Principles and Types of Bioassay

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- Introduction
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- 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?

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**.
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**
  - Matching
  - Bracketing
  - Interpolation
  - Multiple point

- **Quantal**
  - Direct end point assay (DEPA)
  - LD$_{50}$ determination

Quantal

LD$_{50}$ determination
GRADED BIOASSAY
METHODOLOGY: Graded Bioassay

1) Checking of apparatus for proper functioning.
2) Prepare Physiological Salt Solution
3) Arrange the instrument and adjust the water bath.
4) Balance the lever
5) Tissue selection
6) Surgical process and collection of required tissue.
7) Tissue attachment to the water bath
8) Relaxation time given to the tissue
9) Prepare the standard drug (serial dilution)
10) Select lowest possible measurable conc.
11) Prepare DRC for the standard drug.
12) Prepare DRC for the test drug. (serial dilution)
13) Select an assay method (3 point or 4 point assay)
14) Calculation
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)
Conc. of unknown is read from a standard plot of a log dose response curve.
Three Point Assay

$S_1 \quad S_2 \quad T$

$S_2 \quad T \quad S_1$

$T \quad S_1 \quad S_2$

DRC of standard

DRC of test
Three Point Assay

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

\[
M = \frac{T - S_1}{S_2 - S_1} \times \log s_1
\]

- Strength of test solution = \( s_1 \times \text{antilog } M \)

\[t\]
# Four Point Assay

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**DRC of standard**

**DRC of test**

- **S₁** and **S₂** represent standards.
- **T₁** and **T₂** represent test samples.

- **DRC of standard** and **DRC of test** indicate the dilution range for standards and test samples, respectively.
Four Point Assay

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

\[ T \ ( \text{concentration} ) = s_1 \times \text{antilog} \ M \]

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.

\[
\text{Concentration of test} = \frac{TDS}{TDT} \times CSD
\]
**LD$_{50}$ Determination: Graphical & Arithmetic methods**

### Table 3.2 Transformation of percentages to probits

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### LD50 determination by Karber's method

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<th>Group</th>
<th>Dose mg/kg</th>
<th>No. of animals</th>
<th>Dose difference (a)</th>
<th>Dead</th>
<th>Mean mortality (b)</th>
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LD50 = 100 - (190.5/10) = 81mg/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.

Disadvantages

➢ 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