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COMPARISON OF IN VIVO AND IN VITRO TOXIGENICITY TESTS FOR CORYNEBACTERIUM DIPHTHERIAE

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ABSTRACT

This study was undertaken to compare an in vitro and an in vivo toxigenicity tests of Corynebacterium diphtheriae. 17 out of 18 strains were mitis type (94.7%) and one strain was intermedius type (5.3%). All gave positive results to the in vitro and in vivo toxigenicity tests. Therefore, there was a complete correlation between the two methods.

It was suggested that serum substitute should be used in the in vitro toxigenicity test because of the simplicity and economics when compared with pooled rabbit serum.

INTRODUCTION

Toxigenicity test for C. diphtheriae can be performed in 2 methods :

(1) In vivo toxigenicity test. Two technics are commonly used :

(a) Intradermal test. Rabbit or guinea pig can be used by injecting suspension of diphtheria culture intradermally, after 5 hours, diphtheria antitoxin is injected. Another dose of bacterial suspension is injected intradermally in the other site as a control.

(b) Test of single subcutaneously. This technic will be used when the first technic give doubtful reaction. A normal guinea pig is injected subcutaneously with large does of bacterial culture. Another guinea pig is used as a control by injecting first with diphtheria antitoxin, and then with the same bacterial culture. The test animal will develop intoxication, paralysis and death, but the control animal will be normal.

(2) In vitro toxigenicity test. This test was developed by Elek in 1943 (1). The principle of this test is the develop-

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ment of lines of flocculation which are caused by the reaction between diphtharia toxin and its homologous antitoxin in the agar medium. Most of these agar media contain whole rabbit, human or house sera. A serum substitute which compose of commercial glycine, tween 80 and casamino acid can replaced the whole serum (5).

Our study is trying to compare the serum substitute with the whole rabbit serum for in vitro toxigenicity test. The comparison of in vitro and in vivo toxigenicity tests are also studied.

MATERIALS AND METHODS

(1) **Isolation and identification of C. diphtheriae.** Throat swabs are streaked onto cystine tellurite blood agar (CTB). Colonial morphology is recorded and transferred the culture to Loeffler slant media, stained, and examined with microscope.

Culture on Loeffler slant media are used for carbohydrate fermentation including glucose, sucrose, snd starch. Hemolysin production and pellicle formation are also tested.

(2) **In vitro toxigenicity test.** In vitro toxigenicity test of C. diphtheriae are tested according to the methods of Frobisher (6), King (2) and Parsons (4). The toxigenicity test agar composed of two parts:

1) Basal medium (6)

Proteose peptone (Difco)	2 gms
Granular agar	1.75 gms
Sodium Chloride, C.P.	0.25 gms
Distilled water	100.00 ml.

2) Serum substitute (5)

Casamino acids (Difco)	1 gm
Tween 80	1 ml.
Glycerol, C.P.	1 ml.
Distilled water	100 ml.

Three per cent of sterile rabbit serum or serum substitute is added into the basal media before used. The diphtheria antitoxin (Swiss Serum and Vaccine Institute Berne) is 500 units/ml in the paper strip in the toxigenicity test agar. Results of the toxigenicity tests are examined after 24, 48 and 72 hours. The positive test gives precipitin line between the culture lines and the antitoxin soated filter paper.

(3) **Rapid in vitro toxigenicity test.** Artificial throat swabs containing diphtheria bacilli were used in this study. The swabs were streaked onto the antitoxin media as described above. After 48-72 hours, precipitin lines occurred if they were positive.

(4) **In vivo toxigenicity test.** The intradermal test method is used in this experiment. Normal albino rabbits are chosen because of the large number of cultures. The back of rabbit is shaved and injected intradermally with bacterial cultures (turbidity = McFarland tube No. 3). After 5 hours, 500 units/ml of diphtheria antitoxin is injected intravenously into the marginal ear vein, then bacterial suspension is

injected intradermally into another position as a control.

After 24, 48 and 72 hours, a necrotic lesion can be seen at the first injection and no necrotic lesion at the second point of the injection when it is a toxigenic strain. If no reaction in both sites of injections, it is a nontoxigenic strains.

RESULTS

A total of 18 strains of C. diphtheriae were isolated, 17 strains were mitis type, one strain was intermedius type. One strain of C. xerosis and one strain of C. pseudodiphtheriticum were also isolated.

In the hemolysin production test, 13 strains (76.5%) of mitis type are positive, 4 strains (24.5%) are negative.

For the in vitro toxigenicity test, all 17 strains of mitis type gave positive for both serum and serum substitute agar in 48 hours. The intermedius type gave positive for both media after 72 hours. C. xerosis and C. pseudophtheriticum showed negative result in these media (see Table 1).

All 17 strains of mitis type and one strain of intermedius type gave precipitin line after 48 hours and better results after 72 hours. When the normal throat swabs, mixed with diphtheria bacilli, are streaked on CTB agars. The diphtheria bacilli could be isolated in all specimens.

The intradermal toxigenicity test is positive for all 17 strains of mitis type with the diameter of necrotic lesion 5-8 mm. One strain of intermedius type also gave positive result with a diameter of necrosis 4.5 mm. However, C. xerosis and C. pseudodiphtheriticum are negative for this test (see Table 2).

DISCUSSION AND CONCLUSION

Test for hemolysin production using human red blood cells revealed that 13 strains (76.5%) of mitis type were positive and 4 strains (24.5%) were negative. Therefore, hemolysin production was not the characteristic property of mitis type, it can be found in the other types (7).

The isolated diphtheria bacilli from infected patients in Nakorn Chiang Mai Hospital from 1968 - 1969 were mostly mitis type (94.4%) and only 5.6% were intermedius type.

All 17 strains of mitis type and one strain of intermedius type gave positive in vitro toxigenicity test on both serum and serum substitute antitoxin agar. The disadvantage of using pooled rabbit serum is the hemolysis of some erthrocytes giving rise to high concentration of iron. When the concentration of iron more than 1.4 mcg/ml. will decrease the production of diphtheria exotoxin (3, 5). Therefore, serum substitute is recommended for in vitro toxigenicity test.

All strains of diphtheria bacilli in artificial throat swabs gave positive tests

for rapid in vitro toxigenicity test in 48 hours. It is suggeted that a rapid in vitro toxigenicity test may be performed in order to reduce the time for isolation.

When compared the in vitro test with the intradermal test, it was noticed that the in vitro test was more practicle in a small laboratory than the in vivo one.

Table 1.

Comparison of the used of serum and serum substitute in In vitro toxigenicity test.

Type	No. of test strain	Serum			Serum substitute		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Mitis	17	−	+	+	−	+	+
Intermedius	1	−	+	+	−	+	+
C. xerosis	1	−	−	−	−	−	−
C. pseudodiphtheriticum	1	−	−	−	−	−	−

Table 2.

Comparison of in vitro and in vivo toxigenicity test.

Type	No. of test strain	No. positive in vitro toxigenicity	No. positive intradermal test
Mitis	17	17	17
Intermedius	1	1	1
C. xerosis	1	−	−
C. pseudodiphtheriticum	1	−	−

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