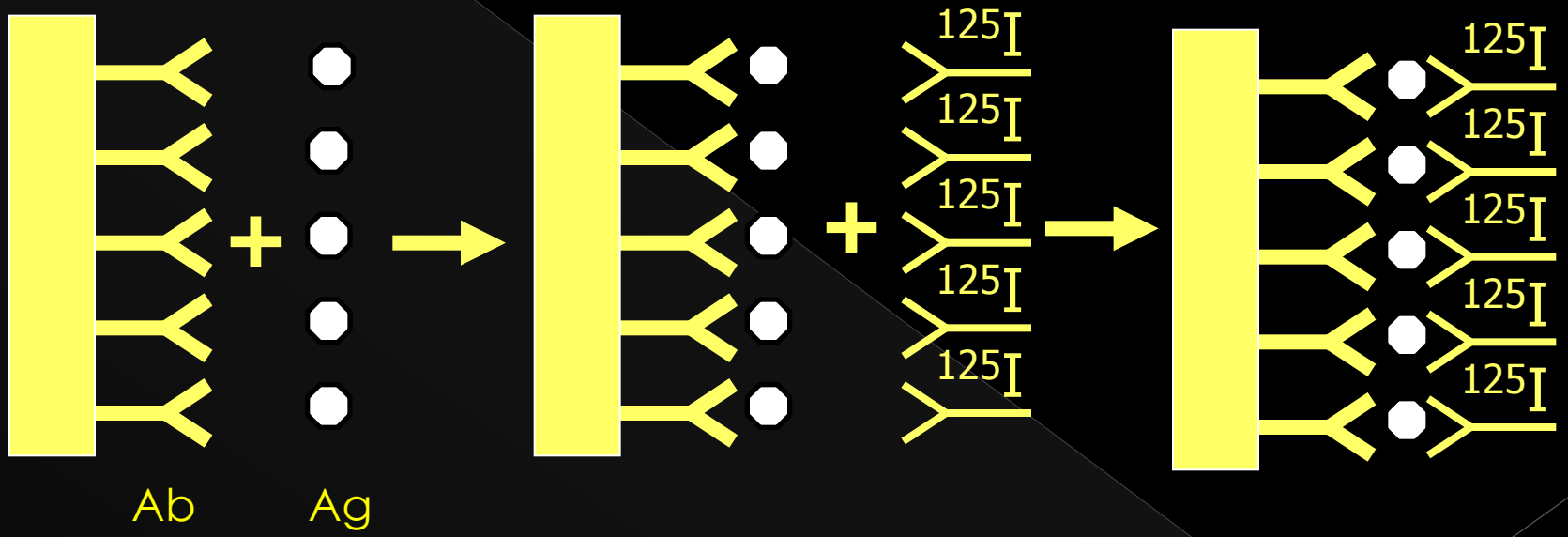
IRMA's advantage in comparation with RIA:

Ab is added in excess, while in RIA, the added volume is limited. Working volume is from 10 to 1000 µL (minor pipetting mistakes cause false results).

Bound Ab is proportional wtih Ag concentration!

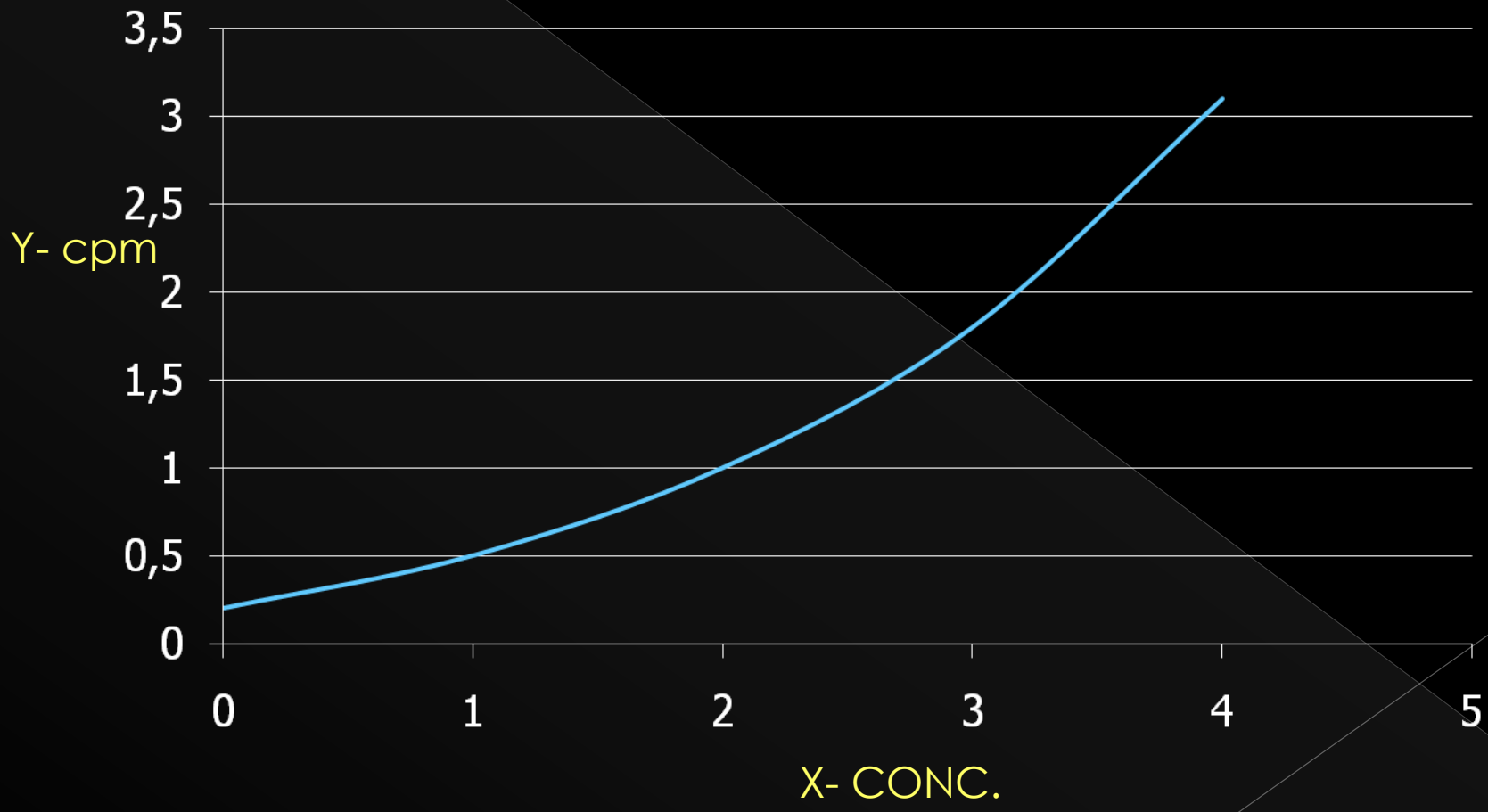
HOOK's effect!!!

IRMA (SANDWICH)



Ab is bounded on the solid phase

IRMA



QUALITY CONTROL

- Control sample in comercial kits
- Intra-laboratory control sample (“innner”)
- Inter-laboratory control sample (“outer”)

METHOD RELIABILITY

1. SENSITIVITY
2. SPECIFICITY
3. ACCURACY
4. PRECISION

SENSITIVITY

The analytical sensitivity indicates to what extent a value changes depending on the signal of the system to be measured.

DETECTION LIMIT

... of the method is the concentration or activity of an individual test sample by which the test sample can be differentiated with high probability from a suitable sample blank.

SPECIFICITY

The analitical specificity is the ability of a method to detect only the analyte under consideration.

Other components of the sample should not influence the analytical results.

In the case of an immunoassay, the specificity is a criterion of the extent to which the assay responds only to a specified analyte and not to other substances present in the sample.

ACCURACY

ACCURACY is a qualitative term for the degree by which the measurement result approaches the reference value.

PRECISION

Precision describes the mutual approach of measurement results, that are independent of each other, if a method is used repeatedly.

THANK YOU!