Bioassay of hormones using RIA and ELISA

Compared to conventional bioassay which uses the concentration measurements in blood plasma or pituitary extracts, immunoassys using radioimmunoassays and competitive ELISAs both have been found to be more accurate, convinient and reliable. These assays depend on the reaction between the hormone in a sample and a fixed amount of antibody added to it: some of the hormone will bind to the antibody, whereas some remains 'free', in a reaction governed by the law of mass action. When a fixed amount of radiolabelled or enzyme-conjugated hormone is added, it competes for binding to the antibody with the hormone already present, and so less labelled hormone will bind when more unlabelled hormone is present. In radioimmunoassays, the bound fraction is then precipitated, usually with a second antibody that recognises the original antibody. The amount of label in the pellet is measured, and the values are compared with a standard curve generated from known concentrations of unlabelled hormone in assay buffer (processed in the same way as the samples). This is a hyperbolic curve, which, after allowing for nonspecific binding, can be reduced by a logit-log transformation to a straight line. In ELISAs, the bound fraction is retained in the assay plate, and the amount of bound label is measured by optical density, giving a similar hyperbolic standard curve.

An assay system is standardised with known amounts of hormone, a standard curve constructed and the activity of unknown determined by comparing the samples.

RIA protocol

The method of performing radioimmunoassay is as follows:

An antibody that is highly specific for the hormone to be measured is produced.

Kia small quantity of this antibody is mixed with a quantity of fluid from the animal containing the hormone to be measured

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And also mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope (there must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed)

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The natural hormone in the assay fluid and the radioactive standard hormone compete for the binding sites of the antibody (in the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid)

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after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution

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the quantity of radioactive hormone bound in this complex is measured by radioactive counting techniques.

Inference:

If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small.

a. Otherwise if only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites.
b. In order to make the assay highly quantitative, the radioimmunoassay procedure is also performed for "standard" solutions of untagged hormone at several concentration levels anda "standard curve" is plotted. By comparing the radioactive counts recorded from the "unknown" assay procedures with the standard curve, one can determine within an error of 10 to 15 percent the concentration of the hormone in the "unknown" assayed fluid.

ELISA protocol:

Enzyme-linked immunosorbent assays (ELISAs) can be used to measure almost any protein, including hormones. This test combines the specificity of antibodies with the sensitivity of simple enzyme assays. The steps are as follows:

plastic plates that each have 96 small wells are taken

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Each well is coated with an antibody (AB1) that is specific for the hormone being assayed.

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Samples or standards are added to each of the wells, followed by a second antibody (AB2) that is also specific for the hormone but binds to a different site of the hormone molecule.

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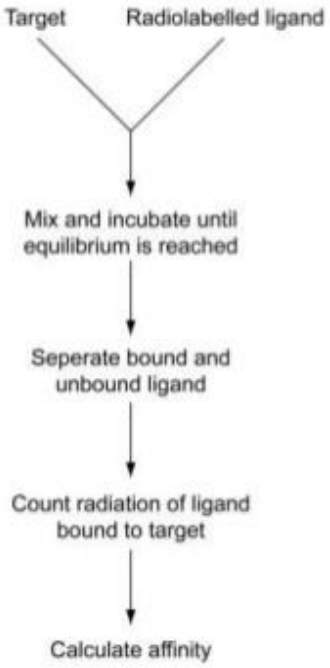
A third antibody (AB3) that is added recognizes AB2 and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods.

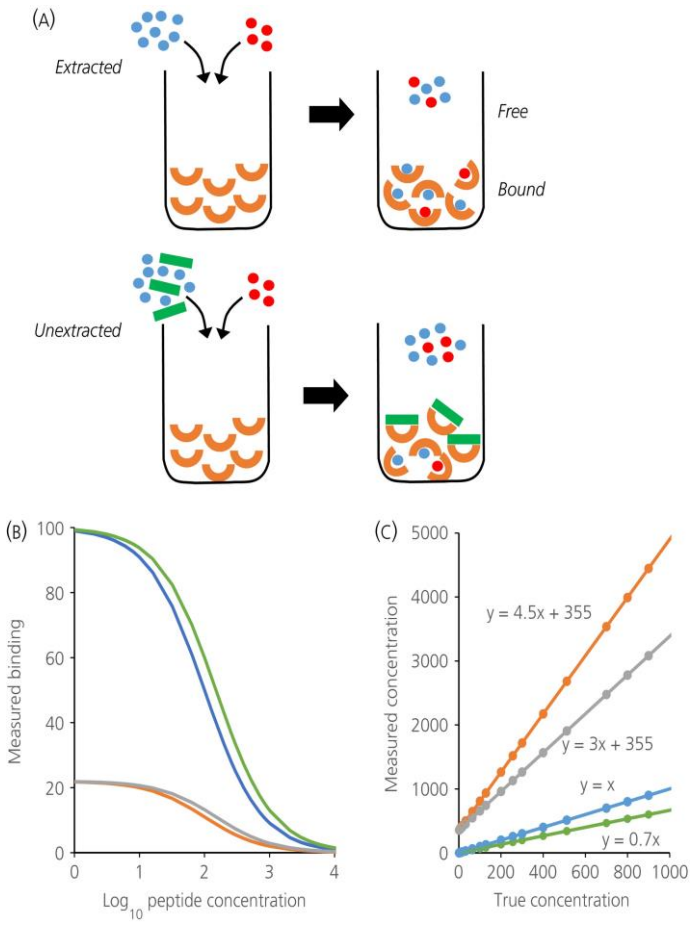
Inference

a. Each molecule of enzyme catalyzes the formation of many thousands of product molecules, therefore even small amounts of hormone molecules can be detected.
b. In contrast to competitive radioimmunoassay methods, ELISA methods use excess antibodies so that all hormone molecules are captured in antibody-hormone complexes. Therefore, the amount of hormone present in the sample or in the standard is proportional to the amount of product formed.
c. Advantages over RIA:
    - The ELISA method has become widely used in clinical laboratories because it does not employ radioactive isotopes.
    - much of the assay can be automated using 96-well plates, therefore it has proved to be a cost-effective and accurate method for assessing hormone levels.

Measurement of binding – Radioligand binding assays



- This is the general process to perform a radioligand binding assay.


(A)
Extracted
Free
Bound
Unextracted
(B)
Measured binding
100
80
60
40
20
0
0
1
2
3
4
Log10 peptide concentration
(C)
Measured concentration
5000
4000
3000
2000
1000
0
y = 4.5x + 355
y = 3x + 355
y = x
y = 0.7x
0
200
400
600
800
1000
True concentration