

## MEASUREMENT OF PROTECTIVE LEVEL OF CLOSTRIDIAL VACCINES BY INDIRECT HAEMAGGLUTINATION TEST

ROUKAY A OSSMAN, DOREYA SHARAF AND A.Z. HUSSEIN

Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre,  
Giza, Egypt.

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### Abstract

The level of antitoxin has been estimated in sera from lambs vaccinated with bivalent lamb dysentery and pulpy kidney vaccine, in vivo by mouse test and in vitro by indirect haemagglutination test. The results showed a good correlation between the toxin neutralization and indirect haemagglutination tests. Studying the immune response of blackleg vaccine in calves and guinea-pigs both by challenge and indirect haemagglutination test revealed that the test may be useful as an aid for the evaluation of the protective potency of blackleg vaccine.

### INTRODUCTION

The British Pharmacopoeia (1965) prescribes toxin neutralization assays in mice for *Cl.perfringens* beta and epsilon antitoxin titration, while, the challenge test in guinea pig is recommended for *Cl.chauvoei*. The replacement of laboratory animals by indirect haemagglutination test (I.H.T.) as an indicator for the response of antibodies in vaccinated animals would allow substantial reduction in the number of laboratory animals required to evaluate the potency of veterinary clostridial vaccines, as well as, saving time.

Dholokia *et al.* (1980), Dholokia and Saxena (1982) used passive haemagglutination test for titration of antitoxin in serum samples of sheep immunized with *Cl.perfringens* type D vaccine and recorded that it gave good results.

Tamura *et al.* (1985 and 1986) found that the results of I.H.T. and the protection test of mice against *Cl.chauvoei* vaccine were equally reliable.

This paper describes studies carried out in an attempt to use indirect haemagglutination test for measuring the antibody response in animals against clostridial vaccines and to evaluate its usefulness as animal protection test.

## MATERIALS AND METHODS

### I. Vaccines

Two types of vaccines prepared in Veterinary Serum and Vaccine Research Institute, Viz. Blackleg vaccine and bivalent vaccine against lamb dysentery and pulpy kidney were used.

### II. Experimental animals

#### A- Cattle

Forty calves were immunized with 2 doses of blackleg vaccine at 4 weeks interval; blood samples were collected 10 days after the second injection. Sera were assayed for I.H.T. and challenge test in mice according to Sinha and Prasad (1973).

#### B- Guinea - pigs

The potency of blackleg vaccine was also assayed in 40 guineapigs. They were given 2 subcutaneous injections 4 weeks apart. Ten days after the second injection, 20 guinea-pigs were challenged with 10 M.L.D. of *Cl.chauvoei* spore suspension in Ca Cl<sub>2</sub>. The other 20 guinea-pigs were slaughtered, and their sera were collected and pooled into 4 groups (5 animals in each), and assayed for I.H.T.

#### C-Sheep

Sixty unvaccinated lambs 10 months old were inoculated with 2 doses of bivalent lamb-dysentery and pulpy kidney vaccines; blood was collected from each animal before injection, and 2 assayed by neutralization test in mice and I.H.T.

#### Titration of lambs sera for Beta and Epsilon antitoxin

Immunized lambs sera were tested by mice neutralization test according to Gadalla *et al.* (1971), and I.H.T. according to Dholokia and Saxena (1982).

After the results were calculated, the serum samples were pooled in 6 groups, each of which contained the same antibody titre. Each sera group samples were then tested by I.H.T. and compared with neutralization test together with positive and negative sera.

### Indirect haemagglutination test

Prior to the test, the same samples were inactivated at 56°C for 30 minutes.

The test was conducted according to Dholokia and Saxena (1982).

### Titration of calves sera

0.5 ml of each serum sample was inoculated in each mouse in a group consisting of four mice, and were left for 48 hours, then, were challenged with 10 M.L.D. of *Cl.chauvoei* spore suspension in 5% Ca Cl<sub>2</sub> solution.

In parallel, each sample was tested by I.H.T., negative serum from non-vaccinated animals and positive *Cl.chauvoei* antisera were used as controls in both tests.

## RESULTS

### Antitoxin titration by indirect haemagglutination and mouse neutralization tests

Table 1 demonstrates the results of toxin antitoxin antitoxin neutralization test in mice and I.H.T. in lambs sera. Results indicated that, serum samples showing indirect haemagglutination titre of high levels (32-64) possessed good levels of antitoxin units (22-28 I.U.). The same pattern was observed with lower titre.

Table 1. The titre of beta and epsilon antitoxin of *Cl.perfringens* by mice neutralization and I.H.T.

Group	No. of sample	Beta		Epsilon	
		titre by NT	titre by IHT	titre by NT	titre by IHT
1	8	25 U/ml	32-64	28 U/ml	16-32
2	16	26 U/ml	32-64	30 U/ml	32-64
3	04	02 U/ml	02-04	04 U/ml	04-08
4	10	28 U/ml	16-32	35 U/ml	32-06
5	10	22 U/ml	16-32	25 U/ml	08-32
6	12	17 U/ml	08-16	12 U/ml	08-16
Positive control	2	50 U/ml	256	40	256
Negative control	8	0.1 U/ml	2	0.2	2

NT = Mice neutralization test.

IHT = Indirect haemagglutination test.

### Results of immune response to black quarter vaccine

#### a. In calves sera

Results illustrated in Table 2, show that, 8 serum samples possessed a high protective ability (100% protection), and indirect haeagglutination antibody producing ability (64-128). A low level of indirect haenagglutinins (IH) was detected in 7 samples and gave 25% protection after challenge exposure.

About 25 samples which induced survival rate of 50-75% had indirect haem-agglutination titre 8-32.

Table 2. The titre of I.H.T. and protection rate in mice for blackleg vaccine.

No. of sample	I.H. titre	No. of injected animals	Challenge test		% of protection
			dead	survive	
7	2-4	28	21	7	25
8	64-128	32	-	32	100
12	8-16	48	24	24	50
13	16-32	52	13	39	75
Positive control	256	5	0	5	100
Negative control	0	5	5	0	0

#### In Guinea-pigs

The group of guinea-pigs (20 animals) immunized with blackleg vaccine showed complete resistance to challenge test. Pooled sera for 20 guinea-pigs, each from a group of 5, when tested with I.H.T., the titres varied as shown in Table 3. Two pooled sera of slaughtered animals gave an I.H. titre of 128, while, the other two gave a titre of 64.

### DISCUSSION

The findings ensued from this investigation demonstrate that, indirect haeagglutination test for estimation of the level of the antitoxin of *Cl.perfringens* types B

and D could be satisfactory. Both in vitro and in vivo tests were in good correlation. This has been proved by Cook (1965), Dholakia *et al.* (1980), Biktimirov (1979), Dholakia and Saxena (1982). Beh and Buttery (1978) stated that, in the haemagglutination test, the serum antibodies react with toxin either by active or as toxoid, while, in toxin neutralization test in mice, antitoxin reacts with active toxin only. Indirect haemagglutination test, being rapid and economic, can be used for measuring the antibody response in a population. However, for quantitative determination, toxin neutralization test in mice would be preferable. The indirect haemagglutination titre of sera against sonic extract of *Cl. chauvoei* paralleled the protection of guinea-pigs immunized with blackleg vaccine. The titres obtained by calves sera correlated with sufficient protection of mice to the challenge exposure.

The conclusion can be made that, indirect haemagglutination test may be useful for evaluation of blackleg vaccine, as an additional aid to the challenged test.

Table 3. Results of I.H.T. in guinea-pigs.

No. of samples	I.H. titre
5 @	128
5	128
5	64
5	64

@ Pooled sera examined from the five guinea-pigs.



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## قياس القوة المناعية للقاحات الكلوستريديا بواسطة اختبار التلازن الدموي الغير مباشر

رقية محمد عثمان، درية شرف ، عبد السلام زكى حسين

معهد بحوث الأمصال واللقاحات البيطرية - مركز البحوث الزراعية - الجيزة - مصر.

تم قياس مستوى الأجسام المناعية (مضادات السموم) لأمصال الحملان المحصنة بلقاح ثنائى دوسنتاريا الحملان والكلوة الرخوة وذلك باستخدام اختبار حقن الفئران واختبار التلازن الدموي الغير مباشر.

أثبتت النتائج أن هناك توافق شديد بين نتائج اختبار التعادل فى الفئران واختبار التلازن الدموي الغير مباشر.

عند دراسة القوة المناعية للقاح التفحم العضلى فى العجول والأرانب الهندية بطريقتى التحدى والتلازن الدموي الغير مباشر وجد أن الطريقة الأخيرة مفيدة جدا لتقييم القوة المناعية فى الحيوانات المحصنة بلقاح التفحم العضلى حيث أعطت نتائج طيبة بديلا عن اختبار التحدى المكلف.