

Radioimmunoassay (RIA)

Radioimmunoassay (RIA) or *radioimmunological assays* include those methods of radioisotope microanalysis based on the immunochemical reaction of an antigen with a specific antibody (Ab), performed in vitro in the presence of a suitable radiolabeled compound as a radiotracer that allows the determination to be quantified by determining the activity distribution.^[1]

History

The method by which the blood level of insulin was first measured in vitro was developed in the 1950s in the USA. It was the first ever quantitative determination of the hormone level in the blood. For this discovery, Rosalyn Sussman Yalow received the Nobel Prize in Medicine in 1977. By being able to accurately measure the level of insulin in the blood, the treatment of diabetes mellitus moved a significant step forward.

Method Principle

It is a competitive immunoreaction, i.e. the labeled antigen competes with the unlabeled antigen for binding sites on the antibody, which is present in a limited amount in the reaction mixture. In this case, the determined substance is an unlabeled antigen, another antigen is labeled, for example, with a radioactive isotope of iodine (¹²⁵I, ¹³¹I) for protein antigens, or with tritium or ¹⁴C for low molecular weight substances. The result of the reaction is the formation of two complexes: labeled antigen-antibody (Ag*-Ab) and unlabeled antigen-antibody (Ag-Ab). The amount of the labeled complex (Ag*-Ab) is inversely proportional to the amount of the determined antigen, i.e. the more the determined substance is in the sample, the smaller the amount of the labeled complex will be and the smaller the resulting signal will be. This can be simply explained by the fact that the labeled antigens do not bind to the antibody due to the lack of binding sites to occupy the unlabeled antigen. It also follows from the above findings that free forms of Ag and Ag* are also found in the reaction mixture. The total reactivity (T) is thus divided into two fractions – bound (B) and free (F), while the following applies: $T = B + F$.

Method Procedure

For the quantitative determination of a certain substance in the examined sample, a calibration curve must be created that reflects the dependence of the resulting signal on the known concentration of the given substance. We prepare the calibration curve from the so-called standards. Controls, where we know the amount of radioactivity in advance, are also an integral part of the determination.

Solid-phase RIA (in tubes)

1. We pipette individual standards and unknown samples into test tubes coated with a specific antibody. Pipette the controls into uncoated tubes.
2. We add the same amount of radioindicator (Ag*) to each test tube.
3. Mix and let it incubate.
4. After a sufficiently long time, aspirate the reaction mixture.
5. We measure bound (B) and free (F) radioactivity on a gamma counter.

Note: Separation of the immunocomplex can also be done in other ways, e.g. using electrophoresis, ion exchange chromatography, etc.

Examples of RIA usage in practice ^[2]

- endocrinology (hormone levels) in the blood
- digitoxin or digoxin in patients taking these drugs
- toxicology: evidence of the presence of drugs
- blood transfusion: presence of hepatitis B surface antigen (HBsAg) in donated blood
- immunology: anti-DNA antibodies in systemic lupus erythematosus (SLE).

Advantages and Disadvantages of RIA

The main advantages of this method are high sensitivity and the possibility of automation. The disadvantage is the necessity of an intermediate separation stage, the expensive equipment required to perform this method and, last but not least, the risks associated with handling the radioactive substance.

Links

Related Articles

- ELISA

External links

- Wikipedia (<https://www.wikipedia.org/>)
- http://biochemie.upol.cz/doc/skripta/imch/9_Radioimmunoanaliza.pdf
- http://immunologie.lf2.cuni.cz/soubory_vyuka/immunoreakce.pdf

References

1. *Incomplete citation of web.* . *Radioimmunoassay (RIA)* [online]. [cit. 2013-05-18]. <<http://orion.sci.muni.cz/virtuallab/dokumenty/pdf/Radioimmunoanaliza.pdf>>. ,
2. *Incomplete citation of web.* . *Immunoreaction with labeled antibodies* [online]. [cit. 2013-05-18]. <http://immunologie.lf2.cuni.cz/soubory_vyuka/immunoreakce.pdf>. ,