BIOASSAY TECHNIQUES

Submitted to

Dr. Sujarani S.

Asst. Prof.

Dept. of VPT, Pookode.

Submitted by

Dr. Sindhu K.

MVSc scholar,

Dept. of VPT, Pookode.

Biological assessment.

- 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.
- The estimation of the concentration or potency of a substance by measurement of the biological response that it produces.
- The structure of bioassay: STIMULUS-applied to subject.

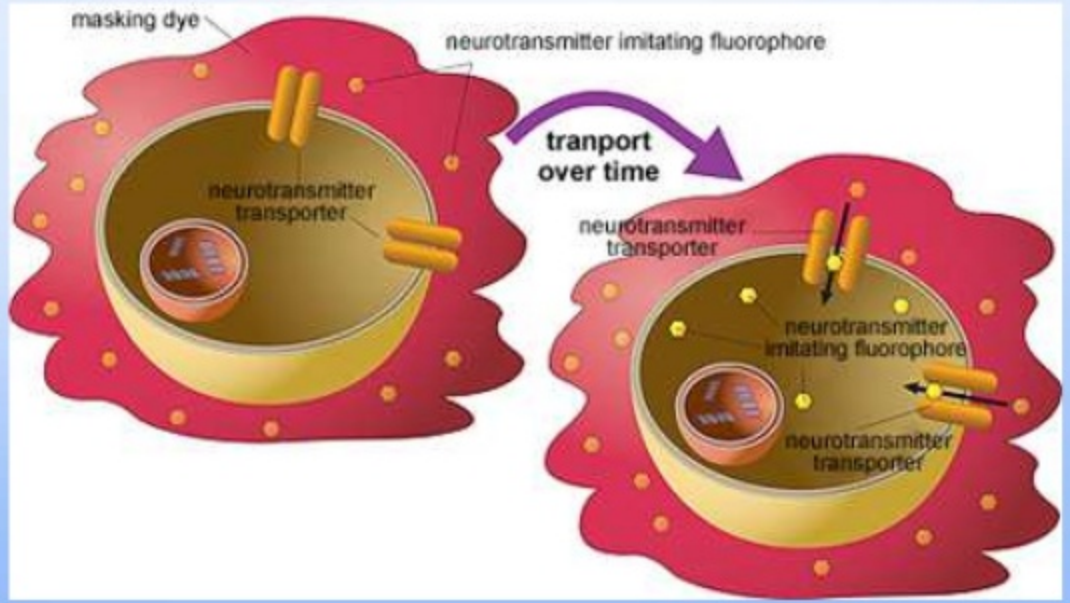
  RESPONSE-of the subject to the stimulus.

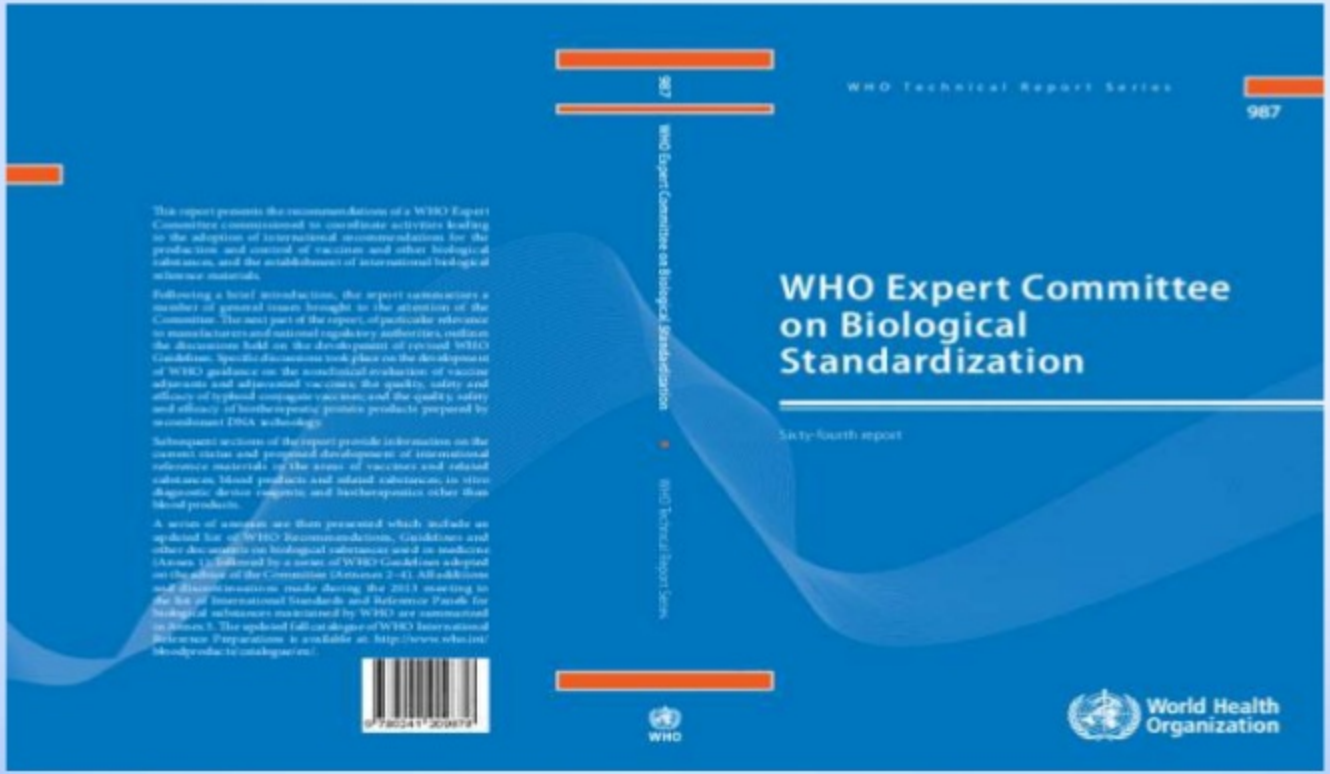
The purpose of bioassay.

- To ascertain the ***potency of a drug*** and hence it serves as the quantitative part of any screening procedure (Research).
- To standardize ***drugs, vaccines, toxins or poisons, disinfectants, antiseptics*** etc., so that each contains the uniform specified pharmacological activity. (***standardization*** required as these are all used over biological system in some or other form.)
- Helps to determine the ***specificity of a compound*** to be used e.g. Penicillin's are effective against Gram +ve. but not on Gram –ve.
- From the clinical point of view, bioassay may help in the ***diagnosis*** of various conditions. e.g. gonadotrophins for pregnancy.
- Sometimes the ***chemical composition of samples are different*** but have ***same biological activity.***
- Certain ***complex compounds like Vitamin B-12*** which can't be analysed by simple assay techniques can be ***effectively estimated*** by Bioassays.
- For samples ***where no other methods of assays are available***.

Principle of Bioassay.

To compare the test substance with the International Standard preparation of the same and to find out how much test substance is required to produce the same biological effect, as produced by the standard.





The standards are internationally accepted samples of drugs maintained and recommended by the ***Expert Committee of the Biological Standardization of W.H.O.***

They represent the fixed units of activity (definite weight of preparation) for drugs.

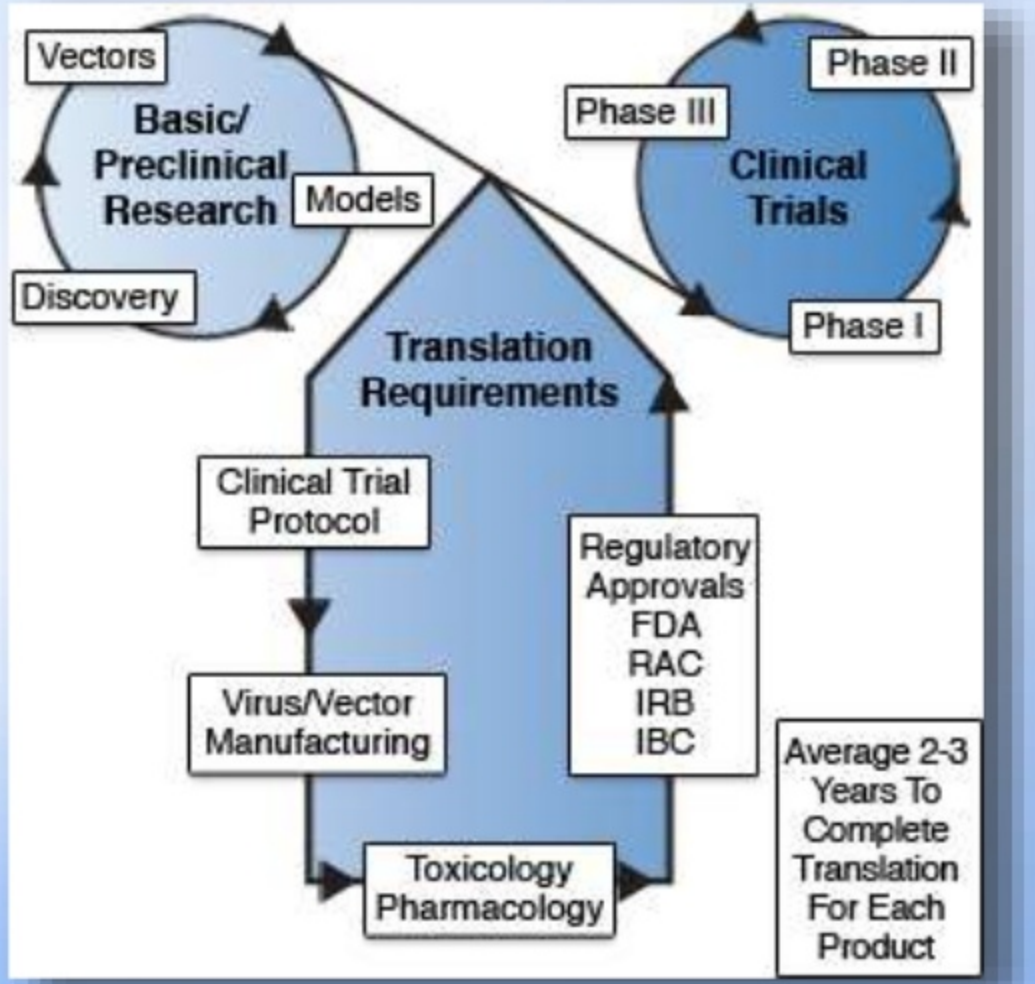
In India

- standard drugs are maintained in Government institutions like

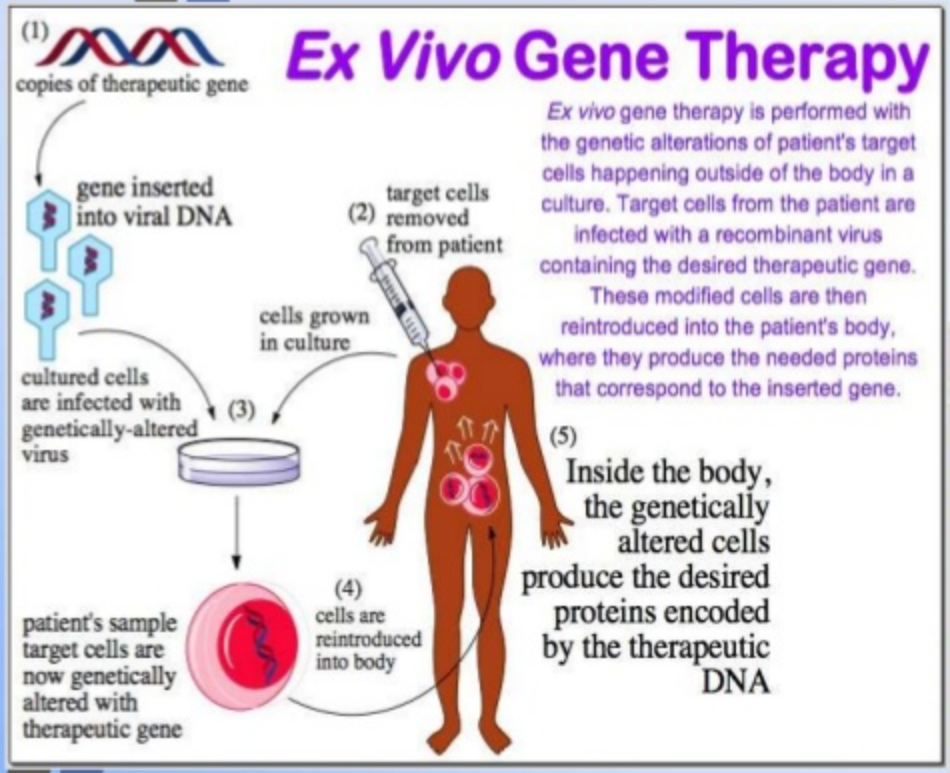
1. ***Central Drug Research Institute***, **Lucknow**
2. ***Central Drug Laboratory***, **Calcutta**.

Classification of bioassay.

- *Invitro.*
- *Invivo.*
- *Exvivo.*



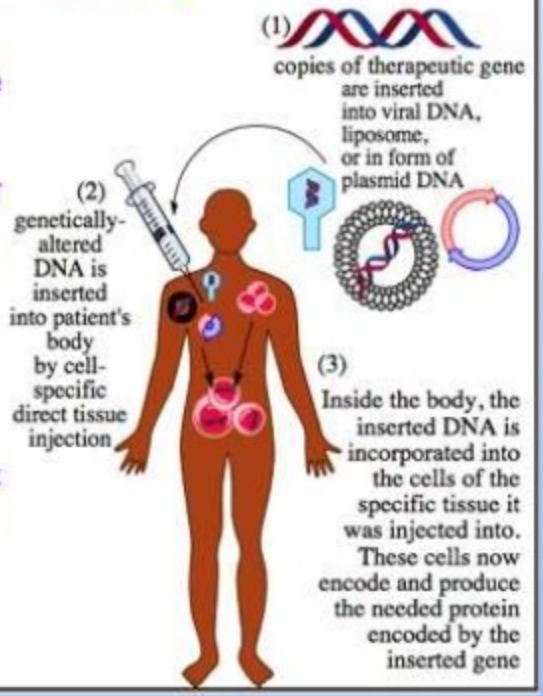
Ex Vivo Gene Therapy



Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.

In Vivo Gene Therapy

In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, but they will likely be the next advancement in therapeutic gene delivery as cell-specific receptor-mediated DNA carriers. Once inside the body and in contact with the specifically targeted cells, the inserted DNA is incorporated into the tissue's cells where it encodes the production of the needed protein.

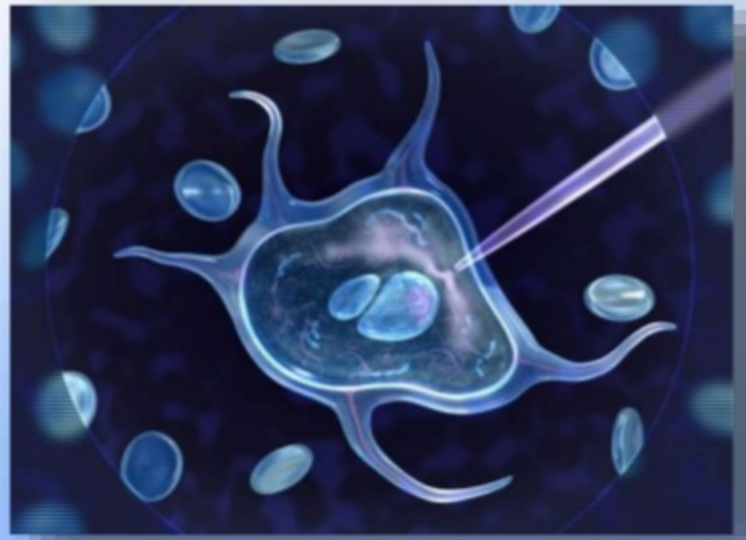


In vitro techniques:

- 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) etc.



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.

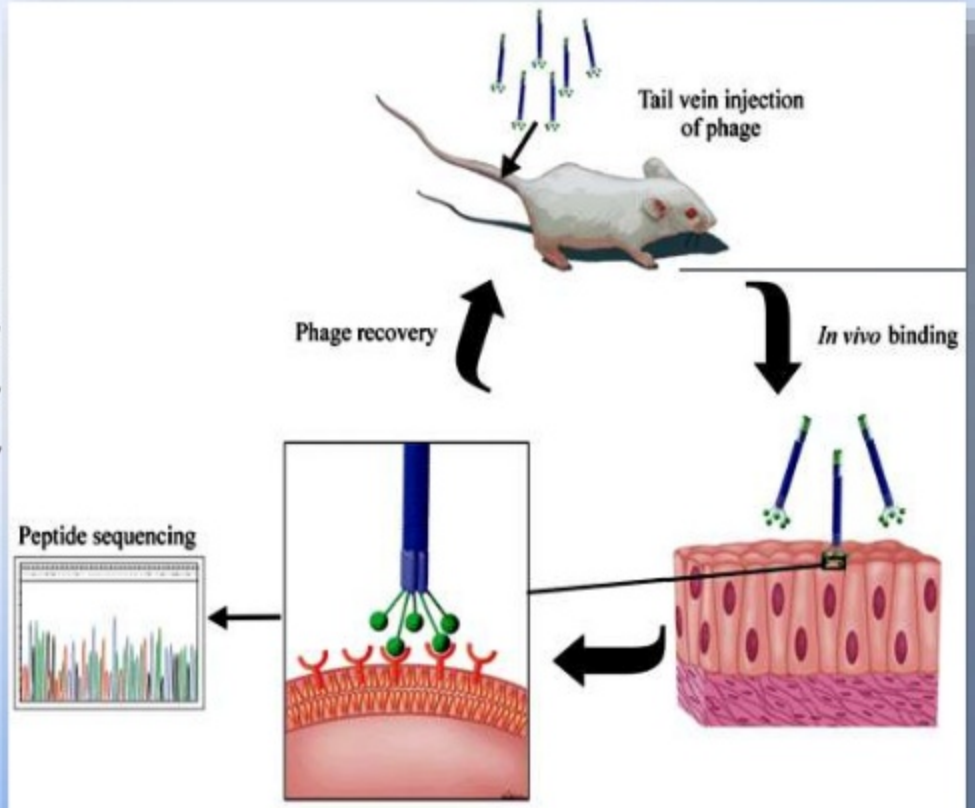


Figure 2 - *In vivo* use of phage libraries. Initially the phage library is injected in the circulation. Next, phage is allowed to circulate for minutes or hours (in this case when internalized phages are to be recovered [1]). Finally [2], after deep anesthesia, mice are euthanized and the organs are dissected for phage recovery and peptide sequencing.

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.***
- 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 integrity.

Types of bioassay.

- **Qualitative bioassay** → is used for assessing the physical effects of a substance that may not be quantified, such as abnormal development or deformity.

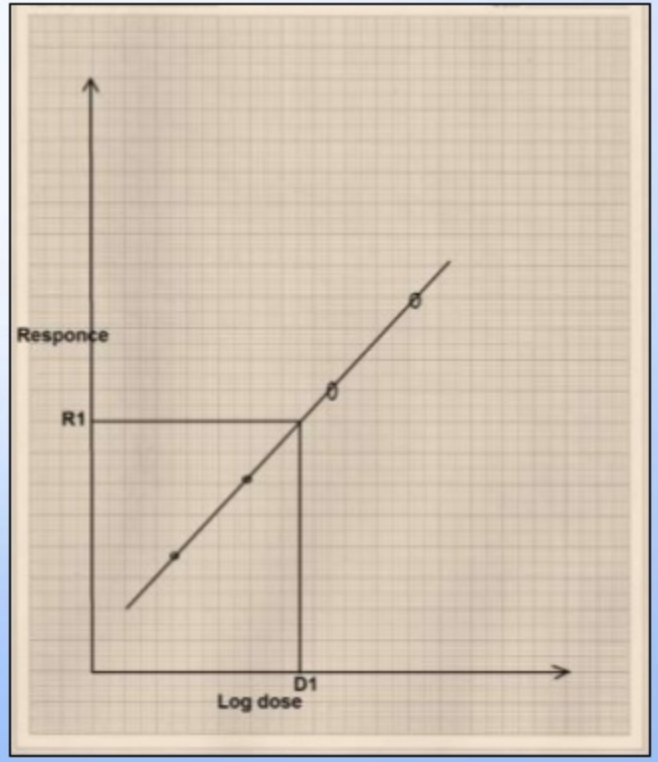
Eg: Arnold Adolph Berthold's famous experiment on castrated chickens. This analysis found that by removing the testes of a chicken, it would not develop into a rooster because the endocrine signals necessary for this process were not available.

- **Quantitative bioassays** → involve estimation of concentration/potency of a substance by measurement of the biological response it produces. These bioassays are typically analyzed using the methods of biostatistics.

Bioassay Methods.

1. 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.

- 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.

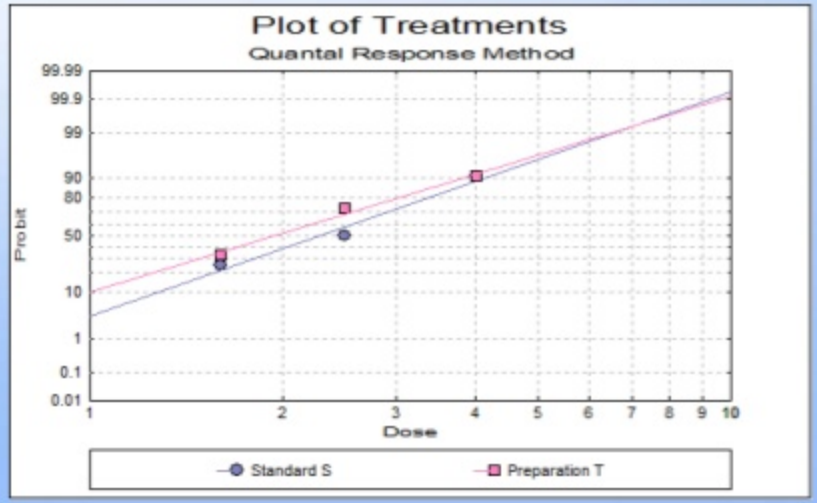


2. End Point or Quantal Assay: As the name indicates, the threshold dose of the sample required to elicit a complete or a particular pharmacological effect is determined and compared with standard.

- E.g., Digitalis producing cardiac arrest.
- Even the Determination of LD50 (LD=Lethal dose) or ED50 (ED= effective dose) is done by this method.

Based on the method used during the grade point assay procedure for determination of Type of activity and Potency of the Sample, four methods of assays are classified as:

- Matching point or bracketing method
- Interpolation assay
- Three point (2+1) assay
- Four- point (2+2) assay



Matching point or bracketing method:

- Here a constant dose of the standard is bracketed by varying dose of sample until an exact matching between the standard dose responses and the particular dose response of the sample is achieved.

This technique is used

- when test sample is too small
- Inaccurate & margin of error

  difficult to estimate

Eg: histamine on guinea pig ileum,

Posterior pituitary on rat uterus.

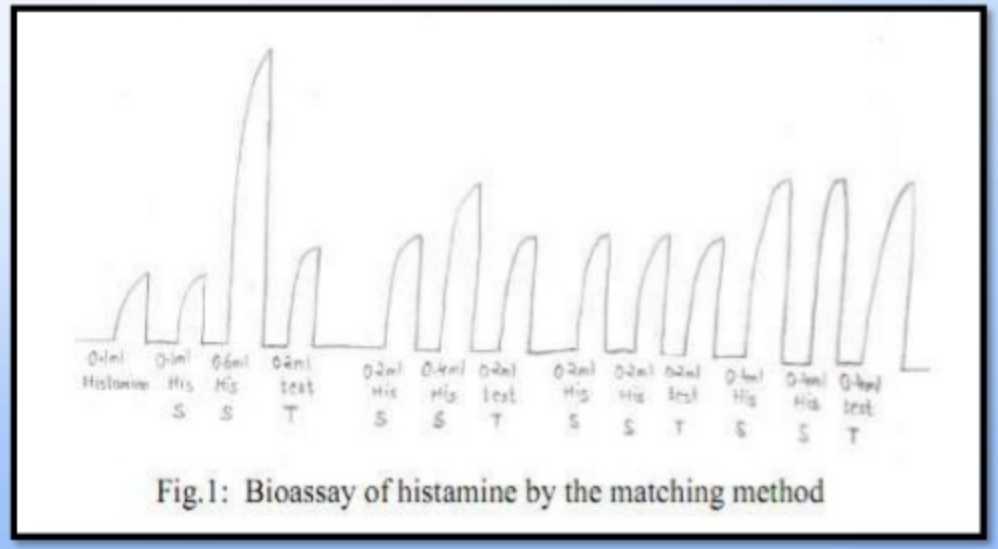
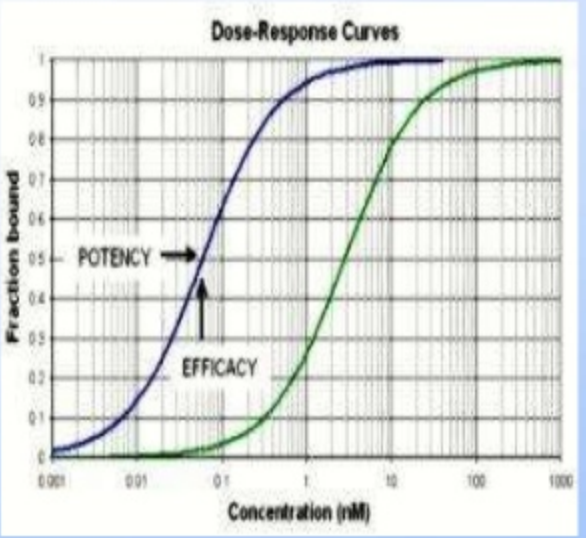


Fig.1: Bioassay of histamine by the matching method

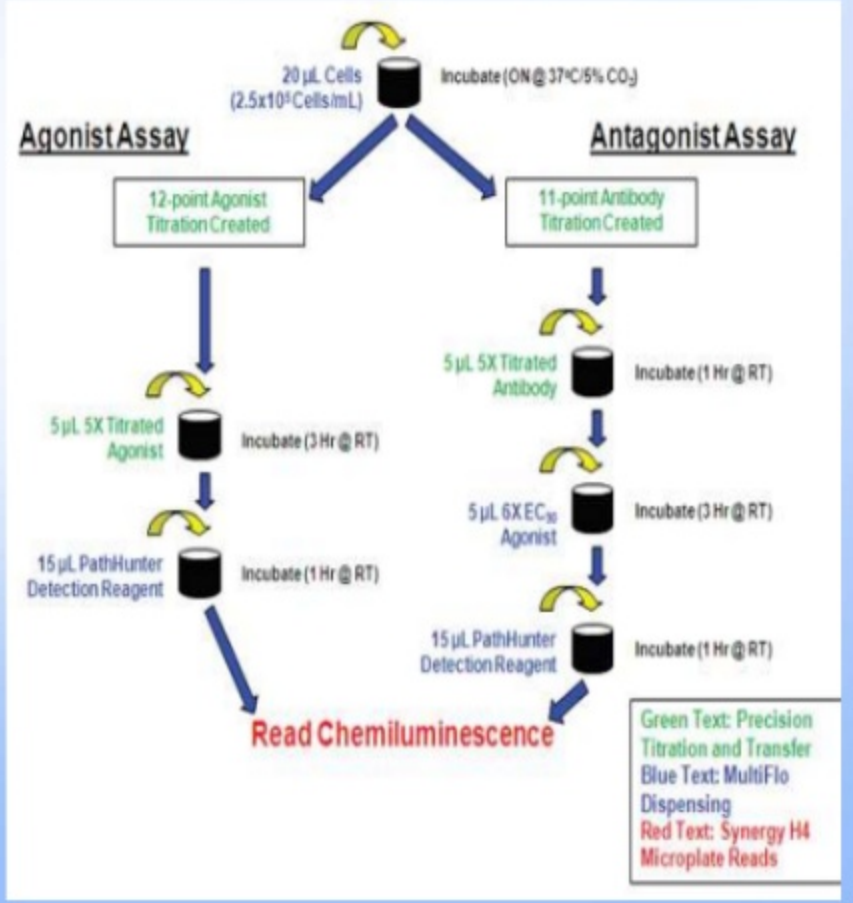
Interpolation assay.

- Bioassays are conducted by determining the amount of preparation of unknown potency required to produce a definite effect on suitable test animals/organs/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.



Multi point Bioassay.

- This method incorporates the principle of interpolation and bracketing.
- 2+1 indicates- Two 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.
- It can further divided as 3 point, 4 point and 6 point bioassay.



Three point assay [2+1 dose assay]

- Fast & convenient:
  - Log dose response [LDR] curve plotted with varying conc of std drug solutions and given test solution
  - Select two std doses s1& s2 [in 2:3 dose ratio] from linear part of LDR [Let the corresponding response be S_1, S_2]
  - Choose a test dose t with a response T between S_1 & S_2
  - Record 4 sets data as follows
    - s1 s2 t
    - t s1 s2
    - s2 t s1
    - s1 s2 t
- Log Potency ratio [M] = $[(T - S_1) / (S_2 - S_1)]$ X log (dose ratio)

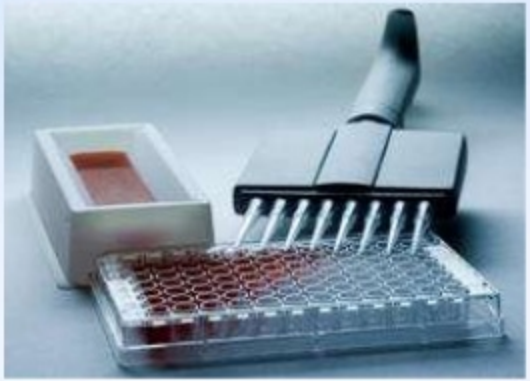
4 point assay [2 +2 dose assay]

- [E.g. Ach bioassay]
  - Log dose response [LDR] curve plotted with varying conc of std Ach solutions and given test solution
  - Select two std doses s1& s2 from linear part of DRC [Let the corresponding response be S_1, S_2]
  - Choose two test doses t1 & t2 with response T_1 &T_2 between S_1 & S_2 ;
  - Also $s_2/s_1 = t_2/t_1 = 2/3$

Record 4 data sets

- s1 s2 t1 t2
- s2 t1 t2 s1
- t1 t2 s1 s2
- t2 s1 s2 t1

Other bioassay's.



- Immunological assay (ELISA).
- Micro-bioassay.
- Radioimmunoassay.
- Biotechnology.