

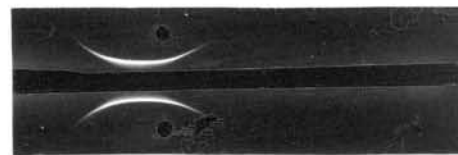


CHAPTER III

RESULTS

A. DETERMINATION THE TYPES OF IMMUNOGLOBULINS ABSORBED ON FILTER PAPER STRIPS BY IMMUNOELECTROPHORESIS

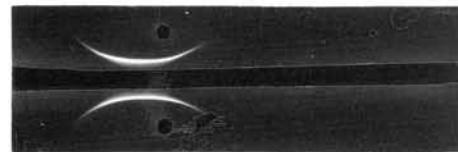
Immunoelectrophoretic analysis of 15 positive paired sera compared between sera eluted from filter paper strips and sera obtained by syringe method from both acute and convalescent stage showed symmetrical precipitation lines with anti-immunoglobulin G and showed nearly absence but symmetrical of immunoprecipitin lines with antiimmunoglobulin M (Fig. 6)



Absorbed acute serum
Anti IgG
Non-absorbed acute serum



Absorbed acute serum
Anti IgM
Non-absorbed acute serum



Absorbed convalescent serum
Anti IgG
Non-absorbed convalescent serum



Absorbed convalescent serum
Anti IgM
Non-absorbed convalescent serum

Fig. 6 IEP patterns of 1 positive paired sera show symmetrical pricipitin lines with anti Ig G and anti Ig M

B. COMPARISON OF THE HI-ANTIBODY TITERS AGAINST DENGUE VIRUS IN BLOOD SAMPLES COLLECTED BY FILTER PAPER METHOD AND SYRINGE METHOD AT DIFFERENT CONDITIONS OF STORING

The titers of antibody of 37 positive dengue cases were compared between different times (2-15 weeks) and different temperature (4°C and 24°C) of storing filter paper strips. The sera obtained by syringe method were used as the control to compare with the filter paper method which was kept at 4°C for about 2 weeks. The results showed that most of the titers of the syringe method were lower than those of the filter paper method which was kept at 4°C for about 2 weeks. At the same time of storing, the titers of the filter papers which were kept at 4°C were higher than those which were kept at room temperature (24°C). The titers of the filter paper were lower and lower according to the time of storing, as shown in Table 1, 2, 3 and 4.

Table 1

No.	SYR	2 WK		4 WK		
		4°C	RT	4°C	RT	
1	>40,960	>40,960	>40,960	20,480	640	
2	5,120	20,480	1,280	2,560	320	
3	5,120	>40,960	640	10,240	640	
4	5,120	20,480	2,560	10,240	320	
5	160	320	80	160	80	
6	2,560	2,560	640	2,560	640	
7	5,120	40,960	640	10,240	320	
8	20,480	20,480	5,120	10,240	320	
9	320	1,280	160	640	80	
10	320	1,280	160	640	80	
11	5,120	10,240	1,280	5,120	160	15 WK 4°C RT
12	5,120	5,120	1,280			14 WK 5,120 20
13	1,280	1,280	1,280			4°C RT 2,560 80
14	2,560	5,120	2,560			5,120 80
15	1,280	5,120	2,560			5,120 160
16	20	40	40			20 <20
17	20,480	40,960	20,480			14 WK 4°C RT
18	2,560			2,560	<20	
19	>20,480			>20,480	320	
20	>20,480			>20,480	2,560	
21	10,240			5,120	40	
22	>20,480			>20,480	640	
23	>20,480			10,240	320	
24	20					12 WK 4°C RT
25	>20,480			>20,480	10,240	
26	>20,480			>20,480	5,120	
27	>20,480			>20,480	320	
28	5,120			2,560	160	
29	2,560			2,560	80	
30	5,120			5,120	80	
31	10,240			10,240	80	11 WK 4°C RT
32	1,280	5,120	640			
33	1,280	2,560	640			
34	80	160	20			
35	>20,480	>20,480	5,120			
36	20,480	20,480	2,560			
37	5,120	1,280	<20			

Table 2 Comparison of the HI-titer between syringe and filter paper method kept at 4° C for 2 weeks

CASE NO.	SYRINGE METHOD	FILTER PAPER METHOD (2 weeks, 4° C)
1	>40,960	>40,960
2	5,120	20,480
3	5,120	>40,960
4	5,120	20,480
5	160	320
6	2,560	2,560
7	5,120	40,960
8	20,480	20,480
9	320	1,280
10	320	1,280
11	5,120	10,240
12	5,120	5,120
13	1,280	1,280
14	2,560	5,120
15	1,280	5,120
16	20	40
17	20,480	40,960

There were 5 cases equal titers, 5 cases 2 fold higher, 5 cases 4 fold higher, 1 case 8 fold higher and 1 case more than 8 fold higher.

Table 3 Comparison of the HI-titer of the filter paper method keep at 4 °C and 24 °C (room temperature) at different time (2-15 weeks)

CASE NO.	TIME (weeks)	TEMPERATURE (°C)	
		4	24 (room)
1	2	>40,960	>40,960
	4	20,480	640
2	2	20,480	1,280
	4	2,560	320
3	2	>40,960	640
	4	10,240	640
4	2	20,480	2,560
	4	10,240	320
5	2	320	80
	4	160	80
6	2	2,560	640
	4	2,560	640
7	2	40,960	640
	4	10,240	320
8	2	20,480	5,120
	4	10,240	320
9	2	1,280	160
	4	640	80
10	2	1,280	160
	4	640	80
11	2	10,240	1,280
	4	5,120	160

Table 3 (cont.)

CASE NO.	TIME (weeks)	TEMPERATURE (°C)	
		4	24 (room)
12	2	5,120	1,280
	15	5,120	20
13	2	2,560	1,280
	15	1,280	80
14	2	5,120	2,560
	14	5,120	80
15	2	5,120	2,560
	14	5,120	160
16	2	40	40
	14	20	<20
17	2	40,960	20,480
18	13	2,560	<20
19	13	>20,480	320
20	13	>20,480	2,560
21	13	5,120	40
22	13	>20,480	640
23	13	10,240	320
24	13	40	<20
25	13	>20,480	10,240

Table 3 (cont.)

CASE NO.	TIME (weeks)	TEMPERATURE (°C)	
		4	24 (room)
26	12	>20,480	5,120
27	12	>20,480	320
28	12	2,560	160
29	12	2,560	80
30	12	5,120	80
31	12	10,240	80
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32	11	5,120	640
33	11	2,560	640
34	11	160	20
35	11	>20,480	5,120
36	11	20,480	2,560
37	11	1,280	<20
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Table 4 Comparison of Hemagglutination-Inhibition (HAI) Titer of Serum and Disk Eluates from the same Person (7)

NO.	SERUM TITER	DISK TITER
1	192	384
2	96	96
3	48	24
4	96	96
5	24	24
6	12	12
7	48	24
8	48	48
9	48	24
10	24	24
11	24	24
12	24	24
13	<6	<6
14	<6	<6
15	<6	<6

C. CORRECTION OF ERROR ON BLOOD VOLUMES ABSORBED
BY FILTER PAPER STRIPS

A. Female Blood Sample: age 24 yrs.

Weight of 100 pieces of blood absorbing parts	=	4.86	gm
Weight of 100 pieces of blood-filled absorbing parts	=	13.07	gm
•• Weight of fresh blood in 100 papers	=	8.21	gm
Volume of fresh blood	=	weight $\times S_B$	
Normal blood specific gravity (S_B) of woman (30)	=	1.052 - 1.060	
•• Volume of fresh blood in 100 papers	=	8.21 \times 1.055	
	=	8.66	ml
•• Volume of fresh blood in 1 paper	=	0.0866	ml
Hematocrit (the average of 3 measurements)	=	35.5	%
Blood volume 100 ml = serum volume (100 - 35.5)	=	64.5	ml
Blood volume 0.0866 = serum volume $\frac{0.0866 \times 64.5}{100}$	=	0.06	ml
•• 1 paper can absorb 0.0866 ml of blood = serum	=	0.06	ml
To obtain a serum dilution 1:20, it must be added a quantity of PBS	=	0.06 \times 20	
	=	1.2	ml
After drying for 24 hours:			
Weight of 100 pieces of dried blood-filled absorbing parts	=	6.88	gm
•• Weight of dried blood in 100 papers	=	2.02	gm
•• Weight of fresh blood in 100 papers	=	2.02 \times 5	
	=	10.10	gm
Volume of fresh blood in 100 papers	=	10.10 \times 1.055	
	=	10.6555	ml
Volume of fresh blood in 1 paper	=	0.10655	ml
Blood volume 100 ml = serum volume	=	64.5	ml
Blood volume 0.10655 ml = serum volume $\frac{0.10655 \times 64.5}{100}$	=	0.07	ml
•• 1 paper can absorb 0.1066 ml of blood = serum	=	0.07	ml
To obtain a serum dilution 1:20, it must be added a quantity of PBS	=	0.07 \times 20	
	=	1.4	ml

B. Male Blood Sample: age 24 yrs.

Weight of 100 pieces of blood absorbing parts	=	4.86	gm
Weight of 100 pieces of blood-filled absorbing parts	=	14.37	gm
•• Weight of fresh blood in 100 papers	=	9.51	gm
Volume of fresh blood	=	weight $\times S_B$	
Normal blood specific gravity (S_B) of man (30)	=	1.057 - 1.064	
•• Volume of fresh blood in 100 papers (1.061×9.51)	=	10.09	ml
•• Volume of fresh blood in 1 paper	=	0.1009	ml
Hematocrit (the average of 3 measurements)	=	45	%
Blood volume 100 ml = serum volume (100 - 45)	=	55	ml
Blood volume 0.1009 ml = serum volume $\frac{0.1009 \times 55}{100}$	=	0.055495	ml
•• 1 paper can absorb 0.1009 ml of blood = serum	=	0.06	ml
•• To obtain a serum dilution 1:20, it must be added a quantity of PBS	=	0.06 \times 20	
	=	1.2	ml

After drying for 24 hours:

Weight of 100 pieces of dried blood-filled absorbing parts	=	7.632	gm
•• Weight of dried blood in 100 papers ($7.632 - 4.86$)	=	2.772	gm
•• Weight of fresh blood in 100 papers (2.772×5)	=	13.86	gm
Volume of fresh blood in 100 papers (13.86×1.061)	=	14.705	ml
Volume of fresh blood in 1 paper ($14.705/100$)	=	0.147	ml
Blood volume 100 ml = serum volume (100-45)	=	55	ml
Blood volume 0.147 ml = serum volume $\frac{0.147 \times 55}{100}$	=	0.08	ml
•• 1 paper can absorb 0.147 ml of blood = serum	=	0.08	ml
To obtain a serum dilution 1:20, it must be added a quantity of PBS	=	0.08 \times 20	
	=	1.6	ml

Table 5 Correction of error on blood volumes absorbed by filter paper strips

	Sex	Studied from	
		Fresh blood	Dried blood
Quantity of PBS added	Female	1.2	1.4
to obtain serum dilution	Male	1.2	1.6
1:20 * (ml)			

* average of 100 papers

D. THE HI-ANTIBODY TITERS OF THE DENGUE INFECTION BLOOD COLLECTED ON FILTER PAPER STRIPS SENT FROM DIFFERENT PARTS OF THE COUNTRY

Forty-two filter paper strips were absorbed with positive dengue blood of one sick child then they were brought to 8 different provinces and mailed back to the laboratory. The titer of antibody of each paper was detected by HI-test in duplicate. It was found that the filter paper strips which took 3-8 days of transportation had no different in titer of antibody, and those which took 11-12 days of transportation had only 2 fold dilution lower, as shown in Table 6 and Table 7.

Table 6

PROVINCE	DAYS *	PAPER NO.				
		1	2	3	4	5
1. CONTROL	3	10,240	10,240	-	-	-
		10,240	20,480	-	-	-
2. U-BOL RACHATHANI	3	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	20,480
3. CHUMPORN	4	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	10,240
4. HAD-YAI	4	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	10,240
5. SU-RAD THANI	4	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	10,240
6. NAKORNSRI THAMARAD	4	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	10,240
7. CHIENGMAI	8	10,240	10,240	10,240	10,240	10,240
		10,240	10,240	10,240	10,240	10,240
8. LAMPANG	11	10,240	10,240	10,240	10,240	5,120
		10,240	10,240	5,120	5,120	5,120
9. NONGKAI	12	10,240	10,240	5,120	5,120	5,120
		10,240	5,120	5,120	5,120	5,120

* Days from they were mailed until backed to the laboratory.

HI-test in duplicate was carried out with each dilution.

Table 7 The average titers of filter papers which sent from different parts of the country with different time of transportation

PROVINCE	DAYS	TITER
Control *	3	10,240
U-Bolrachathani	3	10,240
Chumporn	4	10,240
Hadyai	4	10,240
Suradthani	4	10,240
Nakornsrithamarad	4	10,240
Chiengmai	8	10,240
Lampang	11	5,120 - 10,240
Nongkai	12	5,120

* Control is the absorbed filter paper kept at 4⁰ C in refrigerator until used.

