

# Principles and Types of Bioassay

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# Outline

- Introduction
- Indications of Bioassay
- Principles of Bioassay
- Classification of Bioassay: Graded & Quantal
- Bioassay of antagonist
- Advantages and Disadvantages of Bioassay
- Error in Bioassay
- Human Tissue Bioassay
- Conclusion

# INTRODUCTION

## What is a **Bioassay**?

- Comparative assessment of relative potency of a **test** compound to a **standard** compound on a living tissue.
- **Qualitative** identification & **Quantitative** measurement of the **amount of active principle** in pharmaceutical preparation or biological material.
- Measurement of conc. of a drug from magnitude of its biological effect.

# INTRODUCTION

- Is Bio-standardization same as Bioassay?
  
- Historical aspect: **Paul Ehrlich** – Bio-standardization of Diphtheria antitoxin.

# INDICATIONS OF BIOASSAY

- Active principle **unknown**.
- Active principle **cannot be isolated**.
- To study biological **response of new drug**.
- To **ensure purity & potency**.
- If **chemical assay** not available/ complex/  
**insensitive** to low doses.
- To estimate concentration of **endogenous mediators**.

# PRINCIPLES OF BIOASSAY

- **Compare** potency of unknown substance with standard (including assessment of errors).

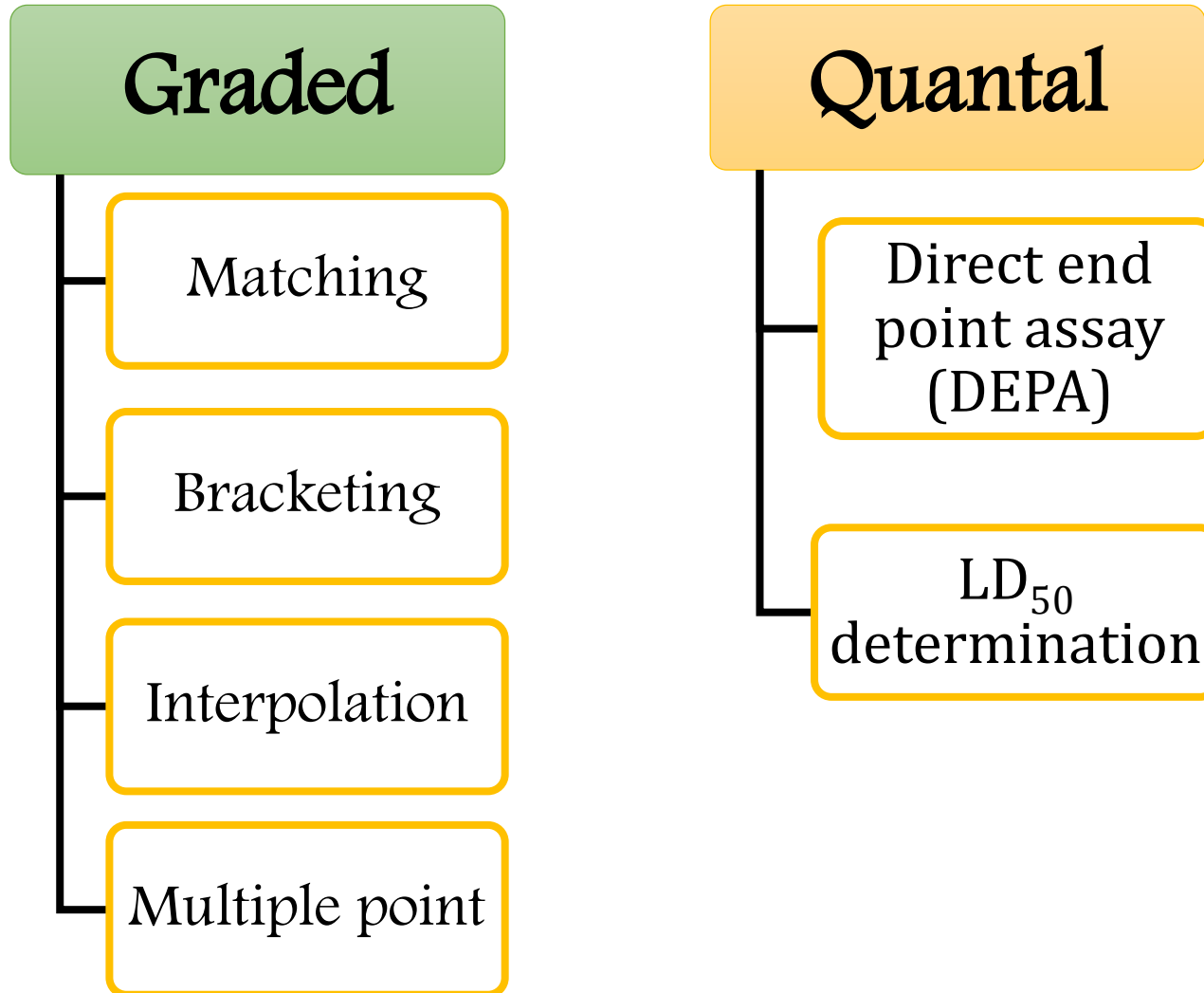


- Standard & test sample should have **same pharmacological effect & mode of action.**

# PRINCIPLES OF BIOASSAY

- The test and standard should be compared using **a specified pharmacological technique.**
- Method selected should **be sensitive, reproducible & should minimize errors** d/t biological variations & methodology.

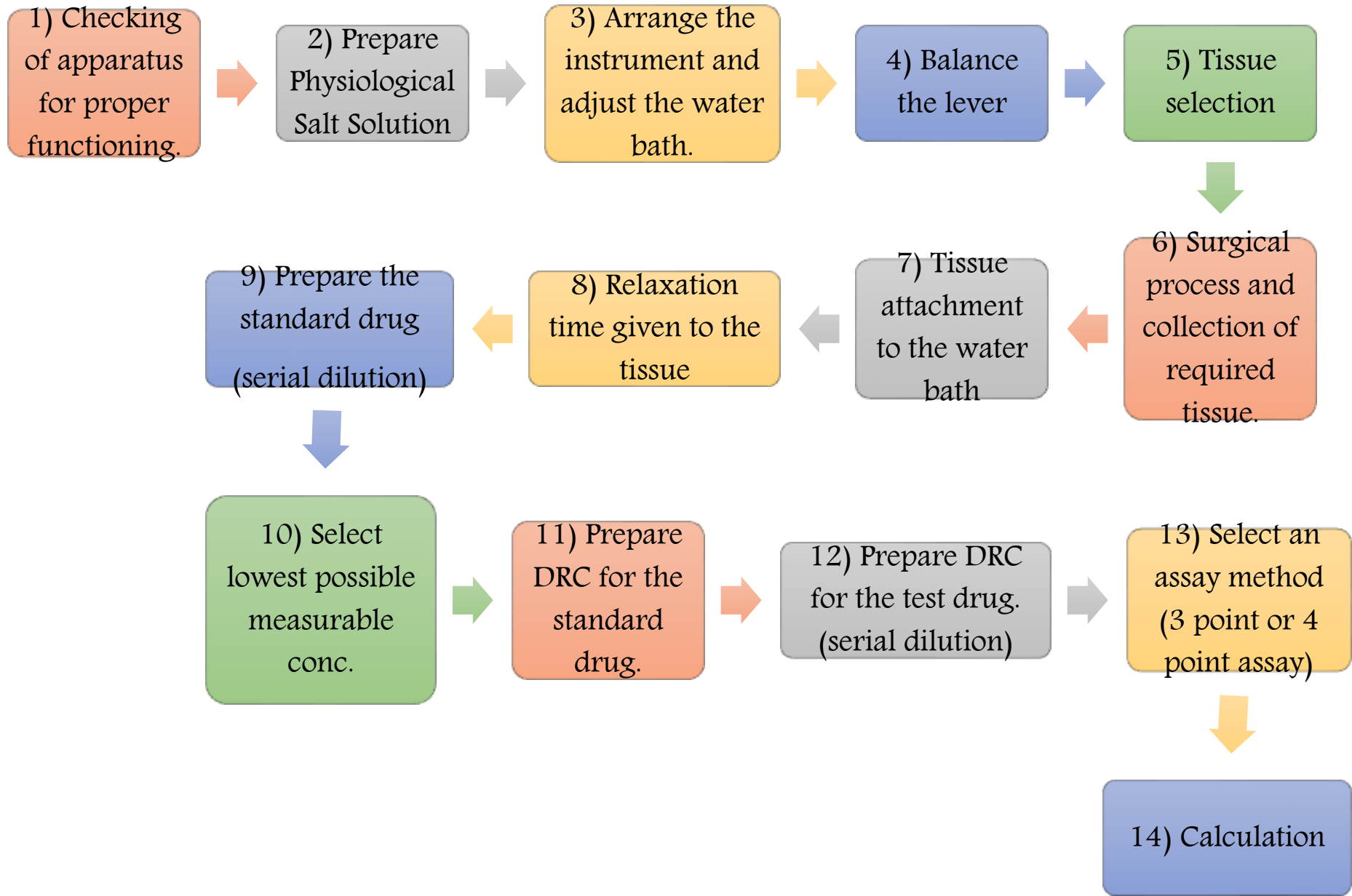
# TYPES OF BIOASSAY





# **GRADED BIOASSAY**

# METHODOLOGY: Graded Bioassay







Graded Bioassay



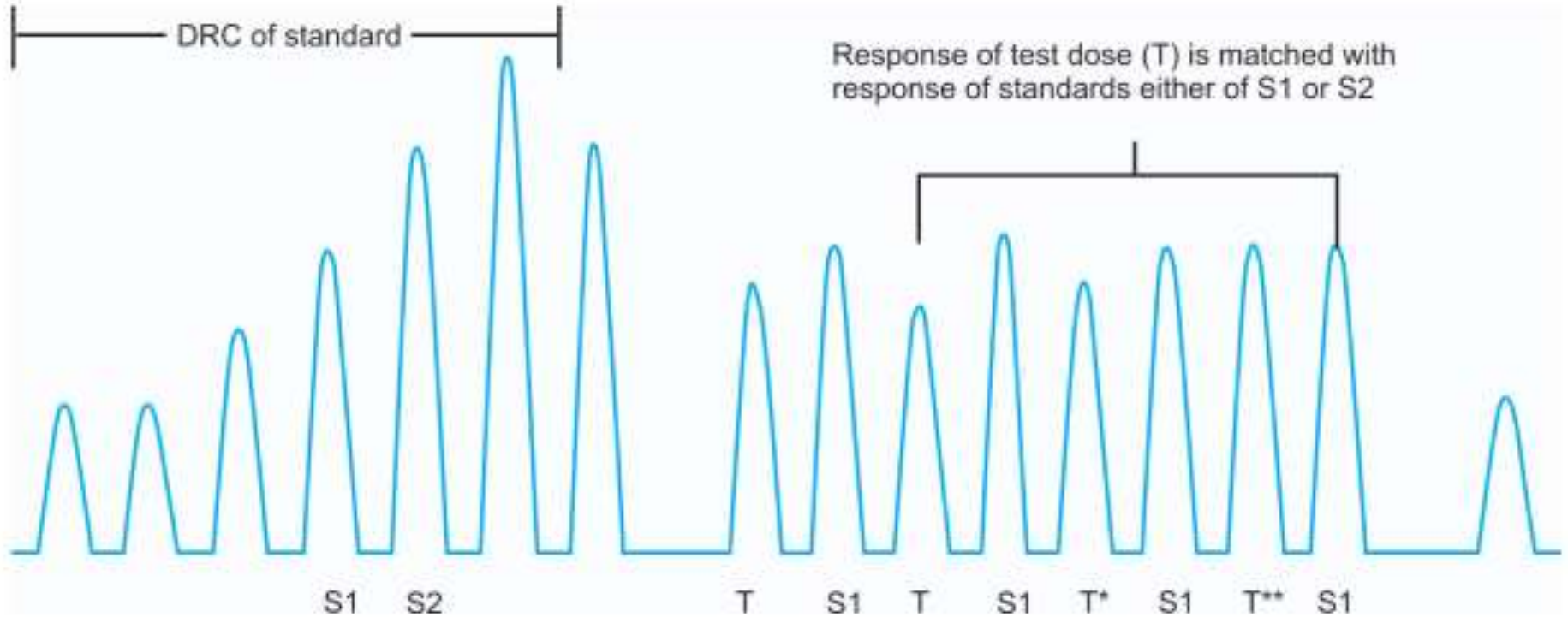
Intermittent dose  
method



Cumulative dose  
method



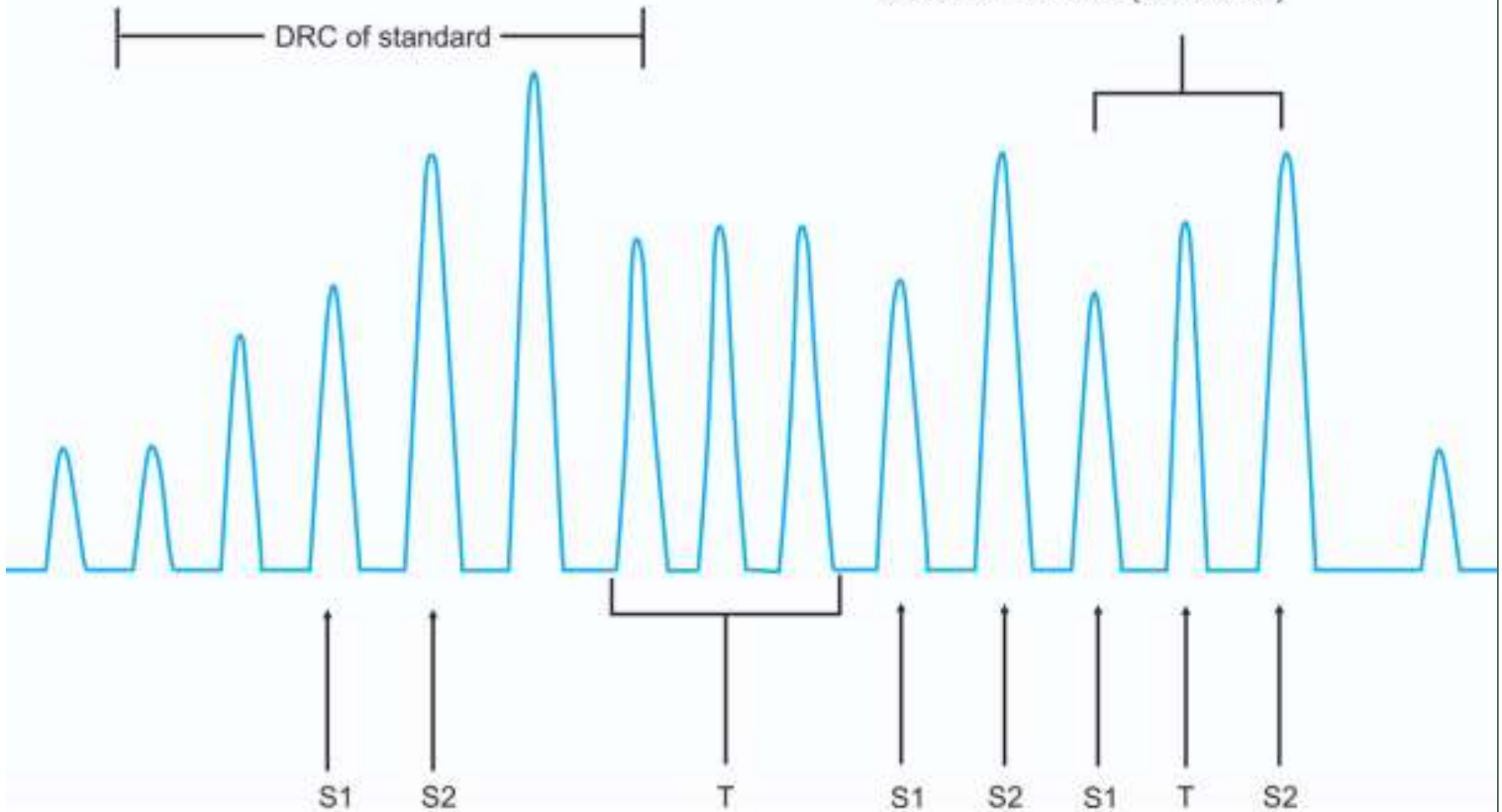
# Matching Assay



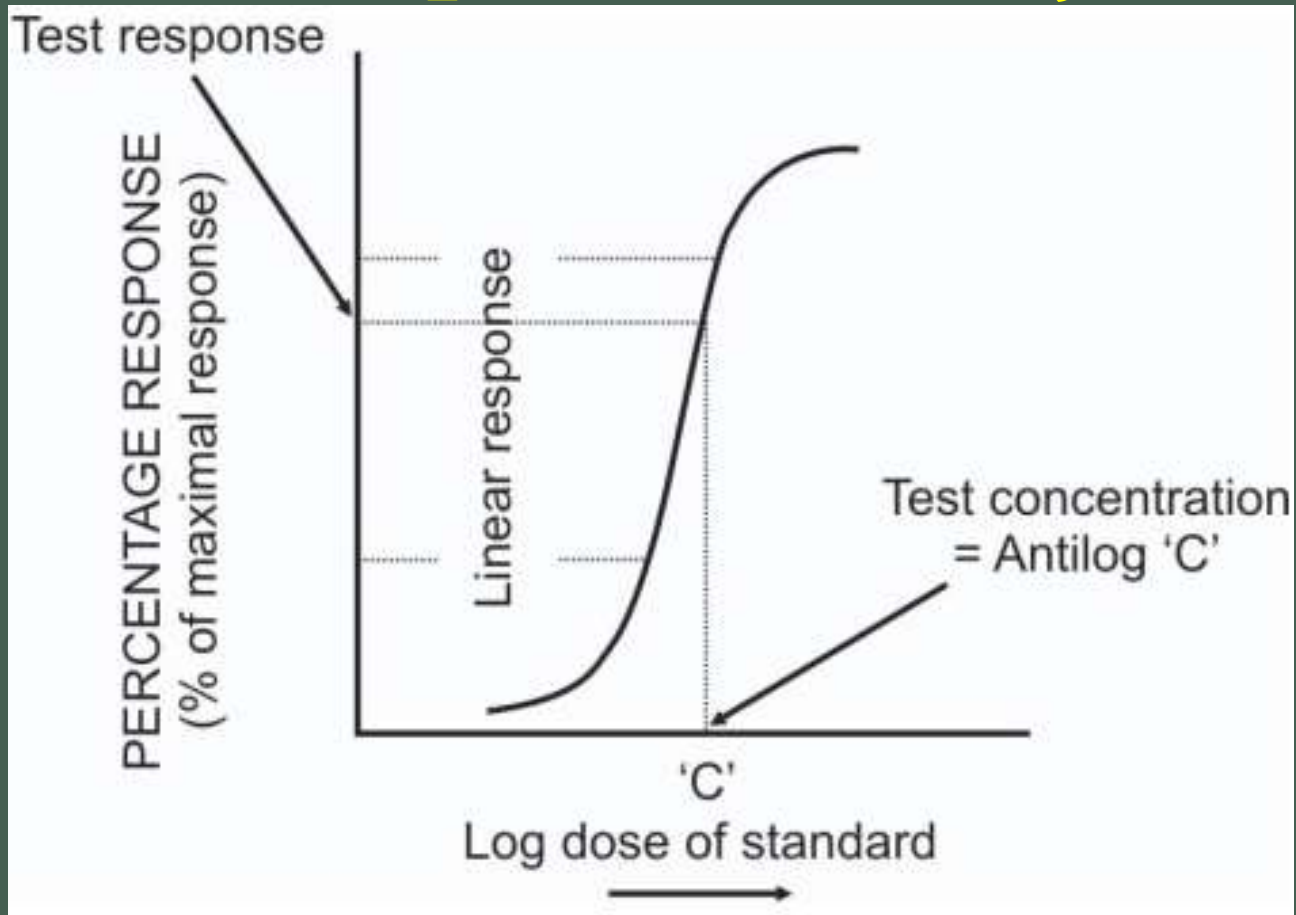
- **Adv:** Test DRC not reqd., small vol., fast.
- **Disadv:** Trial & Error method, poor precision.

# Bracketing Assay

Test response (T) is bracketed between 2 dose of standard (S1 and S2)



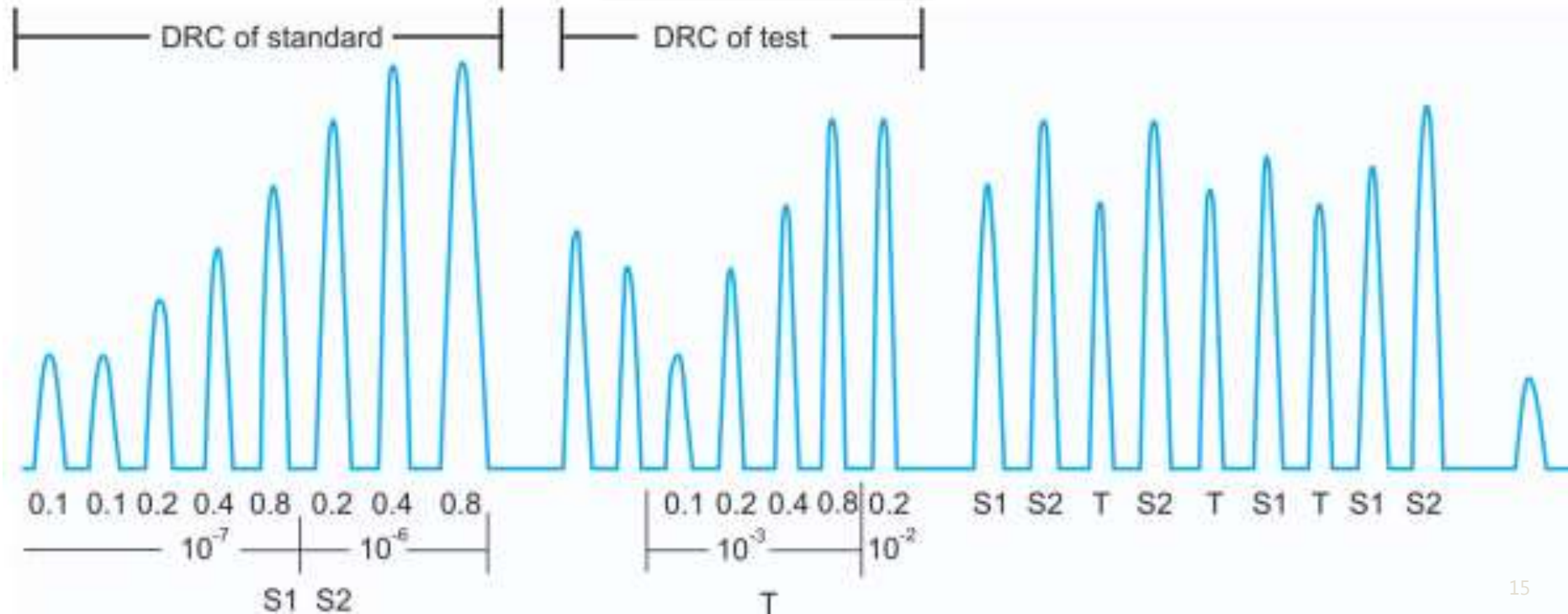
# Interpolation Assay



Conc. of unknown is read from a standard plot of a log dose response curve.

# Three Point Assay

$S_1$	$S_2$	T
$S_2$	T	$S_1$
T	$S_1$	$S_2$



# Three Point Assay

- Mean responses of three sets taken.
- Potency ratio calculated.

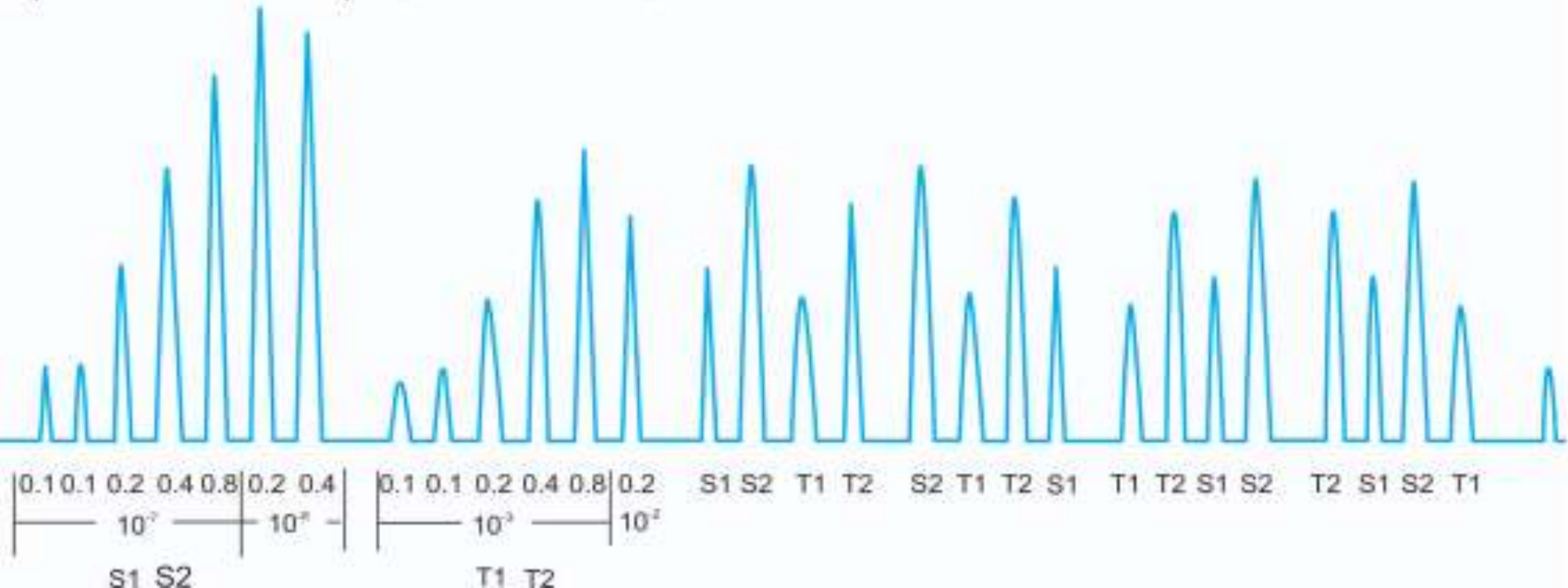
$$M = \frac{T - S_1}{S_2 - S_1} \times \log \frac{s_1}{s_2}$$

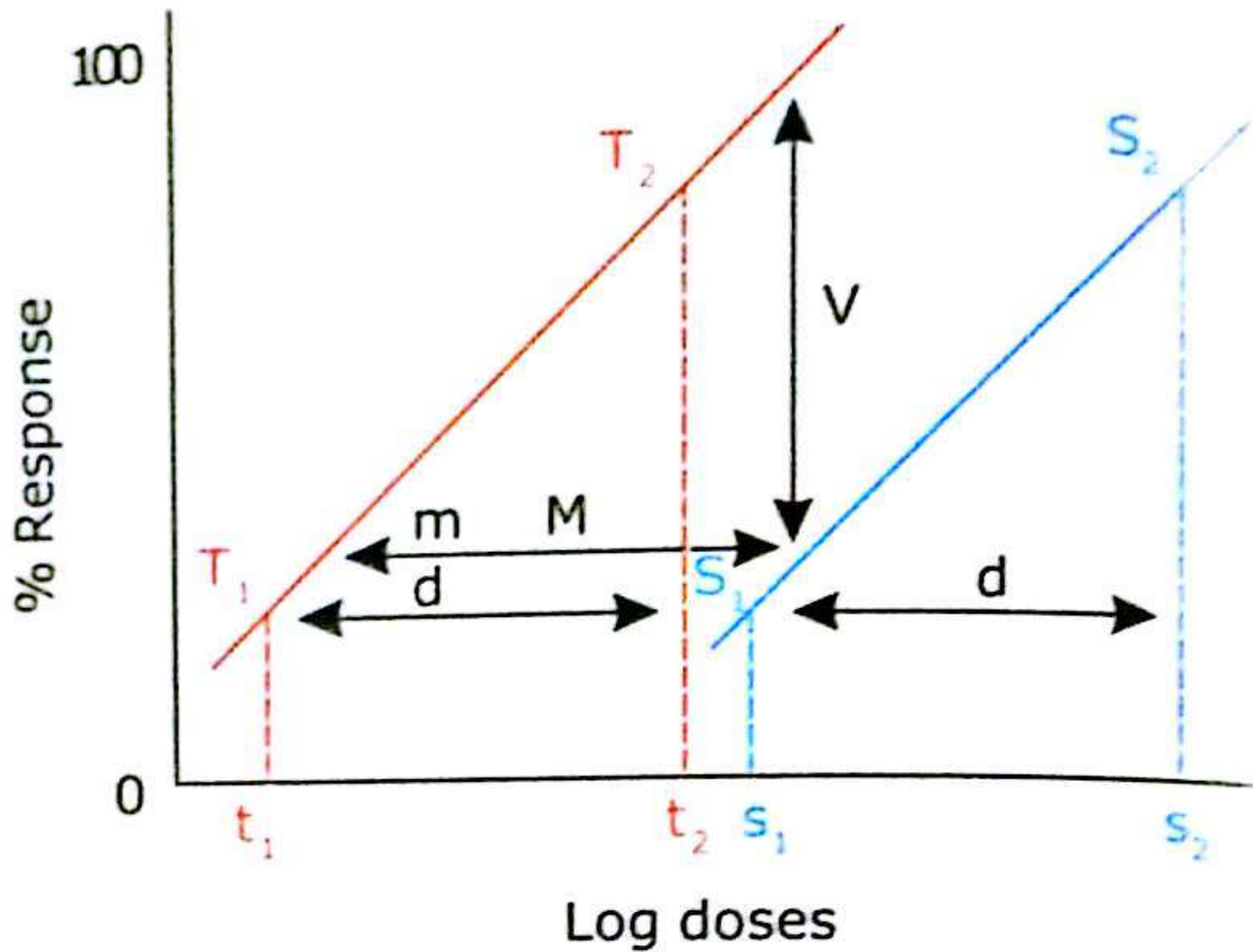
- Strength of test solution =  $\frac{s_1}{t}$  x antilog M



# Four Point Assay

$S_1$	$S_2$	$T_1$	$T_2$
$S_2$	$T_1$	$T_2$	$S_1$
$T_1$	$T_2$	$S_1$	$S_2$
$T_2$	$S_1$	$S_2$	$T_1$





# Four Point Assay

$$M = \frac{[T_1 - S_1 + T_2 - S_2]}{S_2 - S_1 + T_2 - T_1} \times \log \frac{s_2}{s_1}$$

$$T \text{ (concentration)} = \frac{s_1}{t_1} \times \text{antilog } M$$

# Six Point & Eight Point Assay

## Multiple point assays:

- **Adv:** Reduced error, reduced variability.
- **Disadv:** Lengthy, Large amount of test sample required.

# QUANTAL BIOASSAY

# Direct End-Point Assay

- Threshold dose producing a required response is measured on each animal.
- Eg. Bioassay of Digitalis in Cats,  
Hypoglycemic convulsions in mice.
- Threshold dose = Period of infusion X Rate.

$$\text{Concentration of test} = \frac{\text{TDS}}{\text{TDT}} \times \text{CSD}$$

# LD<sub>50</sub> Determination: Graphical & Arithmetic methods

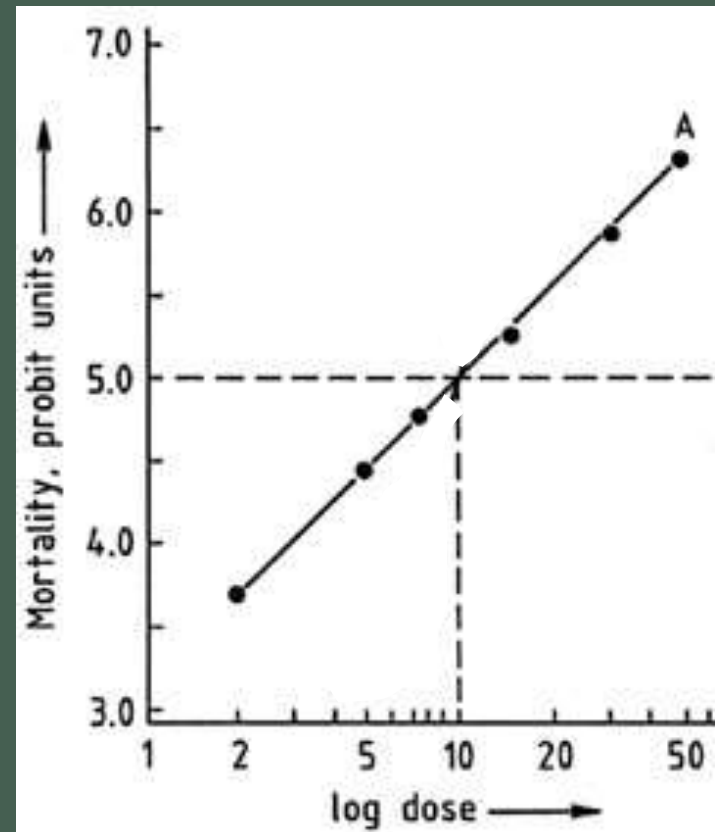
Table 3.2 Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

LD50 determination by Karber's method

Group	Dose mg/kg	No. of animals	Dose difference (a)	Dead	Mean mortality (b)	Product (a × b)
1	64	10	—	0	—	—
2	71	10	7	2	1	7
3	81	10	10	4	3	30
4	90	10	9	9	6.5	58.5
5	100	10	10	10	9.5	95
						190.5

$$LD_{50} = 100 - (190.5/10) = 81 \text{ mg/kg (approx)}$$



# Bioassay of Antagonist

Determination of the type of drug antagonism:

- Parallel shift of the log DRC.
- Double reciprocal (**Lineweaver & Burk**) plot
- **Schild Plot** and  $pA_2$  value.



# Advantages & Disadvantages of Bioassay

# Advantages

- Chemical assay too complex.
- If difference b/w results; bioassay given more importance.
- Toxicity of new drug.

# Disadvantage

- **S**Time consuming.
- Requires much skill.
- Biological variations exist.

# Errors in bioassays

## ➤ **Biological variation**

- Loss of tissue sensitivity.
- Different species/sex/age/weight/health status.
- Laboratory condition may be variable.
- Housing and handling of animals.

## ➤ **Methodological error**

- Lack of standardization of procedure.
- Set-up of apparatus.
- Tissue isolation/preparation for experiment.
- Drug preparation or dilution.

# Human Tissue Bioassay

Animal tissues can't predict accurate outcomes.

**Limitations:** Ethical, costly, take time, cooperation of various specialties required, storage.

- **Vascular tissue:** Veins, cardiac blood vessels, large blood vessels after amputation.
- **Cardiac tissue:** Used fresh, stored at 4°C, functional for 2 weeks.
- **Brain tissue.**
- **Lung tissue.**

**Thank You**