

Efficiency of Peripheral Blood Collected on Filter Paper Discs and One Dengue Antigen in Serologic Diagnosis of Dengue Hemorrhagic Fever

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**BACKGROUND:** For routine serologic diagnosis of dengue infections in this laboratory, serum has been tested against antigens of all four dengue serotypes. This traditional approach has two significant disadvantages in a large scale surveillance program for DHF. First, obtaining blood by venipuncture, aseptic separation of serum and transportation of serum to a central laboratory requires manpower, equipment, and logistical support not often available to smaller provincial hospitals. Second, testing sera against four viral antigens is expensive (cost, \$ 2.00 U.S. per test) and time-consuming, limiting the number of patients which can be tested serologically.

For serologic tests against viruses other than dengue (rubella, coronaviruses), peripheral blood collected on a filter paper disc has proven a practical and economical collection system in extensive field research projects. The purpose of the study reported was to compare filter paper disc specimens with serum specimens for serologic diagnosis of dengue in patients with suspected DHF. A second goal was to compare the efficiency of the HI test in serologic diagnosis of dengue using only one dengue antigen versus all four dengue antigens. This research has important implications in serologic diagnosis of dengue infections under adverse military field conditions.

**METHODS:** Sixty-four patients admitted to Children's Hospital with a diagnosis of DHF were studied. Dengue viruses were isolated from 11 of these patients; all isolates were dengue 2. Acute serum drawn on admission and convalescent serum drawn on discharge on each patient were tested for antibody to the four dengue serotypes by the standard method of Clarke and Casals modified to microtiter; 8-16 units of antigens were used. In addition, peripheral blood obtained by finger prick was collected on two filter paper discs (Schleider and Schuell, No. 740-E, diameter 12.7 mm). One set of acute and convalescent discs from each patient was stored at 4°C and the other at 37°C for one week (to mimic the effect of ambient temperature). Both sets of discs were soaked with 0.4 ml borate saline (pH 9.0) and incubated at 4°C overnight. One half ml of a 25% acid-washed kaolin solution in borate saline was added and incubated at room temperature for 20 minutes with occasional agitation. The eluate was separated by centrifugation at 2500 rpm for 30 minutes, treated with 0.02 ml of packed goose RBC at 4°C for 30 minutes with occasional agitation and then centrifuged. Since the IgG concentrations of several eluates tested approximated 5% of that of companion sera, the final dilution of the eluate obtained was considered to be a 1:20 dilution of whole serum. The eluate was tested for HI antibodies to dengue 2 and dengue 4 antigens by the same technique used for serum specimens.

**RESULTS:** Shown in Table 1 are the differences in HI titer (log 2) between the filter paper technique and serum for 128 individual specimens tested. There is close concordance in the two techniques for dengue 2 HI antibody titers; 86% of the filter paper titers were within one dilution of the serum titer. A different pattern was seen with dengue 4 antigen. Only 61% of filter paper titers were within one dilution of the serum titer; the distribution of filter paper titers was skewed considerably toward lower titers than were obtained in sera. Technical reasons for the discrepancy with the two antigens are not apparent; indeed, identical aliquots of the eluate of each specimen were tested with both antigens simultaneously.

A comparison of titers obtained from filter paper discs stored at 4°C and 37°C for one week against the two dengue antigens is shown in Table 2. Tested were 52 sera from 26 DHF patients. Storage at 37°C did not change HI titers significantly in comparison with storage at 4°C.

Table 3 compares diagnosis of dengue infections using dengue 2 antigen alone by serum and by filter paper disc specimens in the 64 patients studied. Rises in titer were found in 32 patients using serum compared with 29 using discs. Concordance of the two methods was found in 20 patients with titer rises, in 10 with high fixed titers, and in 13 patients with low fixed titers. Discrepancies were limited to variations between titer rises and high fixed titers by both techniques. The results with dengue 4 antigen were similar (Table 4).

The efficiency of serologic diagnosis of dengue infections using the filter disc collection method and one dengue antigen in comparison with serum and four dengue antigens is shown in Table 5 (dengue 2 antigen) and Table 6 (dengue 4 antigen). In this analysis a 4-fold or greater titer rise to any one of the four dengue antigens in serum specimens was considered indicative of dengue infection. Results were similar with both antigens; conventional serologic techniques identified 46 dengue infections by rise in HI titer in the 64 patients studied while filter paper discs detected infections in 29 (D2) and 30 (D4) patients. All infected patients not detected by the filter paper technique had high fixed titers ( $\geq 1:640$ ) to the dengue antigen used. Both methods were concordant in patients with low fixed titers (without evidence of dengue infection). If a rise to either D2 or D4 was used to indicate dengue infection, infections were identified in 31 of the 46 patients who had a serum titer rise to one of the four dengue antigens.

*DISCUSSION:* Little difference in the efficiency of serologic diagnosis of dengue infections with 64 DHF patients studied was found between the peripheral blood filter paper disc specimens and serum specimens when tested with one dengue antigen. Storage of discs at 37°C for one week did not change dengue HI titers. The data suggested that the more convenient filter paper disc can be used for dengue diagnosis with little decrease in diagnostic efficiency.

Considerable decrease in efficiency of dengue serologic diagnosis accrued when one dengue antigen was used instead of all four dengue serotypes. Using serum specimens, only 33 patients (D2 antigen) and 30 patients (D4 antigen) had diagnostic titer rises while 46 patients showed rises when all four dengue antigens were used. By filter paper using one dengue antigen, 29 (D2 antigen) and 30 (D4 antigen) showed rises. All patients with rises detected by four antigens but no rise with single antigens (D2 or D4) had fixed titers of  $\geq 1:640$  against the single antigens. If high fixed titers ( $\geq 1:640$ ) (in addition to four-fold titer rises) are accepted as serologic criteria of recent dengue infections, the use of one dengue antigen results in little decrease in diagnostic efficiency. Patients without serologic evidence of dengue by conventional techniques were correctly identified by the filter paper disc—single antigen test. Although the lack of concordance in titers to dengue 4 antigen between the two tests suggests caution in the application of the filter disc technique for antibody prevalence data, the results suggest that it is an acceptable technique for confirmation of dengue infections in a DHF surveillance program.

Table 1.  
 Concordance between HI titers obtained by filter—paper technique and simultaneous serum  
 in acute and convalescent specimens from 64 patients.

Difference in HI titer (Log 2) of filter—paper method from serum	HA Antigens	
	Dengue 2 (no. sera)	Dengue 4 (no. sera)
-6	0	2
-5	0	1
-4	2	6
-3	1	18
-2	9	21
-1	23	26
0	62	39
+1	25	13
+2	6	2
+3	0	0

Table 2.  
 Comparison of HI titers from eluates of filter paper discs stored at  
 37°C and 4°C (26 patients).

Difference in HI titer (Log 2) of discs stored at 37°C and 4°C	HA Antigens	
	Dengue 2 (no. sera)	Dengue 4 (no. sera)
-3	0	0
-2	3	2
-1	14	14
0	31	30
+1	3	4
+2	1	2
+3	0	0

**Table 3**  
**Efficiency of Serologic Diagnosis of Dengue Infection by Filter Paper**  
**Discs Compared with Serum (Dengue 2 HA)**

Filter Paper Disc		Serum			
		Titer rise	No titer rise		Totals
			$\geq 1:640$ Titer (no. patients)	$< 1:640$ Titer (no. patients)	
Titer rise		20	8	1	29
No titer rise	$\geq 1:640$	11	10	0	21
	$< 1:640$	1	0	13	14
Totals		32	18	14	64

**Table 4**  
**Efficiency of Serologic Diagnosis of Dengue Infection by Filter Paper**  
**Discs Compared with Serum (Dengue 4 HA)**

Filter Paper Disc		Serum			
		Titer rise	No titer rise		Totals
			$\geq 1:640$ Titer (no. patients)	$< 1:640$ Titer (no. patients)	
Titer rise		21	8	0	29
No titer rise	$\geq 1:640$	7	15	0	22
	$< 1:640$	0	0	13	13
Totals		28	23	13	64

Table 5  
 Efficiency of Serologic Diagnosis by Filter Paper Discs; Discs and  
 D<sub>2</sub>HA Compared to Serum and D<sub>1</sub>-D<sub>4</sub> HA

Filter Paper Disc D <sub>2</sub> Antigen		Serum, 4 Dengue Antigens			
		Titer rise	No titer rise		Totals
			≥ 1 : 640 Titer (no. patients)	< 1 : 640 Titer (no. patients)	
Titer rise		28	1	0	29
No titer rise	≥ 1 : 640	17	4	0	21
	< 1 : 640	1	0	13	14
Totals		46	5	13	64

Table 6  
 Efficiency of Serologic Diagnosis by Filter Paper Disc: Discs and  
 D<sub>4</sub> HA Compared to Serum and D<sub>1</sub>-D<sub>4</sub> HA

Filter Paper Disc D <sub>4</sub> Antigen		Serum, 4 Dengue Antigens			
		Titer rise	No titer rise		Totals
			≥ 1 : 640 Titer (no. patients)	< 1 : 640 Titer (no. patients)	
Titer rise		29	1	0	30
No titer rise	≥ 1 : 640	17	4	0	21
	< 1 : 640	0	0	13	13
Totals		46	5	13	64