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EVALUATION OF IMMUNOFLUORESCENCE AND IMMUNOPEROXIDASE METHODS FOR ANTIBODY DETERMINATION AGAINST CHIKUNGUNYA, WEST NILE AND YELLOW FEVER VIRUSES

by

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Summary — The indirect immunofluorescence test (IIFT) and the indirect enzyme linked immunosorbent assay (ELISA) were compared with the haemagglutination inhibition (HI) and neutralization tests (NT) for the detection of antibodies against West Nile (WN), Yellow Fever (YF) and Chikungunya (CHIK) viruses in human sera.

The presence of antibodies against WN and CHIK viruses detected by the NT and HI tests, were all confirmed by both the ELISA and IIFT tests.

For YF however, out of seven sera positive in the NT, only 2 were IIFT positive, but all seven were confirmed by ELISA. Compared with the NT, the IIFT and ELISA detected 2 to 7 times more positives.

For CHIK a good correlation between IIFT and ELISA was observed.

It is concluded that ELISA and IIFT are as specific as the NT test but detect 2 to 7 times as many positives for which it is impossible to know at this moment if they are specific or aspecific.

KEYWORDS : Indirect immunofluorescence; Immunoperoxidase; Arboviruses.

Introduction

The haemagglutination inhibition (HI) test has been used on a large scale for the detection of antibodies against numerous viruses, particularly arboviruses. In recent years the indirect immunofluorescence test (IIFT) has been applied for the detection of antibodies against a wide variety of antigens and in virology especially for those viruses that do not readily produce a haemagglutinin (Wulff & Lange, 1975; van den Groen *et al.*, 1978). This test and the enzyme linked immunosorbent assay (ELISA) are also particularly suited for work with class IV organisms since it requires relatively limited equipment that can be handled more confortably in laboratory isolators. Both the IIFT and ELISA have not been frequently applied to haemagglutinating arboviruses (Tignor & Digoutte, 1976). Since some arboviruses, such as Congo-Krim and Rift Valley fever viruses, may also be responsable for a haemorrhagic fever syndrome, there will be a necessity for handling such agents in laboratory isolators, where the ELISA and IIFT offer considerable advantages.

In this paper we describe the application of the IIFT and ELISA for the detection of antibodies against Chikungunya (CHIK), West Nile (WN) and Yellow Fever (YF) viruses comparing the results with those obtained by the neutralization (NT) and HI tests on the same sera.

Materials and Methods

Human sera collected in Zaire were tested. Twenty eight were collected in February 1977 in Lebela Yalihawo, 38 km South of Yambuku, the center of an Ebola virus outbreak in 1976 (W. H. O. International Commission, 1978). Sixty two sera were collected in February 1978 in 8 villages near Tandala, 325 km West of Yambuku, 10 sera collected in the region of Isiro, North East Zaire.

Viruses used were CHIK, 3th Vero cell passage and WN, 4th cell passage, both after an unknown number of passages in newborn and/or adult mouse brain. YF was a first Vero cell passage of YF 17D vaccine (Wellcome).

IIFT test. Antigen preparation. 20.10^6 Vero cells were inoculated with CHIK, YF or WN viruses at a multiplicity of 0.05 in Plaisner medium.

After 2, 3 and 7 days respectively, the supernatant medium was discarded and the cells harvested by addition of 0.5 per cent trypsin for 1 minute and 0.2 per cent versene for 30 seconds. The cells were suspended in phosphate buffered saline (PBS), pH 7.2 at a concentration of 5.10^5 cells/ml. The suspension was passed through a 25 G needle to remove clumps. Drops of 0.05 ml of the cell suspension were deposited on a 10 well multispot slide (Wellcome), air-dried and fixed in acetone at room temperature for 10 min. The slides were stored at − 20 °C.

Detection of antibody was done as described by Wulff and Lange (1975) using fluorescein labeled sheep antihuman IgG (Wellcome), diluted 1 : 40 in PBS containing Evans blue at a 0.1 per cent final concentration.

HI tests were performed according to the method of Clarke and Casals (1958). Viral antigens were prepared with protamin sulfate, sera were adsorbed with kaolin and packed goose erythrocytes.

For the *neutralization tests* 100 TCD_{50} of the viruses were mixed with serum diluted 1/10, incubated at 37 °C for 30 min. and 0.2 ml of the mixtures inoculated in microtiter plates containing 15.000 Vero cells per well.

Plaque reduction test was performed with CHIK virus only on Vero cells following the procedure previously described (Pattyn, De Vleesschauwer & Brès, 1965). The filter paper discs were impregnated with serum immediately being deposited on the agar.

ELISA test. Viral and control antigens were prepared as described by Mills *et al.* (1978) and the tests performed as described by Voller *et al.* (1976). Goat antihuman peroxidase conjugated was obtained from Nordic. Optimal conjugate and antigen dillutions were determined by chequer board titrations. Results were read visually.

Criteria for positivity were as follows : NT : 1/10, HI : 1/40, IIFT : 1/10, ELISA : 1/10.

Results

These are summarized in table 1. For the HI sera were tested at a dilution 1 : 40. For both the IIFT and ELISA the results obtained at a 1 : 10 dilution were recorded as positive. For each antigen the proportion of positives in the NT and HI are fairly comparable and the correlation between the two tests is 100 per cent CHIK and 85 per cent for WN.

TABLE 1

Comparison of ELISA, HI, IIF and plaque reduction tests for antibodies against CHIK, WN and YF

Antigen	A Number of sera tested	Technique	B Number of positive sera	C Number of sera positive in both tests HI	IIF	ELISA	PRT
CHIK	83	NT	14	14 (100 %) (a)			
	83	HI	12		13 (84 %)	14 (63 %)	3 (83 %)
	83	IIF	25		11 (82 %)	12 (60 %)	3 (83 %)
	83	ELISA	45			24 (73 %)	3 (62 %)
	24	PRT	3				3 (41 %)
WN	40	NT	4	2 (85 %)			
	40	HI	6		4 (32 %)	4 (35 %)	
	40	IIF	21		5 (58 %)	6 (65 %)	
	40	ELISA	20			11 (53 %)	
YF	38	NT	7				
	38	IIF	8		2 (71 %)	6 (58 %)	
	38	ELISA	21			2 (34 %)	

(a) = percentage similarity = $\frac{(A - B_1) - (B_2 - C) + C \times 100}{A}$.

A = number of sera tested.

B_1 = number of positive results in test 1.

B_2 = number of negative results in test 2.

C = number of sera positive in both tests.

Almost all positive results obtained in the NT or HI are confirmed by IIFT and ELISA but the latter two reactions detect 2 to 7 times as many positives as compared with the two former reactions.

All positives (except one) in the IIFT for CHIK were confirmed by ELISA but the latter still detects 20 more positives.

In the case of WN, although the same number of sera were positive for both ELISA and IIFT, the overall similarity of the results is not good. Only 11 sera were positive in both tests.

For YF, there was also a bad correlation between results of ELISA and IIFT.

The plaque reduction test is clearly the least sensitive test.

Discussion

The NT is generally considered to be the most specific test for antibody detection.

Since for the three antigens tested the positive results obtained in the NT were all confirmed by the ELISA and IIFT it may be assumed that ELISA and IIFT are at least as specific as the NT. However, a higher number of positives are detected by IIFT and still a higher number by ELISA, giving rise to rather low similarity percentages between the various results. A similar phenomenon was observed for rubella antibody determination with ELISA by Vejtorp (1978).

A definite answer to the question whether the higher number of positives detected by IIFT and even more by ELISA as compared with NT is the result of a higher sensitivity or a higher number of aspecific reactions will be difficult to obtain.

The correlation between the results of IIFT and ELISA is very good for CHIK antigen. The known broader cross-reactions displayed by WN antibodies with other arboviral antigens may be an explanation for the less favourable correlation between IIF and ELISA for WN virus.

The titers obtained in the ELISA were widely variable but in order to include all NT positives, results obtained at the 1 : 10 dilution had to be retained.

It might be worthwile to study the effect of pretreatment of sera for both the IIFT and ELISA in order to remove aspecificaly reacting substances.

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Evolution des méthodes d'immunofluorescence et d'immunoperoxidase pour la détermination des anticorps vis-à-vis des virus de la Fièvre Jaune, West Nile et Chikungunya.

Résumé — Les méthodes d'immunoflurescence indirecte (IFI) et immunoperoxidase (ELISA) pour la détection d'anticorps vis-à-vis des virus de la Fièvre Jaune (FJ), West Nile (WN) et Chikungunya (CHIK) ont été comparées avec la méthode d'inhibition de l'hémagglutination (IH) et le test de neutralisation (TN).

Les résultats positifs obtenus par le TN et l'IH pour les virus WN et CHIK ont été confirmés tant par l'IFI que l'ELISA.

Pour la FJ, tous les 7 TN positifs ont été confirmés par la méthode ELISA mais deux seulement par l'IFI.

Les méthodes ELISA et IFI fournissaient en général 2 à 7 fois plus de résultats positifs, que le TN.

Il est impossible de savoir à l'heure actuelle, si les résultats positifs, non confirmés par la neutralisation, sont spécifiques ou aspécifiques.

Evaluatie van immunofluorescentie en immunoperoxidase methodes voor de bepaling van Gele Koorts, West Nile en Chikungunya antistoffen.

Samenvatting — De indirekte immunofluorescentie (IIFT) en immunoperoxidase methode (ELISA) werden vergeleken met de haemagglutinatie inhibitie (HI) en neutralisatie test (NT) voor de bepaling van antistoffen tegen West Nile (WN), Gele Koorts (YF) en Chikungunya (CHIK) virussen.

De aanwezigheid van antistoffen tegen WN en CHIK virussen, bepaald met de NT en HI, werden allen bevestigd door zowel de IIFT als ELISA.

Voor het Gele Koorts virus werden alle (zeven) positieve NT resultaten bevestigd door ELISA, door de IIFT slechts twee.

ELISA en IIFT leverden in vergelijking met de NT 2 tot 7 maal meer positieve resultaten op.

De IIFT en ELISA zijn dus blijkbaar even specifiek als de NT. Het is momenteel onmogelijk uit te maken of het verhoogd aantal positieven, het resultaat is van specifiek of aspecifiek reagerende stoffen.

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