

## **PRODUCTION AND STANDARDIZATION OF CLOSTRIDIUM OEDEMATIENS VACCINE IN IRAN (\* \*)**

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Black disease is one of the most important diseases of sheep in Iran. Sporadic outbreaks of black disease have been observed in different parts of the country which cause economical losses in sheep flocks. Many strains of *Clostridium oedematiens* type B have been isolated from liver lesions of affected animals, studied by Ardehali and Darakhshan, 1979 (1). The demand for the vaccine is increasing annually. In 1981, Razi Institute delivered three million doses of black disease vaccine and in 1982 the demand for the vaccine has been increased to 12 million doses.

This paper deals with the preparation and standardization of *Cl. oedematiens* vaccine in Razi Institute.

### **I - PREPARATION OF CLOSTRIDIUM OEDEMATIENS VACCINE**

#### **1) MEDIUM**

The following medium is used for preparation of vaccine.

1. Peptone (Oxoid L52).....	4%
2. Na <sub>2</sub> HPO <sub>4</sub> .....	0.5%
3. Maltose .....	1%
4. L. Cysteine .....	0.05%

PH - 8

The ingredients are dissolved in distilled water and distributed into 20 litre pyrex bottles (16 litres / bottle). The bottles are autoclaved at 115° C for 40 min.

#### **2) PREPARATION OF VACCINE**

For preparation of one batch of vaccine (3000,000 doses), 340 twenty litre bottles of media are used, each one with 16 litres of medium.

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*Clostridium oedematiens* strain is maintained in freeze dried state and the organism is inoculated in a tube of fresh liver medium as seed culture. Then after an active growth, it is subcultured in 500 ml of liver medium. The bottles containing 16 litres of the medium are inoculated with the 0.5–1% (approximately) of the starter. The required time for the growth of the culture is 60-70 hours. The samples are removed from some bottles for determination of minimum lethal dose of toxin in mice. The cultures are checked for sterility by subculturing in broth and also agar media. Those proved to be contaminated are discarded. The volume of 96 ml of 40% of commercial formaldehyde solution is added to each bottle. The formalised cultures are left for 7-10 days more in the incubator to be detoxified and transformed to anaculture-toxoid.

The anacultures after being removed from incubator, samples are taken from each container and mixed together for safety and potency tests of vaccine. Finally the anacultures after being kept for about two months in the cold room and assured the complete detoxification are delivered. The final product consists of 60% of *Cl.oedematiens* anacultures precipitated with 20% of 2% of sterile potash alum solution and with 20% of sterile distilled water.

For preparation of alum precipitated *Cl.oedematiens* vaccine anaculture is treated and mixed well with 2% solution of sterile potash alum as adjuvant and mixed with distilled water to give 0.4% final concentration of alum at pH 6.5–7. Alum and NaOH are added at the same time and mixed well together.

## **II – QUALITY CONTROL OF VACCINE**

The safety and potency of the prepared vaccine is determined according to the British Pharmacopoeia (Veterinary) 1977 (2).

### **1) SAFETY TEST**

Six susceptible healthy sheep are selected for safety test of vaccine. Each group of three sheep are injected subcutaneously with 5 and 10 ml of *Cl. oedematiens* alum precipitated vaccine. All vaccinated animals are observed for two weeks. The injected animals show no severe general reaction, but a local reaction appears at the site of injection which disappears after 3-4 weeks.

### **2) POTENCY TEST**

The following materials are prepared for potency test of *Cl. oedematiens* vaccine.

#### **a / Preparation of *Cl. oedematiens* concentrated toxin**

The highly toxigenic strain of *Cl. oedematiens* type B is grown in a medium consisting of 3% peptone (Oxoid L52), 0.5% Na<sub>2</sub>HPO<sub>4</sub>, 12H<sub>2</sub>O, 1% maltose and 0.5% chopped meat at pH-8 described by Nishida and Nakagawara (1964) (3). After three days incubation, the culture is centrifuged and supernatants transferred in a celophan sac are concentrated by polyethylene glycol compound 20-M (Carbowax). The titre of concentrated toxin obtained is 20,000 minimum lethal dose per ml for 18-20 gram mice. The concentrated toxin is calliberated against *Clostridium oedematiens* antitoxin supplied by Statens Seruminstitut\*.

#### **b/ Preparation of standard antitoxin in sheep**

Two sheep are used for the preparation of standard antitoxin against *Cl. oedematiens*. The animals are hyperimmunized according to the modified method described by Ardehali and Dowran (1973) (4). The prepared antitoxin is calliberated against the International standard antitoxin obtained from Statens Seruminstitut.

Potency test of each batch of *Cl. oedematiens* monovalent vaccine and also combined with other clostridial vaccines is determined according to the British Pharmacopoeia (Veterinaria). A group of twelve rabbits, three to six months old, are injected subcutaneously with 3 ml of vaccine. Four weeks later, a second injection of the same quantity is given. Fourteen days after the second injection, the rabbits are bled, sera are separated from the collected blood and one ml of each serum is taken and pooled. The units of antitoxic value of the pooled sera is determined by standard method for titration of *Cl. oedematiens* alpha antitoxin.

The results of potency tests routinely obtained from different batches of black disease vaccine show that sera contain an average of 15 international units of *Cl. oedematiens* alpha antitoxin.

The reports obtained from the field indicated that the black disease could be effectively controlled by this vaccine in affected area.

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## SUMMARY

### **PRODUCTION AND STANDARDIZATION OF CLOSTRIDIUM OEDEMATIENS VACCINE IN IRAN**

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*In this paper an account of large-scale production of Clostridium oedematiens vaccine in Iran is described. The method of preparation of concentrated toxin, standard antitoxin and quality control of the vaccine is mentioned. The prepared vaccine is highly immunogenic as determined by the laboratory tests according to the British Veterinary Pharmacopoeia and field reports.*

## RESUME

### **PRODUCTION ET STANDARDISATION DU VACCIN CONTRE CLOSTRIDIUM OEDEMATIENS EN IRAN**

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*Les auteurs décrivent les méthodes de production industrielle du vaccin contre Clostridium oedematiens utilisées en Iran. Ils mentionnent les procédés d'obtention de la toxine concentrée, de l'antitoxine standard, ainsi que les contrôles de qualité et d'efficacité du vaccin. Le vaccin produit est hautement immunogène comme l'ont montré les essais réalisés en laboratoire selon la Pharmacopée vétérinaire britannique et les observations faites sur le terrain.*

## REFERENCES

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