BIOLOGICAL ASSAY OF DIPHTHERIA ANTITOXIN

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- **Diphtheria antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralizing the toxin formed by *Corynebacterium diphtheria.***
- **It is obtained from the serum of horses or other mammals, that have been immunized against diphtheria toxin.**

Not less than 1000 IU/ml serum (obtained from horse) and not less than 500 IU/ml serum (obtained from other mammals)

Principle:

The potency of diphtheria antitoxin is determined by comparing the dose necessary to protect guinea pigs or rabbits against the erythrogenic effects of a fixed dose of diphtheria toxin with the quantity of the standard preparation of diphtheria antitoxin necessary to give the same protection.

Standard Preparation:

The standard preparation is the 1st international standard for diphtheria antitoxin, equine ,established in 1934,consisting of the dried hyperimmune horse serum and glycerin, or another suitable preparation the potency of which has been determined in relation to the international standard.

Suggested Method:

- Test Toxin.
- Selection of Test Toxin.
- Determination of test dose of toxin.
- Determination of potency of the antitoxin.

Test Toxin:

- Prepare diphtheria toxin by filtering through bacteria proof filter the medium in which a toxigeneic strain of **Corynebacterium diphtheriae** has grown.
- Store at a temperature of 2^0 to 8^0

Selection of Test Toxin:

In selecting a toxin for use as the test toxin determine the following.

1. Lr/100 dose:

This is the smallest quantity of toxin which when mixed with 0.01 unit of antitoxin and injected intracutaneously into guinea pigs or rabbits causes characteristic reaction at the site of the injection within 48 hours.

2. Minimal reacting dose:

This is the smallest quantity of toxin injected intracutaneously into guinea pigs or rabbits causes characteristic reaction at the site of injection within 48 hours.

A suitable toxin is one which contains at least 200 minimal reacting doses in the Lr/100 dose.

Determination of test dose of toxin(Lr/100 dose):

Standard solution

(0.1 Unit/ 1.0 ml saline)

Test toxin solution

Prepare mixture (1 ml standard solution + one of series of graded volume of test toxin). Finally make up to 2 ml with saline.

Allow the mixture to stand at room temperature, protected from light, for 15 to 60 minutes.

Inject each mixture (0.2 ml, sc) at shaven or depilated flanks of two animals. Observe animal for 48 hours.

- **The test dose (Lr/100) of the toxin is the amount present in 0.2 ml of that mixture which causes at the site of injection a small, characteristic reaction in the skin of the guinea pig or rabbit.**

- **Larger amount of toxin cause larger reaction and necrosis.**

- **Smaller amount of toxin cause no reaction.**

Determination of potency of the antitoxin:

Dilute the test toxin with saline solution so that 1.0 ml contains 10 times the test dose

Test Solution mixture

(1ml of test toxin + series of graded volumes of the preparation under examination – make up to 2 ml saline.

Standard Solution mixture

(1ml of test toxin + 0.1 Unit of anti-toxin) - make up to 2 ml with saline.

Allow the mixture to stand at room temperature, protected from light, for 15 to 60 minutes.

Inject each mixture (0.2 ml, sc) at shaven or depilated flanks of four animals. Observe animal for 48 hours.

- **The mixture of the preparation under examination that contains**

0.01 unit of antitoxin in 0.2 ml is the mixture that produces the same degree of local reaction as that produced by the injection into the same animals of the mixture of the standard preparation that contains 0.2 ml of the test dose (Lr/100) of the toxin and 0.01 unit of anti-toxin.

The limits of error have been estimated to be between 90 – 111 percent

THANK YOU