

STAPHYLOCOCCAL AGGLUTINATION-INHIBITION REACTION: A RAPID AND SIMPLE TEST FOR DENGUE ANTIBODIES

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SYNOPSIS

A rapid and simple test for demonstrating dengue antibodies is described. The test is based on inhibition of agglutination of dengue antibody-coated staphylococci by dengue virus antigen.

INTRODUCTION

Routine laboratory confirmation of a clinical diagnosis of dengue or dengue haemorrhagic fever (DHF) depends on the demonstration of dengue antibodies in the patient's serum by serological tests, most commonly the haemagglutination-inhibition (HI) and complement fixation (CF) tests. A significant dengue antibody rise between acute and convalescent phase sera or, in the case of a single acute serum, a significantly high antibody titre, is usually considered as evidence of a recent or presumptive recent dengue infection. However, these serological tests are time-consuming and require specialized equipment for their performance.

We describe here a rapid and simple procedure for demonstrating dengue antibodies in patient's serum and preliminary results of its use as a routine diagnostic test for dengue infection. This procedure uses dengue antibody-coated staphylococci in an agglutination-inhibition reaction performed in drops on a glass slide. Staphylococci are used because the protein

A component on the cell wall has the property of combining with the Fc portion of human IgG molecule, thereby leaving the antigen-binding sites exposed (Forsgren and Sjoquist, 1966).

MATERIALS AND METHODS

Staphylococcus aureus containing protein A (National Collection of Type Cultures no. 8532) was grown overnight in nutrient broth with added β -glycerophosphate. The washed bacteria were treated with 0.5% formaldehyde for 3 hours and then heated at 80°C for 4 minutes. The bacteria were washed and finally resuspended in phosphate-buffered saline (PBS), pH 7.4, containing 0.1% sodium azide and stored at 4°C until use.

A pooled human serum containing a high level of dengue antibodies (HI titre 1:1,280) was used for coating the staphylococci. Dengue positive sera from individual patients have also been used. For coating, the serum was mixed with the staphylococci and the suspension left at room temperature for 30 minutes. The antibody-coated staphylococci were washed and finally resuspended in borate saline, pH 9.0, containing bovine serum albumin. For controls, uncoated staphylococci and staphylococci similarly treated with a pooled human serum containing no dengue antibody (HI titre < 1:10) were used.

Dengue virus antigens used in the experiments were either a 10% suckling mouse brain suspension centrifuged at 10,000 r.p.m. for 60 minutes or sucrose-acetone extracted according to the method of Clarke and Casals (1958).

The staphylococcal agglutination-inhibition test was carried out on a glass slide. The reagents were added dropwise in the following order: patient's serum, dengue antigen, buffer, and antibody-coated staphylococci. The slide was tilted back and forth for about 1-2 minutes and the reaction observed with the naked eye. In

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TABLE I
STAPHYLOCOCCAL AGGLUTINATION-INHIBITION TEST ON PATIENTS' SERA

Serum No.	HI titre	Original serum (1:10)		Original serum (1:100)		Kaolin-treated serum (1:10)	
		Control	Test	Control	Test	Control	Test
1	1,280	+	+	—	—	—	—
3	10	+	+	—	+	—	+
7	<10	—	+	—	—	—	+
8	1,280	—	—	—	—	—	—
10	10	+	+	—	+	+	+
22	40	+	+	—	+	—	—
27	5,120	—	—	—	—	—	—
S289/2/75	640	—	—	—	—	—	—
S315/2/75	160	—	(1,280)*	—	—	—	—
			(80/160)*				

+ = Agglutination; — = inhibition

* = Staphylococcal agglutination-inhibition titre.

the absence of dengue antibody, the antigen causes agglutination of the antibody-coated staphylococci; in the presence of dengue antibody the agglutination is inhibited. Known dengue positive and dengue negative patients' sera were included in each test as controls.

RESULTS AND DISCUSSION

The accompanying table shows representative results of the staphylococcal agglutination-inhibition test performed on patients' sera. Preliminary findings from many experiments are summarized below:

(1) Some sera (nos. 1, 3, 10, 22) showed auto-agglutination (i.e., in the absence of dengue antigen), especially after storage at -20°C for long periods. Auto-agglutination was prevented when these sera were diluted and used at 1:100. Kaolin treatment also removed auto-agglutination from some of these sera.

(2) The staphylococcal agglutination-inhibition test was negative (no antibody) when the sera showed a HI titre of 1:10 or less; it was

positive (antibody present) when the HI titre was 1:40 or greater.

(3) The staphylococcal agglutination-inhibition titre is very closely correlated with the HI titre (S-289/2/75 and S-315/2/75).

The staphylococcal agglutination-inhibition test appears to be a very suitable screening test for a recent dengue infection. It is rapid (2 minutes) and very simple to perform, and is suitable for use at outpatient clinics.

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