

# Bioassay of Insulin

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## BIOASSAY OF INSULIN



### INSULIN:

- ✓ It is a pancreatic hormone, which release from pancreatic beta-cell and helps to control the blood sugar level by converting glucose into glycogen.
- ✓ It was discovered in 1921, which helped millions of people suffering from type-I diabetes mellitus (Insulin-dependent Diabetes Malitus)
- ✓ In IDDM, capability of insulin production by pancreatic beta-cell is lost
- ✓ In manufacturing company, it is produced by bacteria in **Lilly** and by yeast in **Novo-Nordisk** by genetic engineering.



## BIOASSAY OF INSULIN

**Bioassay:** Biological assays or “bioassays” are a set of techniques for estimating the **Potency** or **Strength** of an “agent” or “stimulus” by utilizing the “response” or “effect” on biological system or experimental living subjects

**Principle of Bioassay:** The basic principle of bioassay is 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

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### Bioassay Experimental Models/Methods for Insulin

- ✓ Rabbit model- observation of hypoglycemia effects
- ✓ Albino Rats/Mice model- observation of hypoglycemia effects and hypoglycemic convulsion effects
- ✓ Isolated rat diaphragm model- observation of glycogen contents
- ✓ Rat's Epididymal Fat mode- observation of glucose metabolism

#### Standard Solution:

- ✓ Standard solution is prepared by using pure, dry and crystalline insulin and One unit contains 0.04082 mg or 40.82 ug. This unit is specified by Ministry of Health, Government of India and is equivalent to international unit.

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### Preparation of Standard Solution for Bioassay:

- ✓ 20 unit ( $40.82 \times 20 = 816.4$  ug) pure insulin is dissolved in small volume ( $< 1$  ml) normal saline and Acidify it with HCl to pH 2.5
- ✓ 0.5% phenol is added as preservative
- ✓ Add 1.4% to 1.8% glycerin.
- ✓ **Final volume should contain 20 units/ml.**
- ✓ Store the solution in a cool place and use it within six months.

### Preparation of Test Solution for Bioassay

- ✓ Same as standard solution

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### 1. Rabbit model:

#### Animal selection:

- ✓ Experimental Protocols should be approved prior to the Experiment by IAEC\* (Institution Animal Ethics committee)
- ✓ Thereafter, healthy rabbits (2-3 kg) are selected and habituated for the experiment in standard laboratory environment condition as per the norms of CPCSEA\* (Committee for the Purpose of Control and Supervision of Experiments on Animals), Govt. of India
- ✓ After that animal are kept in fasting for 18 h before performing the assay
- ✓ Water is withdrawn during the experiment

\*IAEC and CPCSEA are the committee constituted in India

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## 1. Rabbit model:

**Standard and Test sample dilution:** 1 U/ml and 2 U/ml solution is freshly prepared from stock solution (20 U/ml) of standard and test solutins

**Principle:** The potency of a test sample is estimated by comparing the hypoglycemic effect of the sample with that of the std. preparation of insulin.

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## 1. Rabbit model:

### Experimental Protocol/Procedure:

✓ Animals are divided into 4 groups and each containing 3-5 animals.

### 1. First Part of test:

#### A. "Initial Blood Sugar Level"

✓ A sample of blood is withdrawn from marginal ear vein of each rabbit and examine the reducing blood sugar level (...mg/dL) before insulin treatment.

Group No	Group Name	Treatment
I	Std-1	Std dilution-1, s.c.
II	Std-2	Std dilution-2, s.c
III	Test-1	Test dilution-1, s.c
IV	Test-2	Test dilution-2, s.c

## 1. Rabbit model:

### First Part of test:

#### B. "Final Blood Sugar Level"

- ✓ After standard and test insulin injection from each rabbit, a sample of blood is withdrawn up to 5 hrs. at the interval of 1 hr. each. Blood sugar is determined again. This is known as 'Final Blood Sugar Level'.

### 2. Second Part of Test: Cross Over Test

- ✓ The same animals are used for the second part after one week.
- ✓ Again Animals are fasted and initial blood sugar is determined.
- ✓ The grouping is reversed, means, those animals which received the standard are given the test and those which received the test are now given the standard.
- ✓ Those animals which received the less dose of the standard are given the higher dose of the test sample and vice-versa.
- ✓ This test is known as 'Twin Cross Over Test'

## 1. Rabbit model:

### 2. Second Part of Test: Cross Over Test

Group No	Group (First)	Group (Second)
I	Std-1	Test-2
II	Std-2	Test-1
III	Test-1	Std-2
IV	Test-2	Std-1

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## 2. Rats/Mice model:

**Principle:** Mice/Rats show characteristic convulsions after s.c. inj. of insulin at elevated temperatures. The percentage convulsions produced by the test and standard preparations are compared.

**Standard and Test solution:** stock (20 U/ml) solution are prepared similar as discussed previously. For this test, 0.064 U/ml (std dilution-I) and 0.096 U/ml (std dilution-II) are prepared.

### Animals:

- ✓ Albino mice (25-30 g) are selected and habituated under standard environment.
- ✓ Experimental procedures should be approved by IAEC prior to the experiment
- ✓ Animals should be fasted 18 hrs. prior to the experiment.

## 2. Rats/Mice model:

**Experimental procedure and Group:** Animals are divided into 4 groups each contains 20-25 animals.

Group No	Group Name	Treatment
I	Std-1	Std dilution-1, s.c.
II	Std-2	Std dilution-2, s.c
III	Test-1	Test dilution-1, s.c
IV	Test-2	Test dilution-2, s.c



## 2. Rats/Mice model:

### Observation: “hypoglycemic convulsion”

- ✓ Mice are put in an air incubator at 33°C and observed for one and a half hr.
- ✓ The mice which convulse or die are taken out of the incubator and observed.
- ✓ **% Convulsion of test is compared with the standard**

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