**BIOASSAY**

**What is bioassay?**

**Bioassay (biological assay), or biological standardization is a type of scientific** [**experiment**](http://en.wikipedia.org/wiki/Experiment)**. Bioassays are typically conducted to measure the effects of a substance on a living** [**organism**](http://en.wikipedia.org/wiki/Organism) **and are essential in the development of new** [**drugs**](http://en.wikipedia.org/wiki/Drugs) **and in monitoring environmental** [**pollutants**](http://en.wikipedia.org/wiki/Pollutant)**.**

**Both are procedures by which the** [**potency**](http://en.wikipedia.org/wiki/Potency_%28pharmacology%29) **or the nature of a substance is estimated by studying its effects on** [**living matter**](http://en.wikipedia.org/wiki/Living_matter)**. Bioassay is a procedure for the determination of the concentration of a particular constitution of a mixture.**

**Bioassay definition:**

**“ Estimation or determination of concentration or potency of a physical, chemical or biological substance (agent) by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions”**

**Principle of bioassay:**

**The bioassay compares the test sample with a same Internationally applicable standard substance. It determines the quantity of test sample required to produce an equivalent biological response to that of standard substance.**

**Standard samples are accepted by expert committee at international level and they represent fixed units of activity.**

**Purpose:**

1. **Measurement of the** [**pharmacological**](http://en.wikipedia.org/wiki/Pharmacology) **activity of new or chemically undefined substances**
2. **Investigation of the function of** [**endogenous mediators**](http://en.wikipedia.org/wiki/Endogenous_mediator)
3. **Determination of the** [**side-effect profile**](http://en.wikipedia.org/wiki/Adverse_effect_%28medicine%29)**, including the degree of** [**drug toxicity**](http://en.wikipedia.org/wiki/Drug_toxicity)
4. **Measurement of the concentration of known substances (alternatives to the use of whole animals have made this use obsolete)**
5. **Assessing the amount of pollutants being released by a particular source, such as** [**wastewater**](http://en.wikipedia.org/wiki/Wastewater) **or** [**urban**](http://en.wikipedia.org/wiki/Urban_area)[**runoff**](http://en.wikipedia.org/wiki/Surface_runoff)**.**
6. **Determining the specificity of certain enzymes to certain substrates.**

**Bioassay Methods / types of bioassays**

**Basically there are two types of bioassays as per the technique used in determination of the Sample under test.**

**1. Graded Response Assay**

**2. End Point or Quantal Assay**

**Graded Response Assay:**

**In these assays, as the dose increases there is an equivalent rise in response. The potency is estimated by comparing the Test sample responses with the standard response curve.**

**In the graded dose response relationship, relates the size of the response to the drug in a single biologic unit as the dose administered increased the pharmacological response also increases and eventually reaches a steady level called the ceiling effect there will be on further increase in response even with an increase in dose.**

**The graded dose response curve is obtained by plotting a graph with dose on the X-axis and response on the Y-axis. It is usually sigmoid in shape however the log dose response curve is almost a straight line and particularly useful in bio assay.**

**Conc. of unknown= Threshold dose of standard / threshold dose of test x Conc. of standard.**

**e.g. Acetyl-choline producing contraction in the muscle of frog Rectus abdominis.**

**End Point or Quantal Assay:**

**As the name indicates, the threshold dose of the sample required to elicit a complete or a particular pharmacological effect is determined and compared with standard.**

**E.g., Digitalis producing cardiac arrest. Here the effect is identified by the response it produces on the biological system. Digitalis produces cardiac stimulation on further doses it produces cardiac arrest.**

**(+)d TC (Tubocurarine) producing neck relaxation in rabbit, Here as the sample is injected to the neck muscle of the Rabbit, the neck starts to droop. On further doses there is complete hanging of the neck and rabbit has no ability to lift the neck**

**Even the Determination of LD50 (LD=Lethal dose) or ED50 (ED= effective dose) is done by this method.**

**Techniques:**

1. **Matching Bioassay**
2. **Interpolation Method**
3. **Bracketing Method**
4. **Multiple Point Bioassay (i.e.-Three-point, Four-point and Six Point Bioassay)**

**Matching Bioassay:**

 **It is the simplest type of the bioassay. In this type of bioassay, response of the test substance taken first and the observed response is tried to match with the standard response. Several responses of the standard drug are recorded till a close matching point to that of the test substance is observed. A corresponding concentration is thus calculated. This assay is applied when the sample size is too small. Since the assay does not involve the recording of concentration response curve, the sensitivity of the preparation is not taken into consideration. Therefore, precision and reliability is not very good.**

**Interpolation bioassay:**

 **Bioassays are conducted by determining the amount of preparation of unknown potency required to produce a definite effect on suitable test animals or organs or tissue under standard conditions. This effect is compared with that of a standard. Thus the amount of the test substance required to produce the same biological effect as a given quantity the unit of a standard preparation is compared and the potency of the unknown is expressed as a % of that of the standard by employing a simple formula.**

**Three point (2+1) & Four- point (2+2) assay:**

**This method incorporates the principle of interpolation and bracketing. 2+1 indicates- Tow response of Standard and one response of Test respectively. This procedure of 2+1 or 2+2 is repeated 3 times or 4 times based on the method with crossing over of all the samples.**

**Here cross over method is used during the assay as.**

**S1 S2 T, T S1 S2, S2 T S1 doses.**

**Environmental bioassays:**

**Environmental bioassays are generally a broad-range survey of** [**toxicity**](http://en.wikipedia.org/wiki/Toxicity)**. A toxicity identification evaluation is conducted to determine what the relevant** [**toxicants**](http://en.wikipedia.org/wiki/Toxicant) **are. Although bioassays are beneficial in determining the biological activity within an organism, they can often be time-consuming and laborious. Organism-specific factors may result in data that is not applicable to others in that species. For these reasons, other biological techniques are often employed, including** [**radioimmunoassays**](http://en.wikipedia.org/wiki/Radioimmunoassay)**.**

[**Water pollution**](http://en.wikipedia.org/wiki/Water_pollution) **control requirements in the** [**United States**](http://en.wikipedia.org/wiki/United_States) **require some industrial dischargers and municipal** [**sewage treatment**](http://en.wikipedia.org/wiki/Sewage_treatment) **plants to conduct bioassays. These procedures, called** [**whole effluent toxicity**](http://en.wikipedia.org/w/index.php?title=Whole_effluent_toxicity&action=edit&redlink=1) **tests, include acute toxicity tests as well as chronic test methods. The methods involve exposing living aquatic organisms to samples of wastewater.**

**Bioassay systems and techniques**

**The bioassay systems vary based on the biological system used like animals (mouse, rat, guinea pig, rabbits etc), plant bioassay (using plant constituents to evaluate a sample like(haemolytic activity) microbiological or cell based assay (using microbes like bacteria, fungi or cultured cells for anti biotic compound screening etc).**

**Based on techniques they can be differentiated into two broad types like**

1. **In vivo techniques:**

**These techniques employ a living animal recommended for the purpose of assay. The techniques aims to study the biological effect or response of the compound under screening in a living system directly. Ex: By use of rodents, rabbits etc.**

1. **In vitro techniques:**

**These techniques employ a cell culture of recommended biological system to study the effect of compound under standard condition not similar to that of living environment. Here the cell culture survives by utilization of the nutrition in the media. Ex: use of stem cells, cell culture, microbes ([bacteria](http://www.rajaha.com/identification-bacteria-methods/)) etc**

1. **Ex vivo techniques:**

**These techniques employ a tissue or cells of recommended living system to study the effect of compound under test in suitable conditions within the stipulated time of organ survival outside the body. The methods described in the videos employ a living tissue of an animal in an apparatus to study the contractile effect of drugs.**

**Ex: Use of any isolated organ from animals in a glass ware to study the effect of compound within the period of its survival outside the living body with provision of only oxygen, glucose and isotonic salts to maintain cell & [cell organelles](http://www.rajaha.com/cell-organelles-function/)integrity.**