KINETIC COMPLEMENT FIXATION TEST FOR RAPID IDENTIFICATION OF JAPANESE ENCEPHALITIS AND WEST NILE VIRUSES

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ABSTRACT

A kinetic CF test was employed for rapid identification of the viruses of JE–WN complex. Addition of haemolytic system after incubation of antigen, antibody and complement for 2 hours at 4° C gave reactions which could differentiate the two viruses with ease. Two strains of JE and one strain of WN virus were identified in this manner. Identification of the isolates were confirmed by employing absorbed mono-specific immune sera.

A RAPID identification of Japanese encephalitis (JE) and West Nile (WN) viruses is often difficult due to the close antigenic relationship between the two viruses. This difficulty was overcome by Pavri and Shaikh[1] by employing monospecific JE/WN absorbed sera in routine complement fixation tests. A 20% infected mouse brain suspension in normal saline could be used as antigen for rapid identification. Non-availability of monospecific absorbed sera pose problems, as most laboratories are not equipped with a high-speed centrifuge. By changing certain parameters, such as the reaction time, it was possible to establish the identity of the new isolates. The present communication deals with the procedures followed for their identification.

P 20778 strain of JE virus and G 22886 strain of WN virus were employed in the complement fixation tests. Sucrose acetone extracted mouse brain antigens were prepared according to the method described by Clarke and Casals[2]. For rapid identification, 10–20% infected mouse brain suspensions in normal saline were centrifuged at 2500 g for one hour and the supernatants were employed as antigens. Virus strains 724038 (JE) and 724268 (WN) were isolated from mosquitoes collected in Andhra Pradesh (Rodrigues : personal communication) and strain 733690 (JE) was isolated from human brain during JE epidemic in West Bengal (Sarkar)[3]. Normal mouse brain antigen was prepared in a similar manner.

Hyperimmune sera/ascitic fluids were raised in mice by inoculating intraperitoneally five doses of live virus. The mice were bled 4–7 days after the last dose. Immune ascitic fluids were collected from hyperimmunized mice inoculated with Erlich ascitic tumour cells. These cells were maintained in this laboratory after receipt from Dr. J. K. Sarkar of Calcutta School of Tropical Medicine and Hygiene. JE/WN monospecific absorbed sera were prepared according to the method described by Pavri and Shaikh[1],

Complement fixation (CF) tests were performed by the method described by Pavri *et al.*[4]. A kinetic CF test was carried out by varying the incubation period. The extracted antigens or quick antigens, the hyperimmune sera/ascitic fluids, and 2–2·5 units of complement were incubated at 4° C. Sensitized sheep erythrocytes were added at intervals of 0 hour, 1 hour, 2 hours and overnight incubation of the test at 4° C. The plates were then incubated at 37° C and the test was read one hour after the incubation.

The extracted antigens reacted rapidly with the hyperimmune sera against the homologous antigen. There was either no reaction or a low reaction with heterologous sera when the test was incubated for 0 hour to 2 hours. The homologous as well as heterologous titres increased with the increase in incubation period.

Another parameter, the concentration of antigen, also had an effect on the detection of homologous as well as heterologous antibodies in relation to the period of incubation (Table I). A unit of antigen is defined as the highest dilution of the antigen which fixes 2·5 units of complement in the presence of homologous mouse immune serum. Sixteen units of antigens gave more cross reactions with heterologous sera in overnight incubation of the test system and as the concentration of antigens decreased the reactions with heterologous sera were less marked especially when 1–2 units of antigens were employed. The titres of homologous sera were not affected with the decrease in the concentration of antigens in overnight incubation. The reaction was demonstrated in one hour when more than four units of antigens were employed.

The kinetic CF test with the saline extracted mouse brain antigens of 724038 and 733690 strains gave a sharp difference in their reactions with JE hyperimmune sera when 2 hour reaction time was allowed (Table II). Identification of both the strains of JE virus were further confirmed by demonstrating a positive reaction with monospecific

TABLE I

Kinetic CF test effect produced by variable quantity of antigen

Antigen	No. of units of antigen	HOURS* 0 JE imm.	0 WN imm.	1 JE imm.	1 WN imm.	2 JE imm.	2 WN imm.	18 JE imm.	18 WN imm.
JE	32	32	<8	256	16	256	32	512	128
	8	8	<8	256	<8	256	32	512	128
	4	<8	<8	256	<8	256	16	512	128
	2	<8	<8	16	<8	32	<8	UNS	16
	1	<8	<8	<8	<8	<8	<8	512	<8
	<1	<8	<8	<8	<8	<8	<8	UNS	<8
WN	16	8	32	32	128	32	128	256	512
	8	<8	16	32	128	32	128	128	512
	4	<8	<8	16	128	16	128	64	512
	2	<8	<8	<8	16	<8	128	16	512
	1	<8	<8	<8	<8	<8	16	<8	512
	<1	<8	<8	<8	<8	<8	<8	<8	<8
Normal	..	<8	<8	<8	<8	<8	<8	<8	<8

Imm. = Immune sera, UNS = Unsatisfactory, * Different time of incubation at 4° C before addition of sensitized erythrocytes. Normal control sera in all cases yielded titres of <8.

TABLE II

Kinetic complement fixation test identification of new isolates

Immune sera	Antigens 724038 *0	724038 2	724038 18	724268 0	724268 2	724268 18	733690 0	733690 2	733690 18	Homologous 0	Homologous 2	Homologous 18
JE imm.	16	128	≥256	<8	32	64	32	≥128	≥128	8	64	≥256
WN imm.	<8	32	128	256	≥512	≥512	<8	<8	32†	256	≥512	≥512
										†16	16	64
JE imm. (absorbed Sr.)	8	64	128	—	—	8	—	—	128	8	64	128
WN imm. (absorbed Sr.)	<8	<8	<8	—	—	64	—	—	<8	<8	16	≥258
N Sr./PF	<8	<8	<8	<8	<8	<8	<8	<8	<8	—	—	—
Identification of the isolate	JE				WN			JE				

* Hours of incubation at 4° C before addition of sensitized erythrocytes, † Homologous titres of antiserum employed in that particular test. —= Not done. Normal control antigen did not react with the immune sera.

absorbed JE immune serum as well as in neutralization test. The isolate 724268 gave a specific reaction with West Nile immune serum when the sensitized cells were added immediately. Further confirmation was obtained by demonstrating reaction with WN specific absorbed sera.

Hatgi and Sweet[5] found that by varying a number of parameters of the test such as antigen concentration and the reaction period they could profitably apply the test to type dengue viruses and in some cases also establish intratypic strain variations. The kinetic CF test was more specific at lower antigen concentrations. Cross reactions were more marked when excess antigen was employed. In the present studies the titres of the crude antigens were not determined. The checkerboard titrations of JE–WN antigens against homologous as well as heterologous hyperimmune sera indicated that the reactions with heterologous sera diminished proportionately with the reduction in the concentration of antigens, however, reactions with the homologous sera were not affected to the same extent.

The sharp differentiation in reactions of the hyperimmune sera of JE–WN complex in less than

2 hours has made this test a useful tool especially when monospecific immune sera not available.

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DISCONTINUITY IN THE LARVAL DISTRIBUTION OF PHORONIDA AND BRACHIOPODA IN THE INDIAN OCEAN

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ABSTRACT

Distribution of Phoronid and Brachiopod larvae in Indian Ocean was studied on the basis of the zooplankton samples collected during the International Indian Ocean Expedition. The striking feature is the discontinuous distribution of these larvae. The larvae of Phoronida prefer relatively low salinity waters while the Brachiopod larvae have high tolerance to changes in salinity. The abundance of both Brachiopod larvae and Actinotrocha in the Bay of Bengal suggests the richness of the adults in this region of the Indian Ocean.

PHORONIDA and Brachiopoda have free swimming larval stages in their life-histories and these larvae are familiar constituents of plankton. Both Phoronida and Brachiopoda lead a benthonic existence. Phoronids, so far recorded, are limited to the shallow waters of tropical and temperate zones[1]. Brachiopods are exclusively marine forms and occur in all seas from the intertidal zone to depths of 500 m[1]. Their meroplanktonic larvae serve as links and maintain genetic continuity between populations spatially isolated from one another[2].

There are only a few reports on these larvae from the Indian Ocean and adjacent seas. The earlier records of the Brachiopod larvae are the occurrence of *Lingula* larvae in the Gulf of Aden, South of Red Sea, off the Mysore coast, west coast of Sumatra and of *Pelagodiscus* larvae from the southwestern part of India[3-5]. A number of Actinotrocha are known of which the adult has not been identified[1].

During the International Indian Ocean Expedition from 1960 to 1965, zooplankton samples amounting to 1927 were collected from the Indian Ocean between the Lat. 25° N to 46° S and Long. 20° to 120° E. Most of the samples were taken with an Indian Ocean Standard Net from a depth of 200 m to the surface or in the continental shelf from the bottom to the surface[6]. The data obtained from these zooplankton samples form the basis of the present study.

ACTINOTROCHA

The fully developed larva has an elongated body varying from less than 1 to 5 mm in length[1]. The planktonic existence of the larva extends probably to several weeks. Actinotrocha are represented in 4·1% of the samples. Maximum incidence of the larvae was found to be in the Bay of Bengal, off the coast of Somalia and off the southeast coast of Africa (Fig. 1). A striking feature is the discontinuous distribution of the larvae as they were absent or sparsely represented in the Arabian Sea, Central Indian Ocean and eastern part of the Indian Ocean between the Equator and Lat. 30° S and Long. 68° to 120° E. Their seasonal occurrence and other details are given in Table I. The hydrographical data at the stations, from which high abundance of the larvae was recorded, have a temperature range 13·69°–26·48° C, salinity 32·86–35·46‰, oxygen 0·36–5·40 ml/l and phosphate phosphorus 0·16–1·33 µg at/l. With the exception of three records, they were never found at stations where maximum