วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vol. 6 No. 3
September 1973

HEMAGGLUTINATION TEST FOR SHIGELLOSIS

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Abstract

A hemagglutination test for diagnosis of shigellosis was studied. Samples of 104 sera from blood donor and 50 sera from nonshigellosis patients were tested. 115 out of 154 specimens (74.6%) gave negative result to a titer of 1 : 8. Only 16 samples (10.4%) gave the titer of 1 : 32 to 1 : 64. 23 sera (14.9%) gave a titer of 1 : 16. The persons who gave the titer of 1 : 32 or 1 : 64 might be in a recovery phase or in a carrier state of shigellosis.

Introduction

Most laboratory diagnosis of shigellosis is the isolation of the shigella organisms from stool specimens which will take at least three days. Very few serological tests are used for diagnosis of shigella infection. Therefore, serological diagnosis was introduced by some investigators (3,4). Since Specific antibody against shigella is low in titer, hence, passive hemagglutination test, a very sensitive test for detecting antibody in sera is used (3, 4, 5). In 1954, Chun and Park (1) detected shigella antibody by passive hemagglutination test. Later on, there were some investigators (2, 6) studied the application of passive hemagglutination test for detecting shigella antibody. The present study is undertaken to find a passive hemagglutination technique for serodiagnosis of shigellosis.

Materials and Methods

1. Preserved sheep red blood cells (PSC). Sheep blood was collected from the jugular vein and stored in Alsever's solution (1 : 1). The erythrocytes was washed three times with Triethanolamine buffer saline (TBS). The thrice washed 50% red blood calls suspension was added with isotonic formaldehyde to a final of 1% formalin. The mixture was kept at 37°C for 2 hours with constant stirring, then washed with TBS for four times. The preserved

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red blood cells is stored in a refrigerator until used.

2. Shigella flexneri lipolysaccharide antigen. Smooth and nonagglutinable colonies of **Shigella flexneri** were streaked onto trypticase soy agar. After 18-24 hours of incubation, the growth organisms were washed off with normal saline and adjusted the opacity to match with McFarland tube No. 7. An equal volume of 0.1 N. NaOH was added and heated in a boiling water bath for one hour. It was neutralized with 2N HCl, then dialyzed against cold distilled water for 24 hours, centrifuged and the supernatant is kept at -20 °C.

3. Standardization of Shigella flexneri antigen. The lipopolysaccharide antigen was diluted to be 1 : 5, 1 : 10, 1 : 20, 1 : 40, and 1 : 80. Each dilution of antigen was mixed with equal volum of 10% suspension of PSC, incubated at 37 °C for 2 hours, then washed three times with TBS. The sensitized PSC (APSC) were titrated with inactivated hyperimmune rabbit serum. Each 0.5 ml. of 1% APSC was added to 0.5 ml. of various dilution of immune sera, incubated at 37 °C for one hour, then refrigerated at 4 °C overnight. Each titer for each antigen dilution was determined. The bast dilution of antigen was 1 : 10, therefore, sensitization of PSC with antigen would be the 1 : 10 dilution.

4. Study sera. A total of 154 sera were tested. 104 sera were taken from blood donors and the other 50 sera from cases of nonshigellosis persons.

Results

The appropriate dilution of antigen from **Shigella flexneri** for sensitization of PSC is 1 : 10 (Table I). 33 out of 50 nonshigellosis sera (66%) give negative to 1 : 8, 8 samples (16%), 1:16, 8 sera (16%) 1 : 32 and 1 serum (2 %) 1 : 64 (Table II).

82 specimens (78.9 %) gave negative result to the titer of 1 : 8, 15 sera (14.4%) a titer of 1 : 16 and 7 samples (6.7 %) a titer of 1 : 32 - 1 : 64 from a to total of 104 sera of blood donor (Table III).

Table IV showed the hemagglutination test of the total 154 sera; 115 samples (74.6 %) gave negative to 1 : 8, 23 sera (14.9 %) a titer of 1 : 16 and 16 sera (10.4 %) a titer of 1 : 32 to 1 : 64.

Table I Standardization of Shigella flexneri Antigen

Antigen dilution	Final dilution of hyperimmune rabbit sera										Titer
	2	4	8	16	32	64	128	256	512	1024	
1 : 5	+	+	+	+	+	+	−	−	−	−	64
1 : 10	+	+	+	+	+	+	−	−	−	−	64
1 : 20	+	+	+	+	+	−	−	−	−	−	32
1 : 40	+	+	+	−	−	−	−	−	−	−	8
1 : 80	+	+	+	−	−	−	−	−	−	−	8

Table II Hemagglutination Test of 50 sera from Nonshigellosis patients

	Hemagglutination titer						
	Negative	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
Total No.	2	5	11	15	8	8	1
Percent	4	10	22	30	16	16	2

Table III Hemagglutination Test of 104 Sera from Blood Donors.

	Hamagglutination titer						
	Negative	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
Total No.	9	8	28	37	15	4	3
Percent	8.7	7.7	26.9	35.6	14.4	3.8	2.9

Table IV Hemagglutination Test of 154 Sera from Nonshigellosis Patients and Blood Donors

	Hemagglutination titer						
	Negative	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
Total No.	11	13	39	52	23	12	4
Percent	7.1	8.4	25.3	33.8	14.9	7.8	2.6

Discussion

The results of standardization of Shigella flexneri antigen was agreeable to the studies of Young et al. (7). Sixty-six percent (33 out of 50) of tested sera give low titer of 0 - 1:8, 16% (8 of 50) 1:16, 16% (8 of 50) 1:32 and 2% (1 of 50) 1:64

The hemagglutination test of 104 sera of blood donor gave comparable results to the 50 nonshigella sera. 82 sera (78.9%) were negative to 1:8, 15 sera (14.4%) 1:16 and 7 specimens 1:32-1:64.

The tested sera gave titer of 1:32 or 1:64 should contain shigella antibody either from being carriers or convalescent (4).

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