Bioassay

Disclaimer

All the content material provided here is only for teaching purpose.

BIOASSAY:

- **OVERVIEW**

BIOASSAY – Definition/Synonyms

PRINCIPLES OF BIOASSAY

INDICATIONS OF BIOASSAY

TYPES OF BIOASSAY

USES OF BIOASSAY

DRAWBACKS

BIOASSAY IN HUMANS

Bioassay:

Definition: Potency or concentration of an active principle in unit quantity of preparation by measuring its biological response on living tissues.

- Introduced by Paul Ehrlich - biostandardization of Diphtheria antitoxin

Principles of bioassay:

- To compare the test substance with the International Standard preparation of the same
- To find out how much test substance is required to produce the same biological effect, as produced by the standard
- Activity assayed should be the activity of interest
- Standard & test sample - similar pharmacological effects & mode of action
- Both should be compared for their established pharmacological effect using specified technique
- Problem of biological variation must be minimized.

Indications of bioassay

- No chemical method has been developed
- Chemical assay is too complex /not sensitive enough to measure (ex: insulin, Ach)
- To measure the pharmacological activity of new or chemically undefined substances
- For biological standardization of drugs obtained from natural sources as these cannot be obtained in pure form. Eg: Oxytocin,Vasopressin,Insulin,Heparin
- To compare the strength of a drug obtained from various sources due to different compositions (Eg:Cardiac glycosides)
- Chemicals with similar structure, but different biological activity
- Chemical structure of the active principle is unknown
- Chemical structure known; cannot be actively purified. Eg: Peptide hormones

Types of Bioassay

- **QUANTAL ASSAY**

  Quantal response - the response is in the form of "all or none", i.e. either no response or maximum response.

  Drugs producing quantal effect can be bioassayed by *end point method*

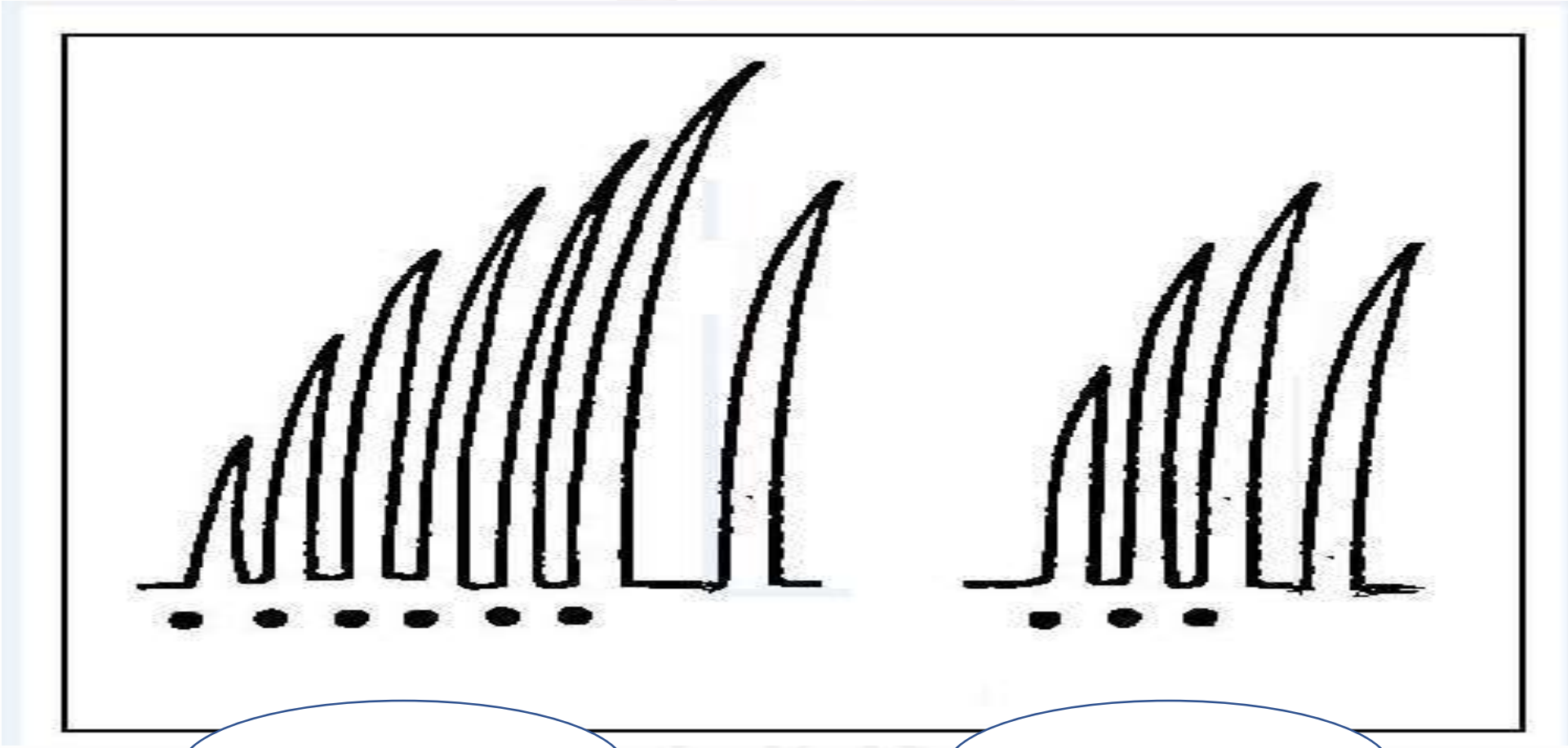
- **GRADED ASSAY**

  Graded response - response is proportional to the dose and response may lie between no response and the maximum response.

Types of Graded Assay:

- **Bracketing /direct matching**
- **Interpolation**
- **Multiple point assays**
- Three point assay
- Four point assay
- Six point assay
- **Cumulative dose response**

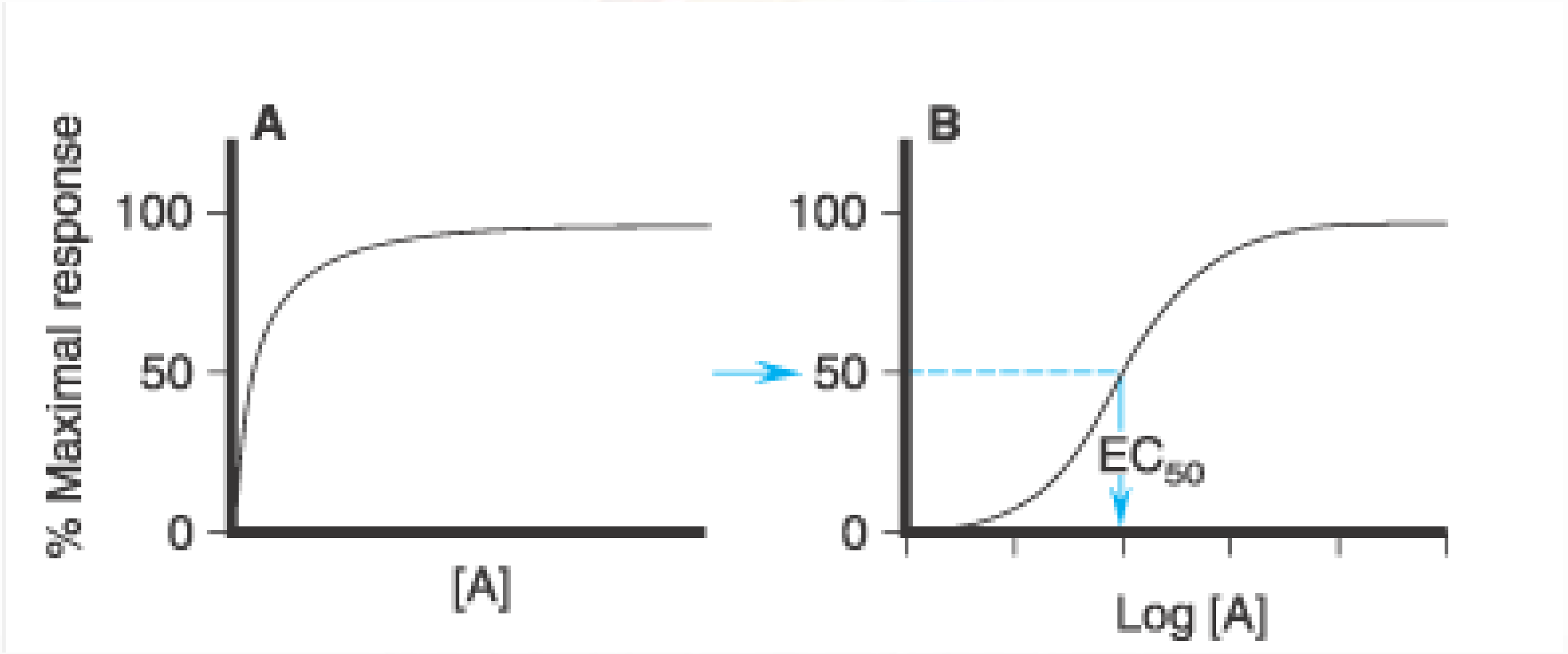
Graded DRC:



standard

Test/unknown

DRC & Log DRC:

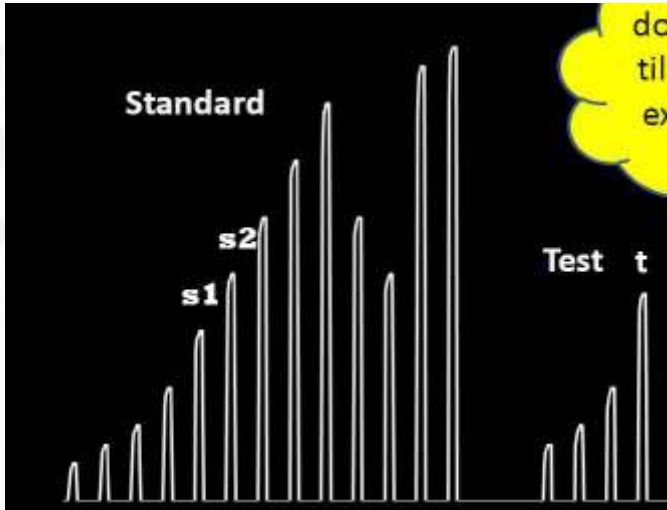


Rectangular hyperbola

Sigmoid curve

Bracketing or Direct Matching:

- A constant dose of the standard is bracketed by varying dose of test sample
- until an exact matching between the response of std & that of the sample is achieved
- Strength of unknowm/test drug can be found by simple interpolation of bracketed response.



Bracketing or Direct Matching

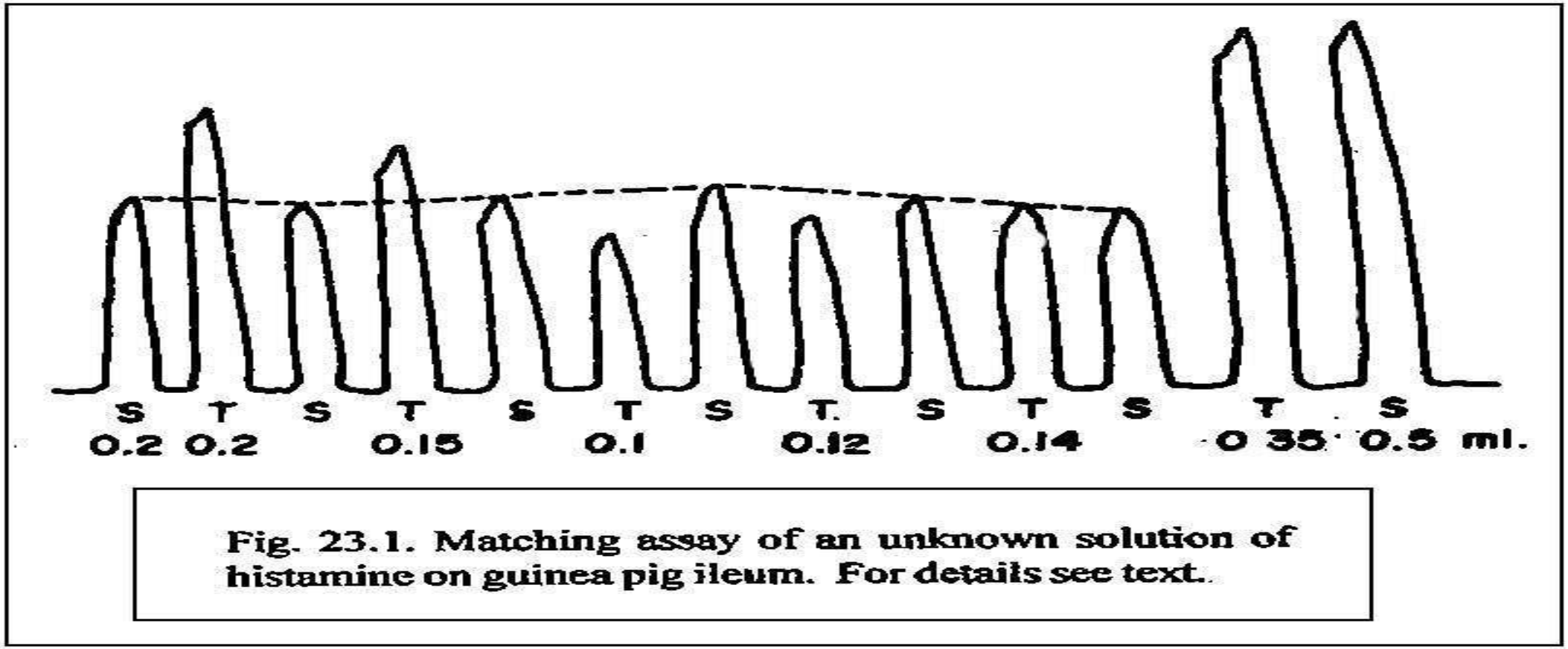
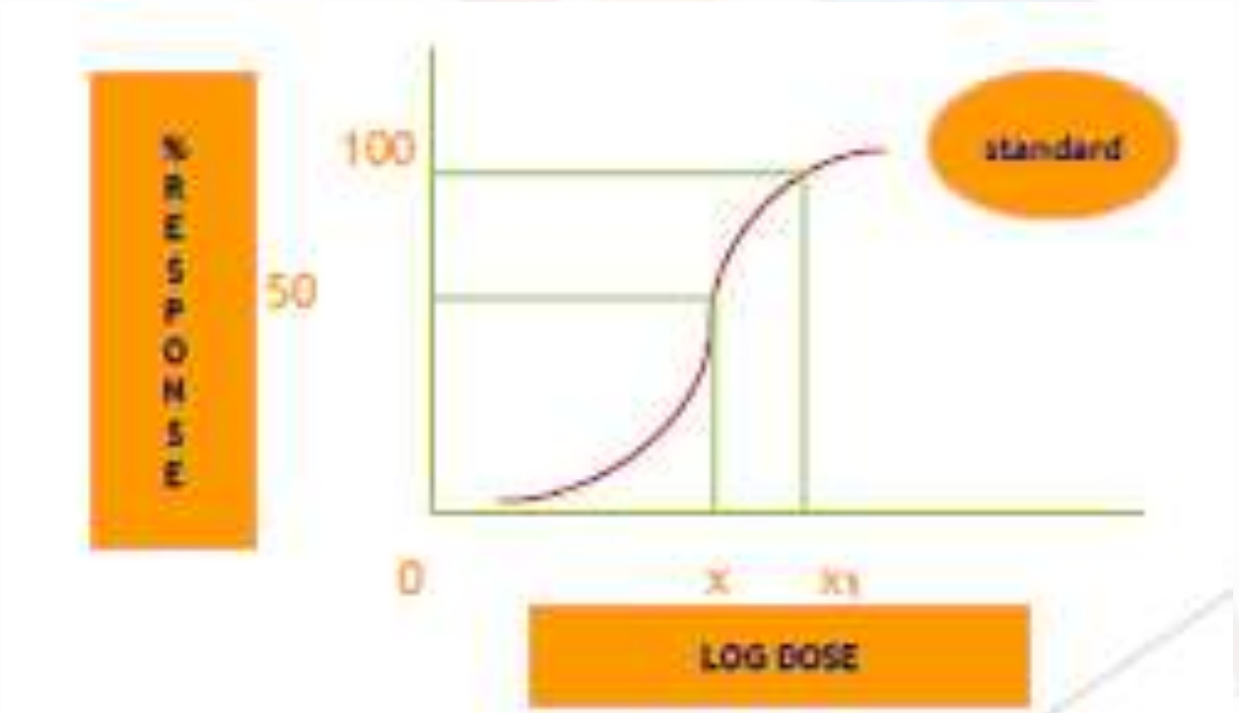


Fig. 23.1. Matching assay of an unknown solution of histamine on guinea pig ileum. For details see text.

Interpolation assay



Multiple point assays

- Responses are repeated several times and the mean of each is taken
- Chances of error are minimized

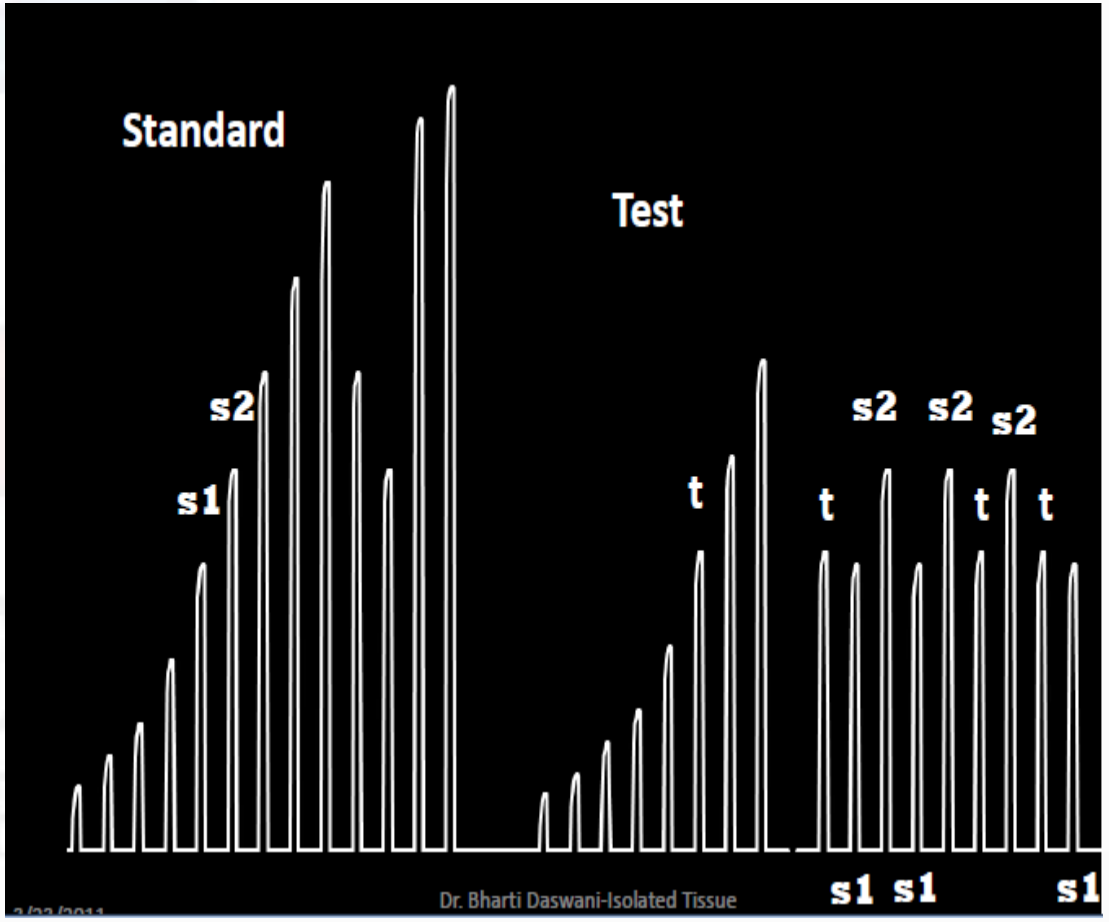
  3 point method - 2 doses of std+1 dose of test

  4 point method - 2 doses of std+2 doses of test

  6 point method - 3 doses of std+3 doses of test

3 - point assay

s_1	s_2	t
t	s_1	s_2
s_2	s_1	t
t	s_2	s_1



CALCULATION

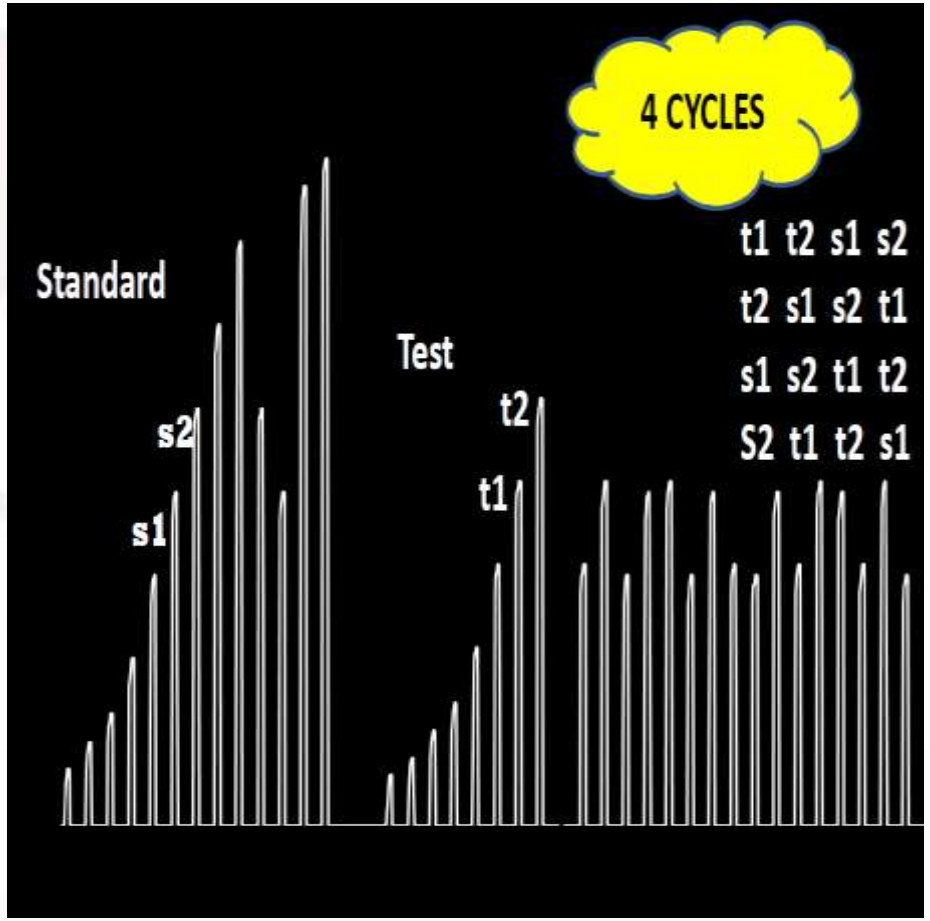
- Mean responses of these 3 sets plotted
- Log potency ratio (M) = (T-S1÷ S2-S1)× log d
- where, d – dose ratio = s2/s1

- Strength of unknown =s1/t × antilog of M

4 - POINT ASSAY

s_1	s_2	t_1	t_2
s_2	t_1	t_2	s_1
t_1	t_2	s_1	s_2
t_2	s_1	s_2	t_1

Latin Square design



Calculation

- Mean responses of 4 sets plotted

  Log potency ratio (M)

$$\frac{(T2-S2)+(T1-S1)}{(S2-S1)+(T2-T1)} \times \text{Log d}$$

where, d-dose ratio = s2/s1

Strength of unknown = s1/t1 × antilog of

Six point assay

- 3+3 dose assay
- 3 conc each of std & test drug are used
- 6 sets of experiments using 6 doses in each set
- More time consuming,lesser in use
- Reliability is excellent

References

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