

## **USING OF IFAT (IMUNOFLUORESCENCE ANTIGEN TEST) FOR VALUATION OF ANTICORPS LEVEL AGAINST BACILLUS ANTRAXIS IN VACCINE ANIMALS DURING (1994-1995)**

As/ Prof. Dr. Pellumb Muhedini , Kapllan Sulaj

Institute of Veterinary research, Tirana 10, Albania

### **INTRODUCTION**

Studies for application of Immunofluorescence Antigen Test (IFAT) to detect level of anticorps against *Bacillus Antraxis* relise with direct method. In some studies like as (Fetenau.A) showed that typospecific serum expouse a clear flouresence wich is valueted with two cross in titre 1/128. Referncing ( Ivins B.E ), (Klein F. , Linclon R.F) exist the possibility of application the indirect methods (IFAT), that is our subjects in this study.

### **MATERIALS AND METHOD**

Place: Laboratory of Bacterial Vaccine Production in Istitute of Veterinary Research Tirane, Vaqarr , Tapize.

Samples: Are colected taking their blood as a samples.by goats and sheeps (> 1 year) that was inoculed before acapsular vaccine and capsular vaccine of *Bacillus Antraxis* in diffrent time .

Antigens: To realise this study used two type of antigens , acapsular antigen and capsular antigen with mezogene virulence. Procedure for preparing was consisted in the first soughting after that centrifugation and rinsing with PBS.

Serological diagnose: Is realised with Indirect Immunofluorescence Antigen Test (IFAT) that is now rutine test for fixation of antigen. In our work for fixation procedure have been used acetone and incubation is done in cameras with humidity.

Using Fluorescence Microscope with amplification 1000 time is readed the reaction.

### **RESULTS**

To realise this test as samples analised serums of goats (> 1 year) and sheeps (> year) that have resisted the infection 100% with 10 m.l.d of virulent strain ( *B. Antraxis*) after 21 days and 12 month of vaccination with acapsular vaccine, type 'Stern'.

*1.Determination of anticorps level with IFAT in vaccinated goats (> 1year) with acapsular and capsular vaccine after 21 days of vaccination.*

Type of serums	Antigens	DILUTIONS OF SERA						
		(1:2)	(1:4)	(1:8)	(1:16)	(1:32)	(1:64)	(1:128)
acapsular	acapsular	+++	+++	+++	+++	+++	++	++
capsular	acapsular	+++	+++	+++	+++	+++	++	++
control	acapsular	-	-	-	-	-	-	-
acapsular	capsular	+++	+++	+++	+++	+++	++	++
capsular	capsular	+++	+++	+++	+++	+++	++	++
control	capsular	-	-	-	-	-	-	-

*2.Determination of anticorps level with IFAT in vaccinated goats (> 1year) with acapsular and capsular vaccine after 12 months of vaccination.*

Type of serums	Antigens	DILUTIONS OF SERUMS						
		(1:2)	(1:4)	(1:8)	(1:16)	(1:32)	(1:64)	(1:128)
acapsular	acapsular	+++	+++	+++	+++	++	+	-
capsular	acapsular	+++	+++	+++	+++	++	+	-
control	acapsular	-	-	-	-	-	-	-
acapsular	capsular	+++	+++	+++	+++	++	+	-
capsular	capsular	+++	+++	+++	+++	++	+	-
control	capsular	-	-	-	-	-	-	-

Positive titre considered up to ++.

Results of table showed that when used tow antigens (acapsular , capsular) the frecueny of fluorensence for dilution (1:128) , (1:32) in vaccinated goats (>1year) after 21 days and 12 months valueted with ++.

*3.Determination of anticorps level with IFAT in vaccinated sheeps (> 1year) with acapsular and capsular vaccine after 21 days of vaccination.*

Type of serums	Antigens	DILUTIONS OF SERA						
		(1:2)	(1:4)	(1:8)	(1:16)	(1:32)	(1:64)	(1:128)
acapsular	acapsular	++++	++++	++++	++++	+++	+++	++
capsular	acapsular	++++	++++	++++	++++	+++	+++	++
control	acapsular	-	-	-	-	-	-	-
acapsular	capsular	++++	++++	++++	++++	+++	+++	++
capsular	capsular	++++	++++	++++	++++	+++	+++	++
control	capsular	-	-	-	-	-	-	-

*4.Determination of anticorps level with IFAT in vaccinated sheeps (> 1year) with acapsular and capsular vaccine after 12 months of vaccination.*

Type of serums	Antigens	DILUTIONS OF SERA						
		(1:2)	(1:4)	(1:8)	(1:16)	(1:32)	(1:64)	(1:128)
acapsular	acapsular	++++	++++	++++	+++	++	+	-
capsular	acapsular	++++	++++	++++	+++	++	+	-
control	acapsular	-	-	-	-	-	-	-
acapsular	capsular	++++	++++	++++	+++	++	+	-
capsular	capsular	++++	++++	++++	+++	++	+	-
control	capsular	-	-	-	-	-	-	-

Results of table showed that when used tow antigens (acapsular , capsular) the frequency of fluorensce for dilution (1:128) , (1:32) in vaccinated sheeps (>1year) valueted with (++) , (+++) after 21 days and (-) , (++) after 12 months .

Serums of control group showed negative results.

## DICUSION

Results of this experiment telling that IFAT is specific to ditect level of anticorps in vaccineted animals against B.antracis and sheeps(> 1year) are the more sensible as goats(> year), it was showed with hight frequency of fluorensce.

IFAT is a best method as a seroneutralisation method beceause we are not be able to valuet the imunity power of organism this for reasen the ability of seroneutralisation serum after detected time will go to zero level.Goats (> 1year) resist in 100% the m.l.d (maximal lethal dose) of vaccination with virulent strain of B.antracis. We think that IFAT is a good test to determine imunity power of vaccine and duringtime of it.

## SUMMARY

In this study using IFAT (Indirect Imunofluoresece Antigen Test) determine imunity power of apcapsular vaccine to B.antacis in diffrent interval of the time after vaccination. Telling results are in corelation with imunity power of vaccine.

## REFERENCES

- 1.Feteanu A. L'identification et la differencation de B. antracis dans les cultures et les tissus des autres germes du germe bacillus a l'aide des anticorpes fluorensce. Arkiva Vetrinari 1967.
- 2.Ivins B E.Immunistion studies with attenuated strain of B.antracis. inf. Immunity.1986
- 3.Klein F.,Linclon R.F. Immunologic studies of anthrax.J.Immunol.



## **УПОТРЕБАТА НА ИФАТ (Имунофлуоресцентен Антиген Тест) ЗА МЕРЕЊЕ НА НОВОТО НА АНТИТЕЛАТА ПРОТИВ Б. АНТРАКС КАЈ ВАКЦИНИРАНИТЕ ЖИВОТНИ, ВО ПЕРИОДОТ 1994-1995**

Асс. Проф. Д-р Пелумб Махедини Бактериолог Каплан Сулај

ИНСТИТУТ ЗА ВЕТЕРИНАРНИ ИСТРАЖУВАЊА, ТИРАНА 10, АЛБАНИЈА

При ова истражување се користеше ИФАТИ индиректен имунофлуоресцентен антиген тест) за да се одреди имунолошката моќ на акапсуларната вакцина во однос на Б. антракс во различни интервали по вакцинацијата. Добиените резултати се во сооднос со имунолошката моќ на вакцината.