Hormonal Essays

What is a hormonal assay ?

A hormonal assay test is performed to give an indication of metabolic processes and conditions, or ‘hormone imbalance’. Many hormones can be measured (assayed) in the blood, including male and female sex hormones (oestrogen, progesterone, testosterone) and hormones secreted by other glands such as the parathyroid and adrenal glands.

How are hormonal assays performed?

Hormonal assays are a blood test and require a few millilitres of blood from a vein.

When would you need a hormonal assay?

Specific hormones or sets of related hormones may be tested in a variety of clinical situations. Commonly, hormonal tests may be related to issues of fertility, menstrual irregularities, or menopause.

Your doctor may request specific hormonal tests if there is a suspicion of an endocrine problem, relating to the function of many glands in the body, including the pituitary, thyroid, parathyroid, adrenals, ovaries and testes.

Test results, explained

Hormone tests give the concentration of specific hormones in the bloodstream. These results are compared to a reference range of ‘normal’ values, obtained from testing well people without symptoms. The specific hormone level may therefore be low, normal, or high.

Each of these results needs to be carefully interpreted by a doctor who understands the complex interactions of the endocrine system, in the context of the limitations of biochemical testing of hormones. A high or low level may not indicate disease, and similarly, a normal test does not always rule out an abnormality.

Radioimmunoassay (RIA)

A **radioimmunoassay** (**RIA**) is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies.

Although the RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing, since radioactive substances are used.

In contrast, an immunoradiometric assay (IRMA) is an immunoassay that uses radiolabeled molecules but in an immediate rather than stepwise way.

A radioallergosorbent test (RAST) is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.

Method

Classically, to perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine, such as 125-I, attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the

unbound ones, and the radioactivity of the free(unbound) antigen remaining in the supernatant is measured using a gamma counter.

This method can be used for any biological molecule in principle and is not restricted to serum antigens, nor is it required to use the indirect method of measuring the free antigen instead of directly measuring the captured antigen. For example, if it is undesirable or not possible to radiolabel the antigen or target molecule of interest, a RIA can be done if two different antibodies that recognize the target are available and the target is large enough (e.g., a protein) to present multiple epitopes to the antibodies. One antibody would be radiolabeled as above while the other would remain unmodified. The RIA would begin with the "cold" unlabeled antibody being allowed to interact and bind to the target molecule in solution. Preferably, this unlabeled antibody is immobilized in some way, such as coupled to an agarose bead, coated to a surface, etc. Next, the "hot" radiolabeled antibody is allowed to interact with the first antibody-target molecule complex. After extensive washing, the direct amount of radioactive antibody bound is measured and the amount of target molecule quantified by comparing it to a reference amount assayed at the same time. This method is similar in principle to the non-radioactive sandwich ELISA method.

ELISA

What is ELISA?

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents

that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

ELISA Basics/ELISA Principle

Enzyme-linked immunosorbent assays (ELISA) principles are very similar to other immunoassay technologies. ELISA's rely on specific antibodies to bind the target antigen, and a detection system to indicate the presence and quantity of antigen binding. In order to maximize the sensitivity and precision of the assay, the plate must be carefully coated with high-affinity antibodies – a process that Boster Bio has mastered.

General ELISA Procedure

*This is conceptual explanation for how ELISA works.

Unless you are using a kit with a plate that is pre-coated with antibody, an ELISA begins with a **coating** step, in which the first layer, consisting of a target antigen or antibody, is adsorbed onto a 96-well polystyrene plate. This is followed by a **blocking** step in which all unbound sites are coated with a blocking agent. Following a series of washes, the plate is **incubated with enzyme-conjugated antibody**. Another series of washes removes all unbound antibody. A **substrate** is then added, producing a calorimetric signal. Finally, the plate is **read**.

Because the assay uses surface binding for separation, several washes are repeated in each ELISA step to remove unbound material. During this process, it is essential that excess liquid is removed in order to prevent the dilution of the solutions added in the next assay step. To ensure uniformity, specialized plate washers are often used.

ELISAs can be quite complex and include multiple intervening steps, especially when measuring protein concentration in heterogeneous samples such as blood. The most

complex and varying step in the overall process is detection, where multiple layers of antibodies can be used to amplify signal.