# Faculty of Pharmacy- University of Tripoli

# Bioassay Practical

## Lab.4

## Methods of bioassay

# Experiments in bioassay may be performed on:

## Whole animal:

- anesthetized animal to study effect of drug on BP.
- un-anesthethetized animal: Behavioral studies.

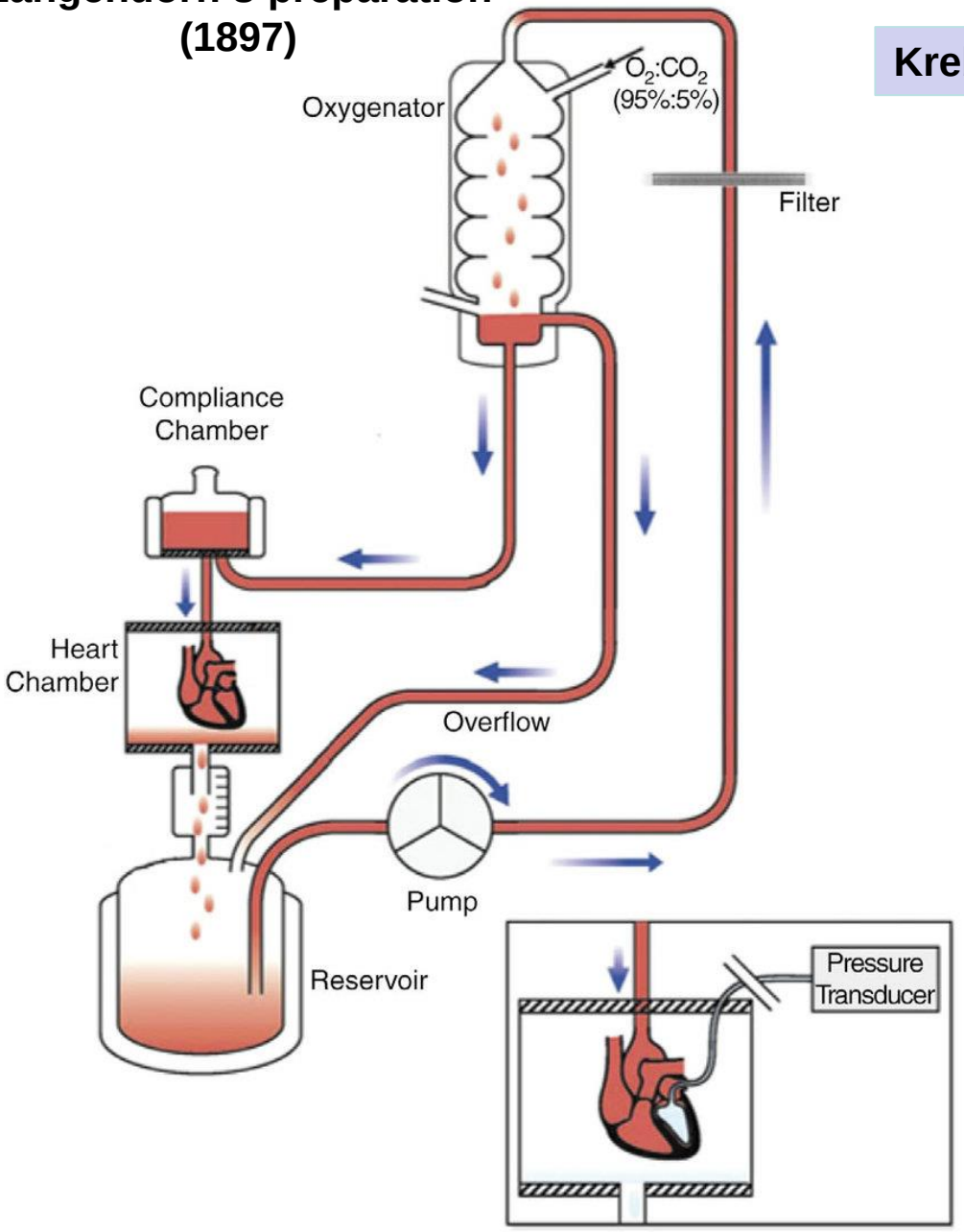
## Isolates organ:

- isolated perfused heart (Langendorff's preparation)

## Isolated tissue:

- isolated smooth muscle: guinea pig ileum

**Langendorff's preparation (1897)**




**Krebs–Henseleit solution or Tyrode's solution, O2**

**Drugs are added (via the perfusate) and observation of their effect on the heart without the complications involved with *in vivo* experimentation, such as ***neuronal*** and ***hormonal*** effects from living animal or human.**

# Methods of Bioassay

**1. Direct Assay Method:** (*End-point method*),

- (Usually 12-16 animals used; half for test & other half for standard)
- In this method **the threshold dose** producing a positive effect is measured on each animal and the comparison between the average results of two groups of animals (one receiving standard and other the test) is done.

Most simple type of assay, where drug is administered to the animal until a clear-cut end-point response is obtained.

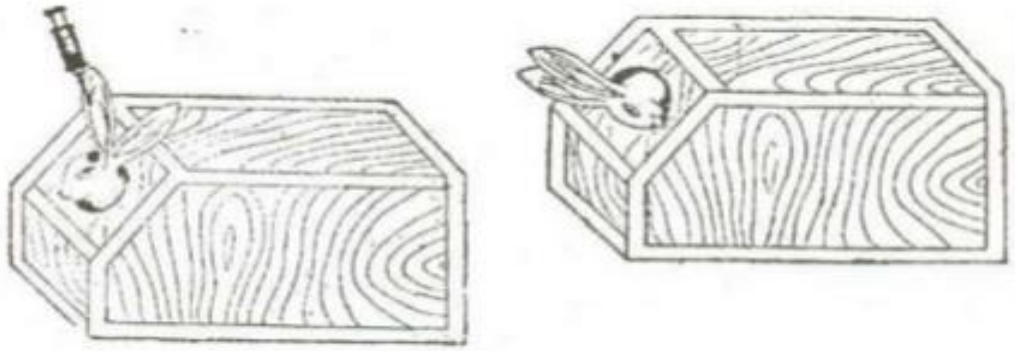
The dose required to produce this response is called “Threshold dose”.

**e.g:** *IV infusion of digitalis to guinea-pigs until → “cardiac arrest.

*Infusion of d-tubocurarine in rabbit →head-drop.

***Experimental Procedure:***

- Rabbit is placed in a holder with its head protruding outside.
- The head should be freely movable.
- Minimum 8 rabbits are used.



**D-tubocurarine is injected (iv) into the marginal vein of the rabbit until rabbit's neck muscles are relaxed to the point that the animal cannot hold its neck up.**

# Methods of Bioassay

**2. Indirect Assay Method:**

A. Graded response method:

- The response directly increased when dose is increased ➔ max response
    1. matching method
    2. bracketting method
    3. multiple point assay

B. Quantal response method: many drugs cannot produce clear cut end point

i.e. there is **all or none response**.

- the response is not graded response

ex. if you assayed insulin ➔ the mouse may either develop convulsion or not.

## Why we use isolated preparation?

- Isolated preparations are very good preparation for the following reasons:

1. Simple and quick
2. Isolated preparations produce direct action

   i.e there is no reflex action

1. No pharmacokinetic disturbance
2. No hormonal disturbance

**Indirect method**

**matching method (*in vitro* studies, isolated tissue)**

- we use Ach on isolated rat ileum (with known concentration, **standard**).
- Ach is an endogenous neurotransmitter concerned with autonomic nervous system
- Ach released in both ganglion (sympathetic and parasympathetic)
- Ach has short duration of action
- metabolized by acetylcholine esterase enzyme (AchE)
- we start with dose-response curve
- we use physiograph, consisting from water bath, tissue bath, transducer and recoding pen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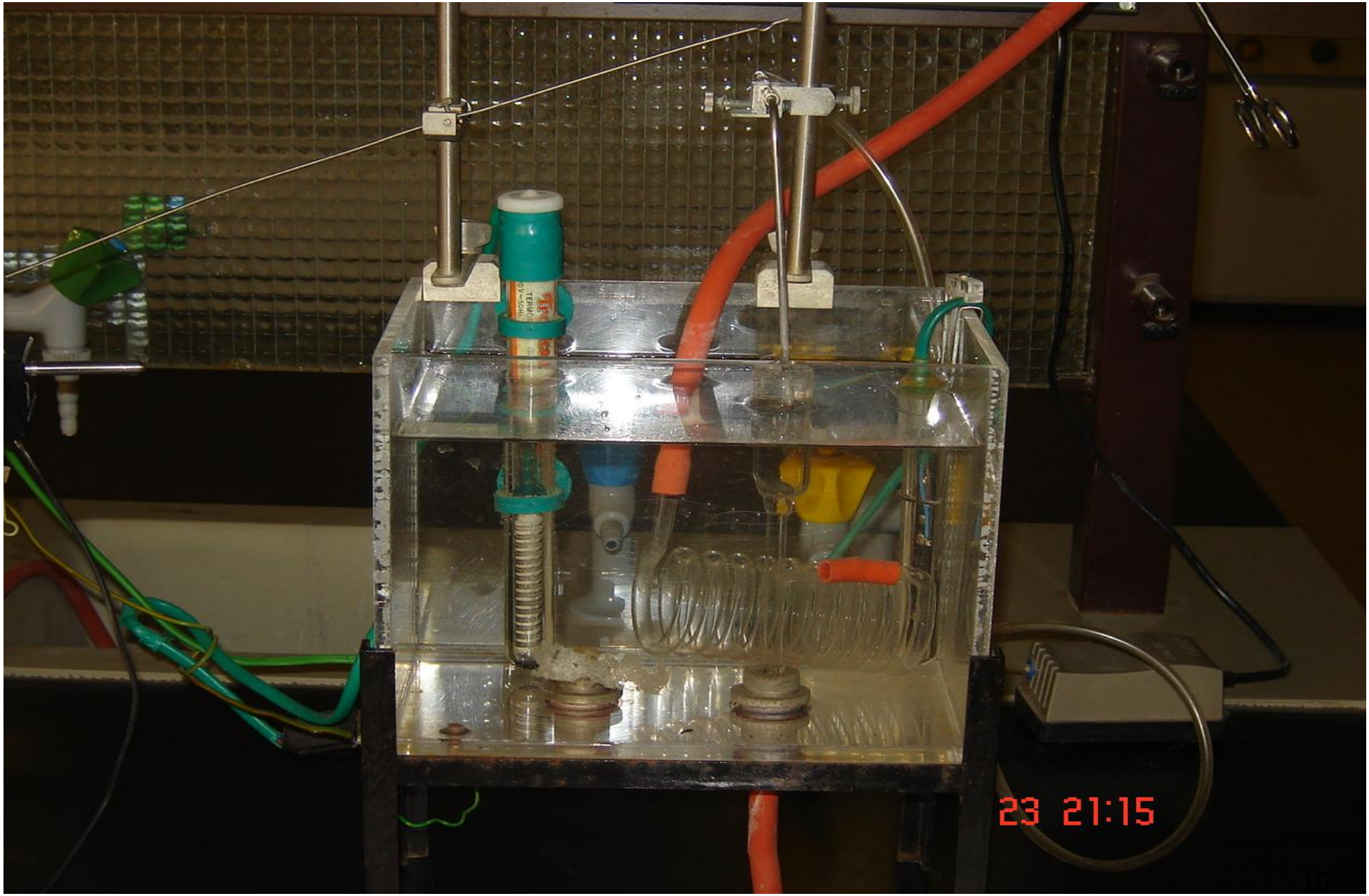
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AUX IN 1 MON OUT
CARDIAC COUPLER
TYPE 7176
INPUT
SENSITIVITY
POSITION
RECORD
AUX IN 2 MON OUT
IMPEDANCE PNEUMOGRAPH COUPLER
TYPE 7173
DIRECT
CAPACITIVE
INPUT
BALANCE
TRACE RESET
CALIBRATE
+ 5 OHMS
SENSITIVITY
POSITION
RECORD
AUX IN 3 MON OUT
TYPE 7173
INPUT
BALANCE
SENSITIVITY
POSITION
RECORD
AUX IN 4 MON OUT
TEMPERATURE COUPLER
TYPE 7174
THERMISTOR PROBE
TEMPERATURE
TEMPERATURE OUTPUT
SENSITIVITY
POSITION
RECORD
TIME MARKS
OFF
TIMER
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1
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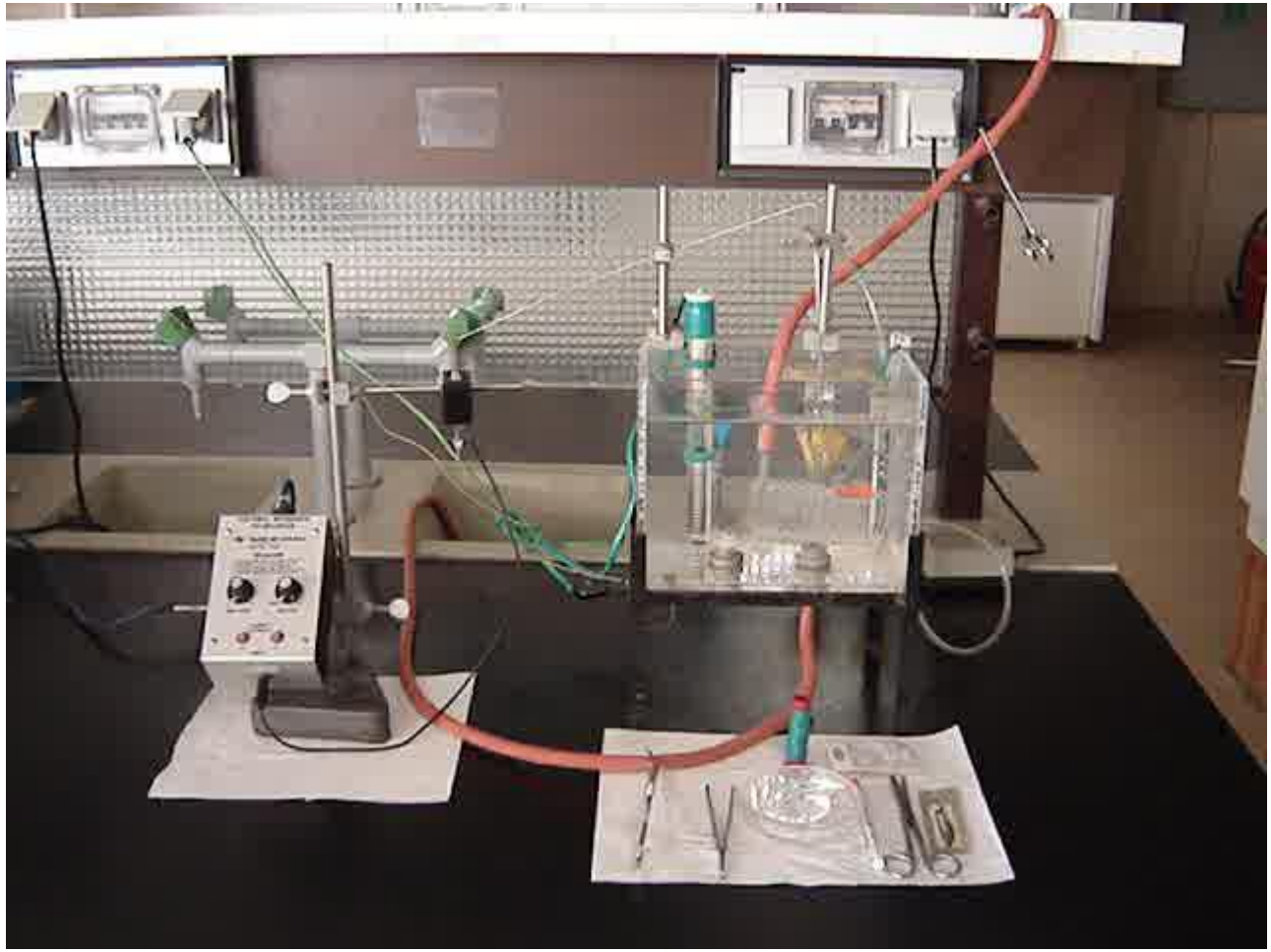
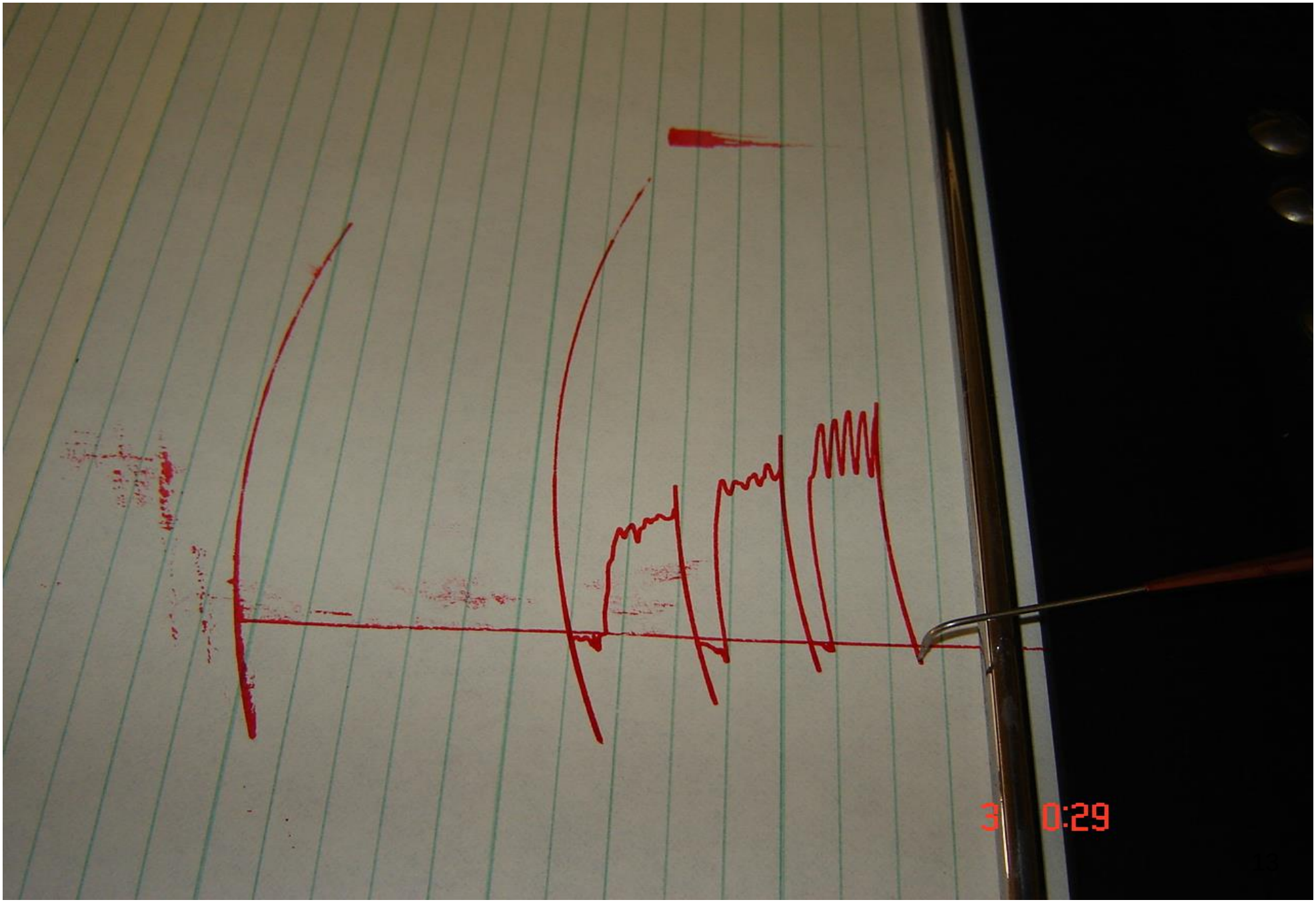
TIME & EVENT
EVENT MARKER
EXT. MKR.
PHYSIOGRAPH® MK-III-S
NARCO BIO-SYSTEMS
A HEALTHDYNE COMPANY
23 21:18

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- we do the same dose-response curve for **<u>test</u>** compound until reach the max response

- we compare the sub-max response of test drug with that of standard reference

# Procedure

1. A piece, about 2 cm of the ileum of freshly killed rat is suspended in organ bath containg Tyrode solution which is aerated with air and maintained at 37°C
2. The proximal end of the intestine is attached to a lever connected to an isotonic transducer that converts changes in the lenght of the muscle into electric current that is recorded on chart paper
3. Drugs to be tested are added – solution in the organ bath in ascending doses in the following way (drug- cycle):

a) leave the drug in contact with the tissue for 30 sec

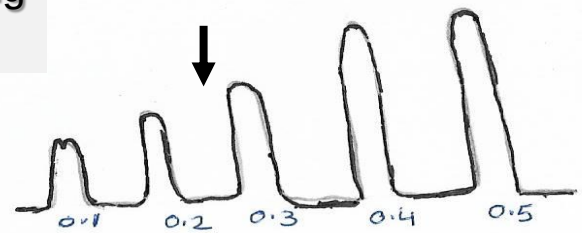
b) wash the dug out using Tyrode solution, leave the tissue about 1 min.

wash again to be sure that the effect of the drug has been eliminated (the tissue must return to the original lenght and the response should reach to the base line before addition of new dose of the drug.

4. use the same procedure described above for the standard drug solution
5. compare the sub-max response of test compound with that of standard response
6. make the dose-response curve of the test drug and the standard drug by blotting log of the concentration against the percentage of the maximum response

# Matching method

1. (DR) test drug
Ach (??????)

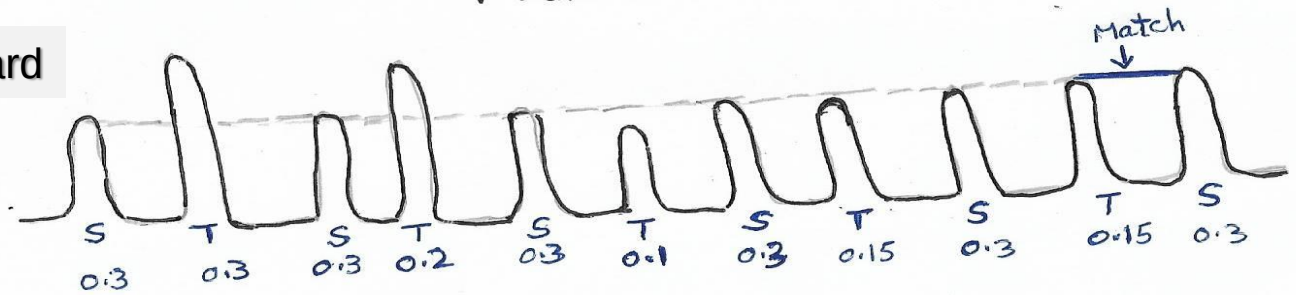



2. (DR) standard reference
Ach (10 µg/ml )

$$RP = \frac{\text{Volume of standard}}{\text{Volume of Test drug}}$$

$0.3/0.15 = \frac{2}{=}$ RP >1

3. Compare test & standard




Concentration of test drug = 2 X 10 = 20 µg/ml

We calculate the relative potency (RP)

- RP ➔ is the response of the test compound related to that the same standard reference
- The advantage of RP ➔ tell us how many time the test compound more or less potent than reference standard

RP = 1 RP>1 RP <1

$$RP = \frac{\text{volume of standard}}{\text{volume of test drug}}$$

Then we calculate the

real concentration = RP x concentration of the standard

# Simple matching

- 
- **Example:** an unknown conc. Of Ach in which a volume of the test of 0.4ml produced an equal response of 0.2 ml of 60 μg/ml Ach solution.
- **RP**= 0.2/0.4 = 0.5
- Concentration of test solution = 0.5 x 60= 30 μg/ml

## Bracketting method

- the response of test compound is bracketting between two closely related responses of the standard reference

## When we use this method

1. the tissue should be very sensitive and produce very good discrimination response between doses

ex. in the bioassay of histamine is better to use guinea pig ileum than rat ileum

2. the quantity of the test compound is very small
3. the sensitivity of the tissue is not remained stable for long time

## Procedure

- we start with dose-response curve of standard reference

- then we do inject one or two doses of the test compound

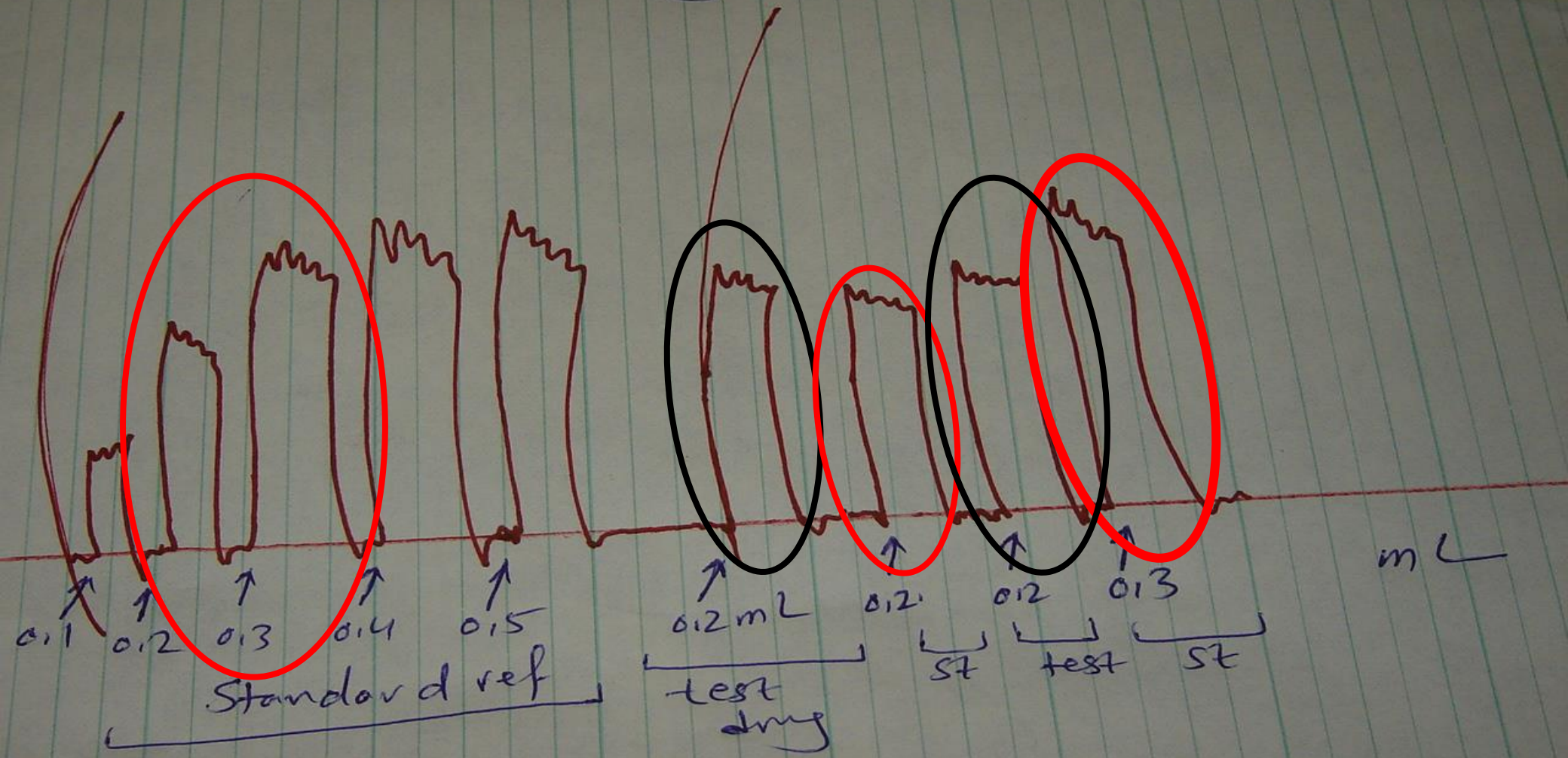
- we insert (bracket) the response between two closely related responses from the dose-response curve of standard reference

- $$RP = \frac{\frac{(\text{vol. } S1 + \text{vol. } S2)}{2}}{\text{vol. } T}$$

- concentration of test solution =

  RP x con. of standard drug

# Bracketting Method

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# If conc. of standard was 20 μg/ml

- RP = $\frac{\text{(vol. S1 + vol. S2)}}{2}$ / Vol. T

RP = $\frac{0.2 + 0.3/ 2}{0.2}$ = 0.25/ 0.2 = 1.25

- concentration of test solution = RP x con. of standard drug

- Conc. Test = 1.25 x 20 = 25 μg/ml.

# Multiple point assay

## Objective

To determine the relative potency of a test solution using the multiple point technique

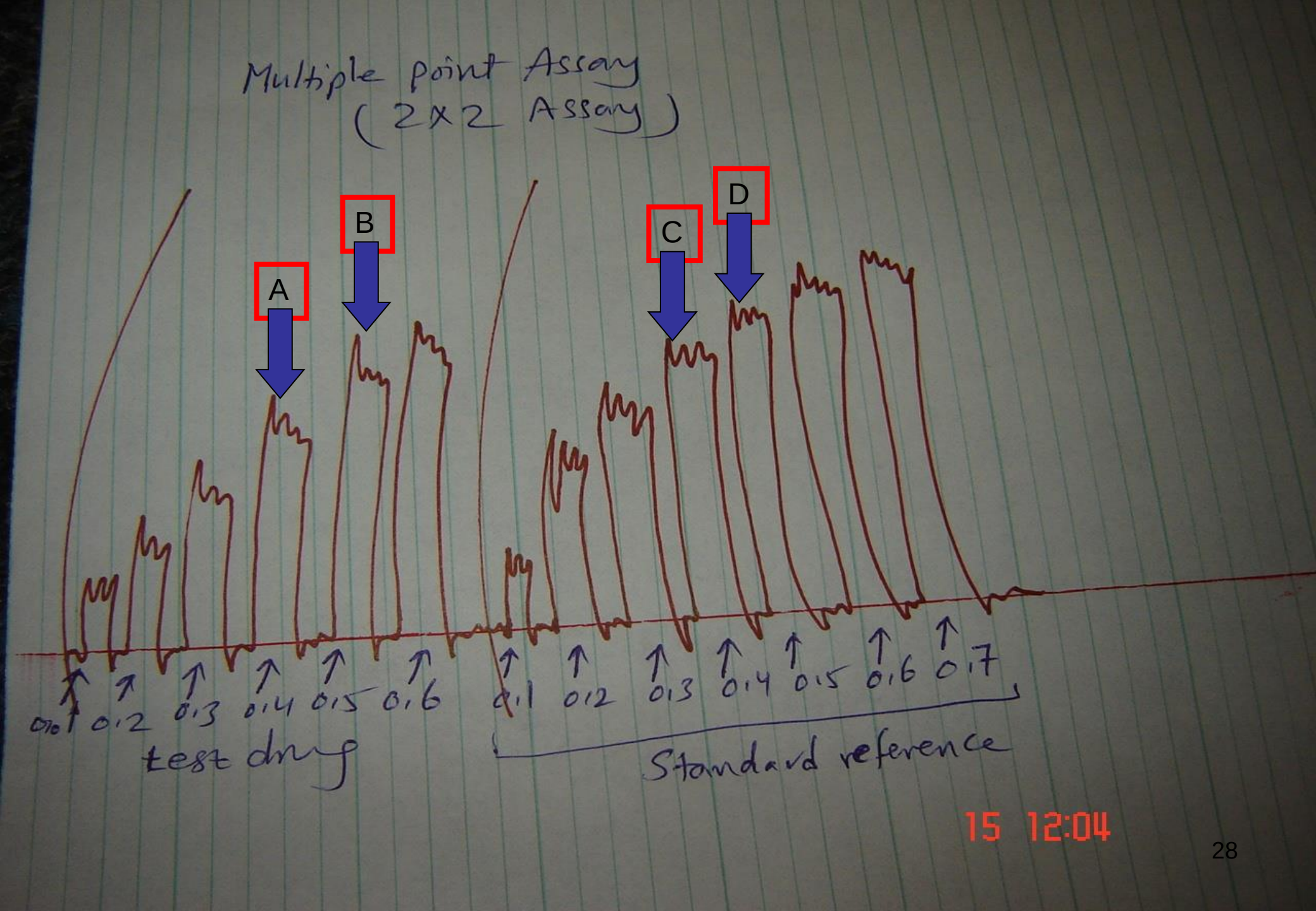
- 4 point assay or 2x2 assay (when we use two doses (referred as points) from standard reference and two doses from test drug).
- some times we use 3x3 assay (6 point assay)
- this method is the **most accurate indirect method** and is used when the drug is available in large quantity and the sensitivity is stable for a long period of time
- we do first dose-response curve with test drug.
- then we do a dose-response curve with standard reference.

- we select two different sub-maximal doses of test solution (one small and one large).

  ex. dose A ➔ small dose of test drug

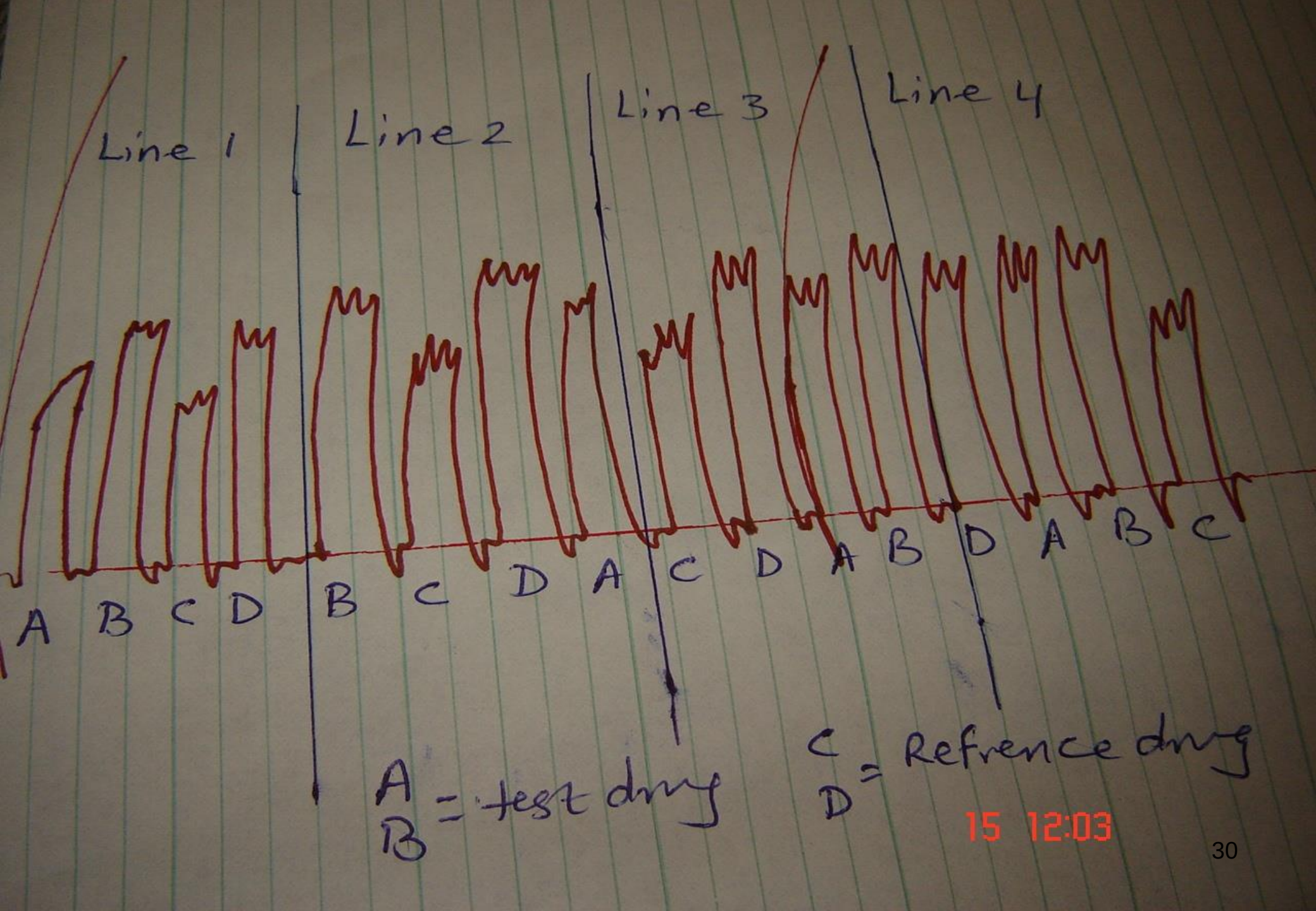
  dose B ➔ large dose of test drug

- Also, we do select two different sub-maximal doses fro the dose-response curve of standard solution (C and D)
- the ratio of volumes of A/B and C/D should be as close as possible.
- the responses to these 4 doses are obtained in a random fashion using a Latin square design


Multiple point Assay
(2×2 Assay)
A
B
C
D
0.1
0.2
0.3
0.4
0.5
0.6
0.1
0.2
0.3
0.4
0.5
0.6
0.7
test drug
Standard reference
15 12:04

# The Latin square design

| Line | | | | |
|---|---|---|---|---|
| 1 | A | B | C | D |
| 2 | B | C | D | A |
| 3 | C | D | A | B |
| 4 | D | A | B | C |


Line 1
Line 2
Line 3
Line 4
A B C D B C D A C D A B D A B C
A = test drug
B = test drug
C = Refrence drug
D = Refrence drug

- we calculate the RP by two methods either by graphic method or by algebraic method

- RP = anti-log (M)

  M is the difference between two lines

- real concentration of test solution =

  RP x con. of standard drug

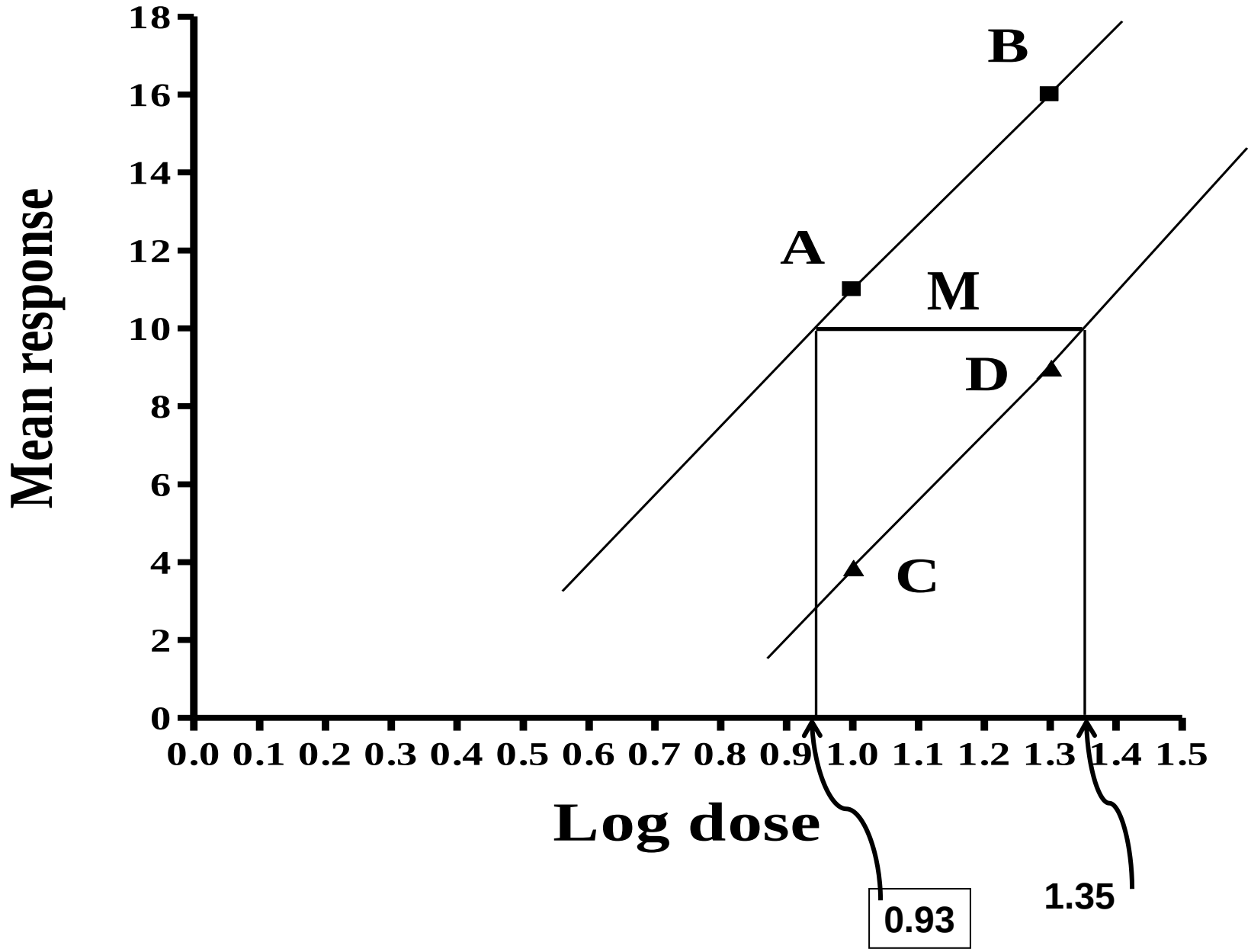
**Example**

- The following are responses obtained in 2x2 point assay of test compound solution using rat ileum.
- Concentration of the standard solution is 100 μg (C & D).
- Calculate the relative potency and the concentration of unknown solution (A & B)?

| Line | Point A (mm) | Point B (mm) | Point C (mm) | Point D (mm) |
|---|---|---|---|---|
| 1 | 10.5 | 15 | 4 | 9.5 |
| 2 | 9.5 | 17 | 3.5 | 8.5 |
| 3 | 12.5 | 18 | 5 | 9.5 |
| 4 | 11.5 | 14 | 3.5 | 8.5 |
| | | | | |
| Volumes (μl) | 10 | 20 | 10 | 20 |

Calculate the relative potency and the concentration of unknown solution ?

| point | dose | Log dose | Line 1 | Line 2 | Line 3 | Line 4 | Mean (mm) |
|---|---|---|---|---|---|---|---|
| A | 10 | 1 | 10.5 | 9.5 | 12.5 | 11.5 | 11 |
| B | 20 | 1.3 | 15 | 17 | 18 | 14 | 16 |
| C | 10 | 1 | 4 | 3.5 | 5 | 3.5 | 4 |
| D | 20 | 1.3 | 9.5 | 8.5 | 9.5 | 8.5 | 9 |


Mean response
18
16
14
12
10
8
6
4
2
0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5
Log dose
A
B
C
D
M
0.93
1.35

M = 1.35 – 0.93 = 0.42

RP = anti-log (M)

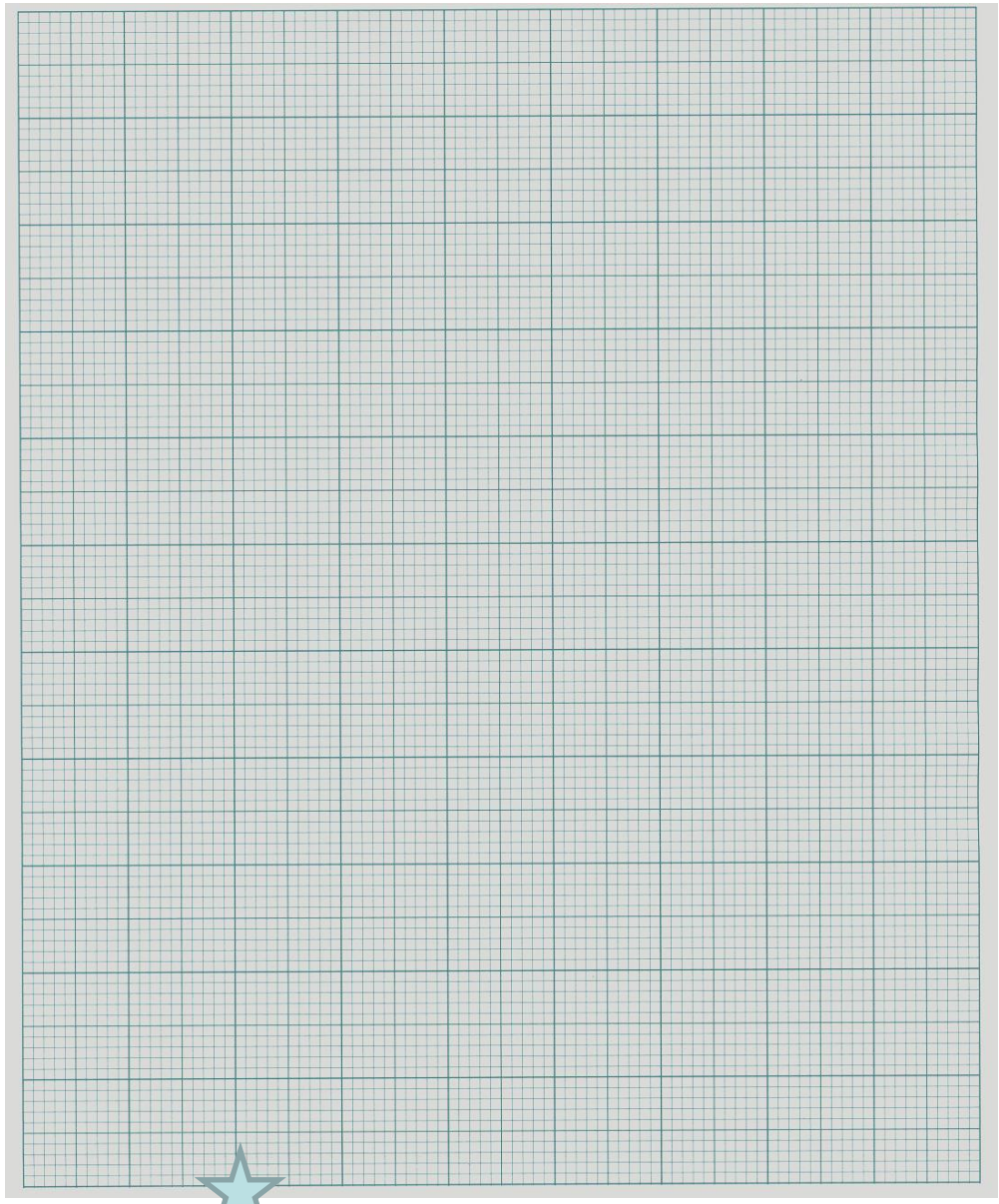
= 2.6

The test compound is more potent than the refrence stndard

Conc. of test compoud = RP x conc of stand

= 2.6 x 100 = 260 μg/ml

Concusion?



1.0 1.1 1.2 1.3