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Invited Article

APPLICATION OF COMPLEMENT C1q FOR SERODIAGNOSIS IN CLINICAL MICROBIOLOGY

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Clinical diagnostic technology developed in the theory and techniques from tests for the diagnosis of bacterial infection and eventually developed into a science almost exclusively during the last 20 to 30 years. The first microbiological diagnostic laboratory became just a part of central diagnostic test facilities, while clinical chemistry laboratory which developed later is equipped with fully automated instruments and recurrently occupied the core of central laboratory facilities.

Even in tests for infectious disorders, there have been some improvements and discoveries of new techniques. However, with the exception of recently developed new methods such as enzyme immunoassay, radioimmunoassay or in situ hybridization technique, almost no progress has been made in the general principles of serodiagnosis of infectious diseases.

I am currently in the development of new antigen and antibody detection system using a labelled complement component C1q. Fortunately, it has become possible to produce peroxidase labelled C1q of sufficiently good quality for use in research and clinical laboratories. Using this complement, it is possible to measure complement fixing antibody or virus neutralizing antibody rapidly,precisely and quantitatively. Fully - automated procedures for the diagnosis of viral infections become also possible in near future. Before going into the main part of the today's presentation, I would like to talk a little bit of the brief outline of the history of serology.

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The mini history of serology

As you may know, the founder of Kitasato Institute and our Kitasato University is Late Dr. Shibasaburo Kitasato. He succeeded in the pure culture of Clostridium tetani under the anerobic condition in 1889, and in the next year of 1890, he discovered that Clostridium tetani produced lethal exotoxin, and the antitoxin was present in the serum from the animals which were administrated with the stepwisely increased amounts of the toxin, and finally, that when serum containing the antitoxic antibody and the toxin are mixed together in vitro, the toxic activity of the exotoxin was neutralized with its immune serum. These great findings results in the establishment of the serum therapy. In 1891, Emil von Behring successfully treated diphtherial patients with immune serum after the Kitasato's advise, and then Behring received the first Nobel prize for his work in serum therapy, and immune serum was recognized as the first agent or medicine to be able to cure intractable diseases.

In 1897, Widal reported the serum of typhus patients agglutinated typhus bacteria. In 1901,Bordet and Gengou developed complement fixation test using fresh guinea pig serum as complement. In 1906, Wassermann applied the CF test for the diagnosis of syphilis. These steps led to the establishment of serodiagnosis for infectious diseases caused by bacterial and viruses. Therefore, serology started with the discovery of the neutralization of bacterial exotoxin with its antibody by Dr. S. Kitasato in 1889, and serology is a long standing with a history of one century among the biological sciences.

Insoluble problems for serological reactions

Modern sciences have developed remarkably in the last 30 years. Although serology has a history of 100 years, during the last 30 years, however, the principles concerning the measurement of antibody titers by the serological tests have remained almost unchanged. Insoluble problems remain in this field.

For the quantitative analysis of antibody present in patient's serum or immune serum, the general principle of the serological reaction is based on a continuous two-fold stepwise dilution of the sample. What is the reason for or purpose of this continuous two-fold

stepwise dilution of serum ? Is it really possible to measure the amounts of antibodies by means of dilution ? Is the two-fold increase of antibody titer from 16 to 32-fold really equivalent to the two-fold increase from 128 to 256-fold?

The problem involved in dilution can be explained more concretely using the assay of virus neutralizing antibody in the serum from two patients #1 and #2, as an example. We can assume that the serum samples are identically diluted to 32-fold, one unit volume of the diluted serum samples is mixed with an equal volume of a virus suspension, for example HSV as a model, and allow to neutralize virus. 90% of the virus is neutralized (10% of the virus remain unneutralized) with the serum #1 at the dilution of 32-fold, and 10% of the virus is neutralized (90% is unneutralized) with the serum from the patient #2 at the same dilution of 1:32. In these two cases, the amount of virus remaining unneutralized is absolutely different, i.e. 10% and 90%. However, when the remaining virus is allowed to replicate in cultured cells, the infected cells with the infecting virus which has actively replicated, and then induce the cytopathic effect (cell degeneration). As a result, the neutralizing antibody titer in both sera is evaluated as presumable 1:16 since neutralizing antibodies in both sera could not neutralized the virus at the dilution of 1:32.

Therefore, in the case of the amounts of neutralized virus is 0.1% or 99.9%, the same conclusion would be obtained just like an example of 100% of the virus. The reason of this is that this conventional method only evaluates whether or not unneutralized virus is present qualitatively and does not qualitate the amount of antibody. In spite of this defect, every textbook including the textbooks using in Chiang Mai University and also in my class that antibody titer can be determined by using two-fold stepwise dilutions of serum samples.

Reconsideration of antibody assay method

I am fully aware of the importance of quantitative determination of antibody titers, but I have strong doubt about the principle of antibody titration by means of serum dilution. Is it impossible to assay the precise amount of antibody directly, continuously, and ideally as the

number of antibody molecules without dilution of the samples ?

There are many substances which bind to IgG or IgM immunoglobulins such as, for example, concanavalin A of lectins, protein A of staphylococcus, rheumatoid factors, antibodies to IgG or IgM and complement. Among these, only complement binds to antibody immunoglobulins which have specifically reacted with its antigen and complement does not bind to intact antibody immune globulins. This indicates that complement can bind only to antigen-antibody complex.

Then, I firstly consider the possibility of the use of an enzyme-labelled complement for this purpose more than 10 years ago.

Complement and complement fixations

As already you know, complement is the complex serum protein with 20 different types of components from C1 to C9. These 20 proteins each react in the correct fashion. When C1 component first binds to antibody molecules fixed on its antigens, a chain reaction as far as C9 occurs on the immune complex. Therefore, if binding of the C1 component can be proven, complement fixation can be represented by the C1 binding reaction alone.

Meanwhile, the complement component C1 consists of three subcomponents of C1q, C1r and C1s. The C1q component of complement component C1 first binds to the immune complex. Therefore, I considered first labelled of the C1q with biological active substance such as peroxidase.

The C1q protein is a macromolecule with a molecular weight of about 400,000 which consists of a head portion in the shape of a glove with six fingers and a long handle shaped of a tail portion. Two of the six fingers of one molecule of C1q bind with two molecules of IgG at the site of Fc portion in the C_{H2} domain.

The tail portion of C1q is a very special collagen like protein in which three different peptide chains are united by several S-S bonds. These S-S bonds are presented only in the end of the tail portion. These S-S bonds of C1q are reduced by the treatment with mild reducing reagent and certain enzyme is inserted in the open bond. Hence, it becomes possible to preferential label only the end portion of C1q molecule with the enzyme. As a result, peroxidase labelled C1q (designated as to P*-C1q) was synthesized by binding one molecule of peroxidase to one

molecule of C1q protein.

The use of this active type of P*-C1q as a biological sensor for the detection of immune complex was investigated. I will show you the examples of application of P*-C1q in the measurement of CF antibody and virus neutralizing antibody, and also identification and typing of herpes simplex virus.

A. ELISA-CF

Objective

Since the introduction of CF test in 1901 by Bordet and Gengou, the determination of CF antibody titers has been based conventionally upon the indirect principle that complement is consumed by its binding to antigen-antibody complex and becomes thereafter unavailable for the hemolysis of sheep erythrocytes by hemolysin. Development of a direct, single-stage CF test acceptable to all investigators has been an ideal yet to be achieved. In view of the fact of sequential binding of complement components, we prepared active enzyme-labelled C1q, which is the first complement component binding to immune complex, and thereby have developed a new CF test system, tentatively designated as ELISA-CF, for the rapid and quantitative determination of CF antibodies to viruses.

Materials and Methods

1. **C1q** (Figure 1)

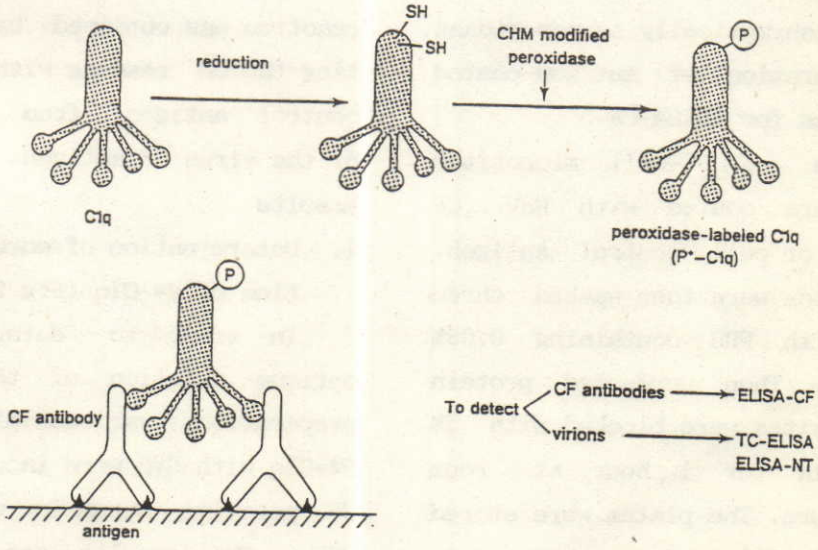

Figure 1 Schematic representation of peroxidase-labelled C1q and its application to diagnosis of viral infections.

C1q subcomponent of component was isolated from goat serum and purified by cation exchange chromatography, and then by gel filtration. The fractions containing C1q protein were reduced with dithiothreitol and allowed to react with horseradish peroxidase which had been modified with maleimide, a cross-linking reagent.

Biological activities of peroxidase-labelled C1q, designated as P*-C1q, were confirmed by demonstrating its functions as follows :

1) hemolytic activity as a mixture of P*-C1q and C1q-depleted guinea pig serum to sheep erythrocytes,

2) binding affinity for BSA and anti-BSA complex,

3) enzymatically as peroxidase.

2. **Preparation of antigen-coated plates for ELISA-CF**

Wells of 96-well microtiter plate were coated with HSV CF antigen or cell control antigen. The plates were then washed three times with PBS containing 0.05% Tween-20. Then unreacted protein binding sites were blocked with 5% skim milk for 1 hour at room temperature. The plates were stored at -20°C until used.

3. **Procedure of ELISA-CF**

Fifty microliters of heat-inactivated serum samples and an equal volume of P*-C1q were diluted with GVB and then added simultaneously to each well. Positive or negative control sera were always included on all plates. The plates were then incubated for 90 minutes at room temperature. After washing all the wells 3 times with Tween-PBS, 100 microliters of a substrate solution (0.04% ABTS containing 0.175% H_2O_2 in citrated buffer at pH 4.0) were delivered to each well, followed by incubation for 30 minutes at room temperature in the dark. The enzyme reaction was stopped by addition of 0.01% sodium azide. The color developed was measured photometrically at a 414 nm.

The ELISA-CF value for specific reaction was obtained by subtracting the OD reading with the cell control antigen from OD reading of the virus CF antigen.

Results

1. **Determination of working dilution of P*-C1q** (Fig 2)

In order to determine the optimum dilution of the P*-C1q preparation, various dilution of P*-C1q with GVB were incubated with CF positive serum in wells with HSV. The results are shown in Figure 2 and indicate that a linear relationship between CF titers by

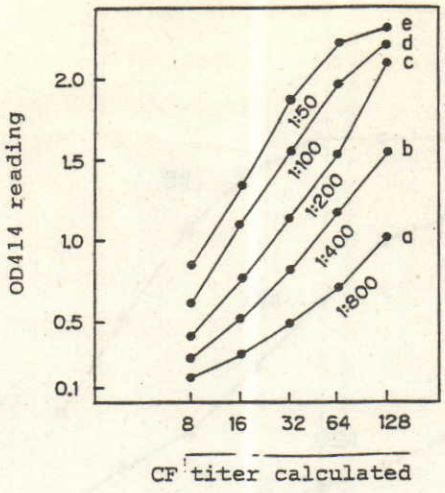

Figure 2 Determination of working dilution of peroxidase-labelled C1q (P*-C1q)

conventional CF test and OD reading by ELISA-CF test was observed primarily at a dilution of 1:200 of the P*-C1q solution. Thus a working dilution of 1:200 was chosen for P*-C1q in subsequent experiments.

With constant concentration of P*-C1q and HSV antigen, the enzyme activity of P*-C1q bound was dependent upon the dilution factors of anti-HSV immune serum. This confirmed the suitability of the P*-C1q for the determination of CF titers of antibodies.

2. **Determination of working dilution of HSV antigen** (Figure 3)

In order to determine the working dilution of HSV antigen prepared, HSV CF antigen at CF titer of 1:40 was serially diluted from 1:5 to 1:40. Blocked titration analysis of HSV antigen against CF antibody positive human serum was performed.

Human serum with a HSV CF titer of 1:32 was diluted serially from 1:50 to 1:3200 for each dilution of the antigen. As seen in Figure 3, as the maximum slope of linear regression between OD reading and serum dilution was observed at a dilution of 1:10 of the HSV antigen, for further experiments the dilution of the HSV antigen was fixed at 1:10 for coating plates.

3. **Optimum dilution of serum samples** (Figure 4)

As shown in Figure 3, most linear color development was obtained when the plates had been

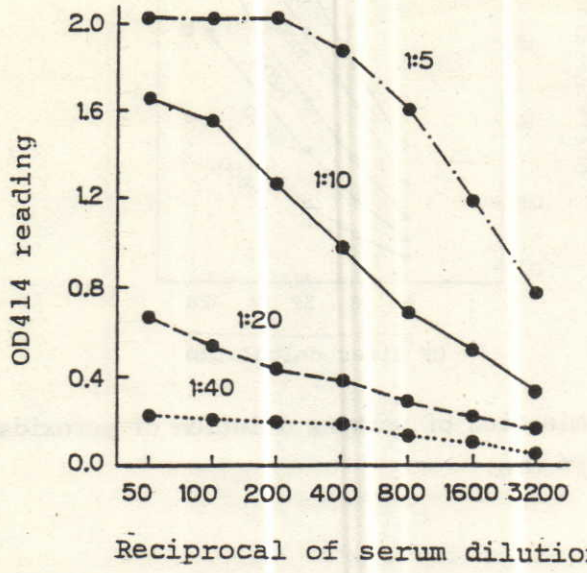

Figure 3 Assessment for optimal dilution of HSV antigen.

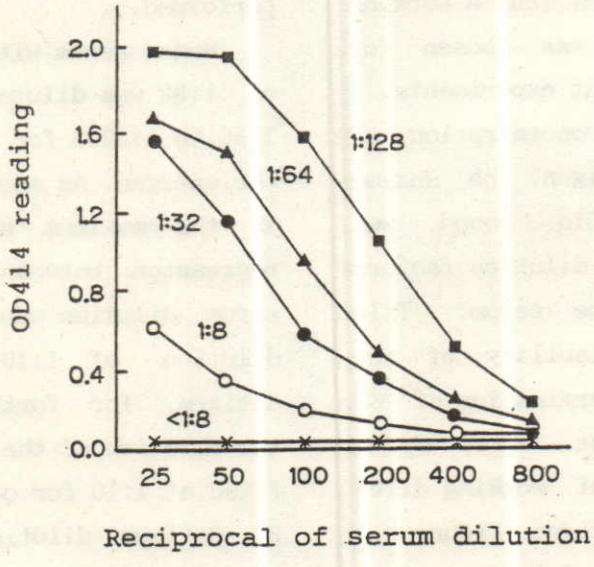

Figure 4 Correlation between dilution and OD reading of antibody-positive sera.

coated with 4 CF units of the HSV antigen and reacted with the P*-C1q at 200-fold dilution.

Then, the optimum dilution of serum samples was determined by camparison of titration curves of human sera with various CF titers to HSV. As shown in Figure 4, virtually linear regression relationship were observed in the case of antibody positive sera from 1:8 to 1:128. On the other hand, no specific reaction was observed with antibody negative serum through the serum dilutions from 1:25 to 1:800 and the color development case at higher serum concentration.

Thus, a dilution factor of 1:50 was considered to be most appropriate for serum dilution.

4. **CF titers and OD reading in the detection of antibody to HSV (Figure 5)**

Experiments were performed to know the correlation between CF titer and OD reading in the detection of antibody to HSV. The relationship of OD reading in ELISA-CF test to conventional antibody titers was explored in 38 human sera varying in CF titer. With elevation of CF antibody titer plotted as abscissa, as shown in Figure 5, the OD reading showed an increase. These two parameters were found to have a high degree of direct correlation with a coefficient of 0.94.

5. **Relationship of CF titer and OD reading in the detection of antibody to VZV (Figure 6)**

ELISA-CF tests were performed on 24 human serum specimens in wells of microplate coated with VZV in the same manner as HSV, and a similar data analysis was made through the sera. Here again, a high degree of direct correlation was noted between CF antibody titer and OD reading with a coefficient of 0.94 although the OD reading showed some variation.

6. **Relationship of HI titer and OD reading in the detection of antibody to rubella virus (Figure 7)**

We also explored the relationship of OD reading in ELISA-CF test for antibody to rubella virus and conventional HI antibody titers in 24 human sera with various HI titers. The ELISA-CF was carried out on 1:50 dilution of test sera as in the detection of antibody to HSV. As can be seen, their correlationship had a coefficient of high as 0.94. However, no detectable color developed in the ELISA-CF with sera having HI antibody titers of 1:32 or lower.

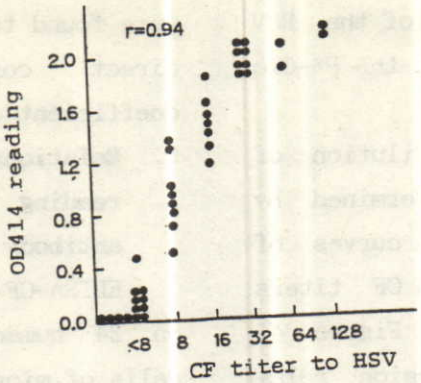

Figure 5 Correlation between CF antibody titer to herpes simplex virus and OD reading

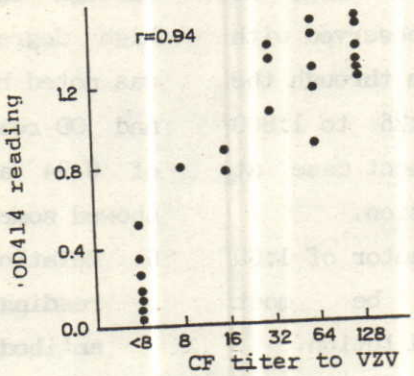

Figure 6 Correlation between CF antibody titer to Varicela-zoster virus and OD reading.

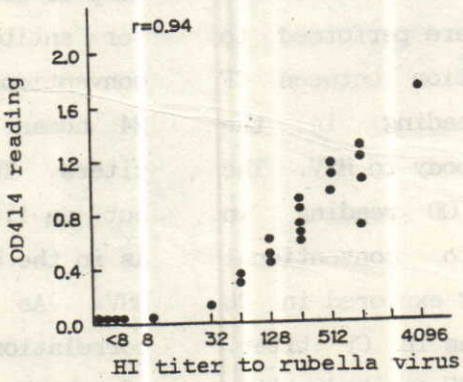

Figure 7 Correlation between HI antibody titer to rubella virus and OD reading.

The good correlation between OD reading in ELISA-CF test and conventional CF or HI antibody titer observed in these studies indicate that the OD reading might be converted to expression in conventional CF antibody titer. In view of this, a standard calibration curve was constructed by plotting ELISA-CF OD reading of reference sera against their known conventional CF antibody titers so that CF antibody titers of test sera, herein after referred to as ELISA-CF titer, could be obtained from OD reading by reference to the standard curve.

7. **Relationship of conventional CF antibody titer and ELISA-CF titers (Figure 8)**

From OD readings in ELISA-CF tests performed on 40 human sera with different CF titers of antibody to HSV, their ELISA-CF titers were obtained by reference to the standard curve. In Figure 8 showed the results, data obtained with 50-fold dilutions of the sera are represented in Chart A, and those with 200-fold dilutions in Chart B. There was a sharp distinction between sera with CF titers lower than 1:8 and those with 1:8 or higher when the sera were used a 50-fold dilution in the Chart A.

When the ELISA-CF was carried out on sera diluted 200-fold, nevertheless, usual subtle differences in antibody titer by the conventional CF test were noted to be expressed as more obvious difference in ELISA-CF titer.

The correlation coefficient therefore at serum dilution of 50 or 200-fold were 0.95 and 0.97, respectively. The ELISA-CF titer obtained from the standard curve showed a concordance rate of 83% with the conventional CF titer at a serum dilution of 1:50 in the Chart A, and 75% at a serum dilution of 1:200. Furthermore, there were no 2-fold or greater discrepancies in antibody titer between conventional CF and ELISA-CF at serum dilution of 1:50.

The data heretofore presented demonstrate that, by reference to a standard calibration curve, an OD reading in ELISA-CF can be converted to an expression in conventional CF antibody titer and that, for a serum showing a conventional CF titer of 1:16 for example, a titer in finer detail between 1:12 and 1:24 is obtained by ELISA-CF.

8. **Virus specificity of the reaction in ELISA-CF**

An attempt was made to ascertain whether the antibody

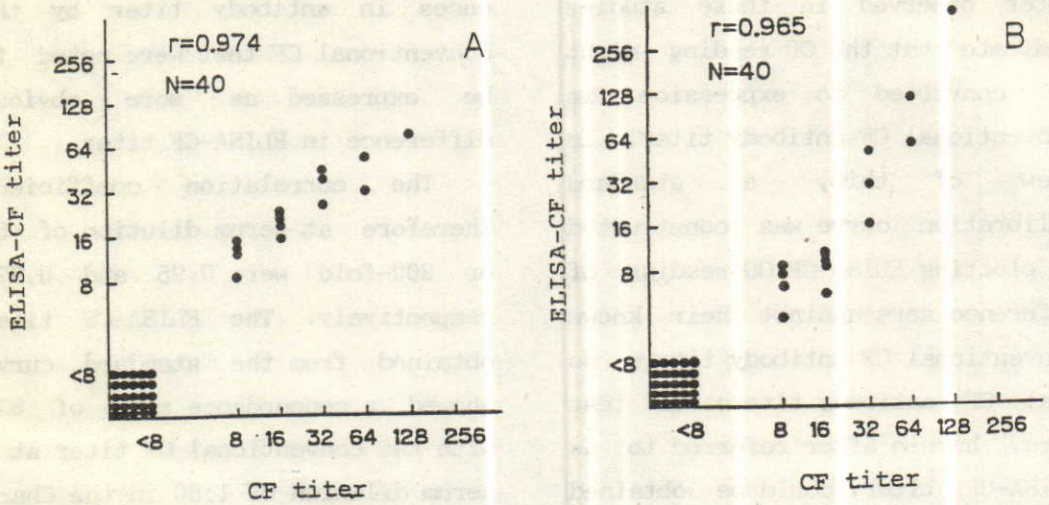

Figure 8 Correlation between CF antibody titer to herpes simplex virus and ELISA-CF titer.

detection by ELISA-CF might be specific of viral antigen. Wells coated with HSV, VZV or CMV were set up, and ELISA-CF tests of sera with known CF titers of 1:8, 1:16 or 1:64 to HSV and free of antibody to the other two viruses were run on these wells to explore virus specificity of the reaction. The test included a serum with a CF titer of 1:64 to VZV and a serum with a titer of 1:64 to CMV. As seen in Figure 8, the sera positive for CF antibody to HSV showed reactions with HSV alone, hence no cross-reaction with the other viruses.

The present investigation has thus demonstrated the ELISA-CF to be a new test system for quantitative detection of antibody to virus, particularly of CF antibody, in the serum of patients with viral infections.

B. **ELISA TC-ELISA**

Objective

We have devised a simple method for rapid identification and serotyping of HSV using commercially available anti-HSV immune sera, hereinafter referred to a TC-ELISA. This presentation briefly describes the new assay system.

Materials and Methods

1. **Materials**

(1) The 2 laboratory reference strains, HF and McIntyre, were used as type 1 standard strains. As type 2 standard strains, strain 196 and UW-268 were employed. Besides 8 HSV type 1 clinical isolates and 14 type 2 isolates were also tested. All these strains had been typed according to cleavage pattern of viral DNA with restriction enzymes as well as by immunofluorescence with monoclonal antibodies to HSV.

(2) Commercially available immune sera to HSV were employed in this study. The anti-HSV type 1 serum was a product of Denka Seiken, for CF test, prepared in guinea pigs. It had a CF antibody titer of 1:64. The anti-HSV type 2 immune serum was obtained from Dako immunoglobulins, Copenhagen; it was a rabbit antiserum for neutralization test. The serum had a neutralizing antibody titer of 1:1024 against homologous type 2 virus and titer of 1:256 against heterologous antigen.

2. **Principle of TC-ELISA**

HSV is propagated, for amplification of viral antigens, over a short duration in Vero cells grown in 96-well microtiter plates. The infected cells are fixed with methanol as soon as a mild degree of CPE has appeared. The test system is based on the principle that HSV antigens are detected and quantitated by one-step ELISA-CF test by incubation with simultaneously added commercial anti-HSV polyclonal immune serum and peroxidase-labelled C1q.

3. **Procedure of TC-ELISA**

(1) HSV is inoculated onto Vero cells.

(2) The plate is incubated at 37 °C for 24 hours or longer, if necessary.

(3) As the cells have become to exhibit a 10% or greater CPE, they are fixed with methanol.

(4) 50 microliters of anti-HSV immune serum and an equal volume of P*-C1q solution are then pipetted directly into each well without prior blocking treatment, and the plate is incubated for 60 minutes.

(5) 100 microliters of a colorgenic substrate solution (ABTS) are

delivered to each well, followed by a further 60 minutes incubation.

(6) The enzymatic activity of P*-C1q bound is measured photometrically with an Immuno Reader at a wavelength of 414 nm.

(7) The absorbance of the mixture in the well of uninfected cells is subtracted from that of the mixture in the well of virus infected cells to obtain the viral antigen specific reaction.

Results

1. **Working dilution of anti-HSV immune serum (Figure 9)**

Cultures of Vero cells were incubated with 100 PFU/well of the HF strain of HSV type 1 and incubated for 24 hours. As 40 to 60% CPE developed in the cultures, appropriate dilutions of antiserum to HSV-1 and HSV-2 were added respectively to wells and the OD reading of each well content was determined.

This picture shows typical results of experiment. From these data it was concluded to adopt a dilution factor 1:15 for Denka anti-HSV-1 and a dilution factor of 1:100 for Dako anti-HSV-2 immune serum in subsequent experiments.

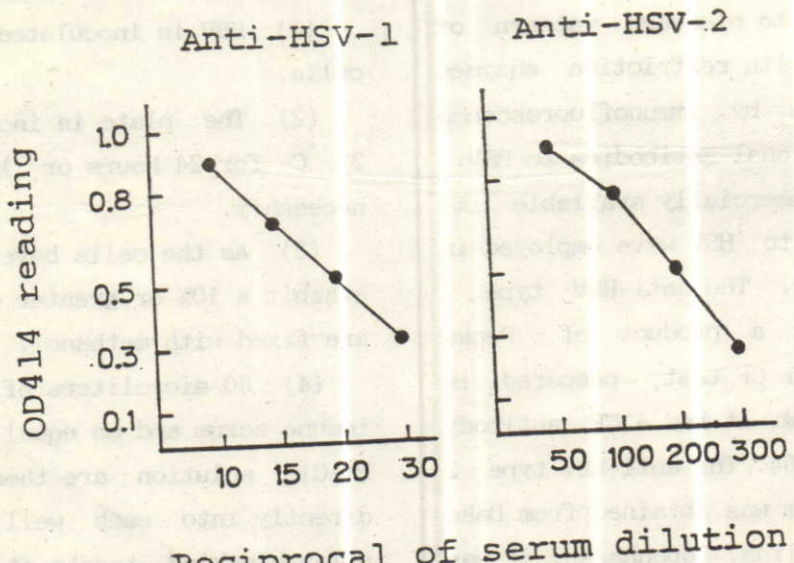

Figure 9 Working dilution of anti-HSV immune sera.

2. Detection of HSV antigen by TC-ELISA (Table 1)

Cultures of Vero cells and MRC-5 cells were inoculated with 100 PFU/well of HSV-1 HF strain of HSV-2 strain 196, followed by incubation for 24 and 72 hours, respectively. Typical findings are presented in this slide.

As seen in column B of this slide, uninfected cell controls showed OD reading of 0.23 or lower. Viral controls of both Vero cells and MRC-5 cells with pronounced CPEs produced by VZV, CMV, measles virus, and mumps virus showed OD reading of less than 0.3. hence essentially the same as the uninfected cell controls.

As seen in column A, a remarkably intense color of 10 or greater OD reading developed with antisera, whether anti-HSV-1 or anti-HSV-2, in cultures infected with HSV-1 HF strain. In cultures of cells infected with HSV-2, on the other hand, OD readings greater than 1.0 were obtained with anti-HSV-2 immune serum. But OD readings were as low as less than 0.37 with anti-HSV-1 immune serum.

The difference in absorbance, that is, intensity of coloration was so obvious that they could be readily appreciable with the naked eye.

Table 1 OD reading of reaction of HSV antigen by TC-ELISA.

Virus	Host cell	with anti-HSV-1 serum (A)	(B)	(A-B)	with anti-HSV-2 serum (A)	(B)	(A-B)
HSV-1	Vero	1.19	0.23	0.96	1.34	0.21	1.13
(HF)	MRC-5	1.03	0.13	0.89	1.09	0.10	0.99
HSV-2	Vero	0.35	0.12	0.12	1.00	0.15	0.85
(196)	MRC-5	0.37	0.13	0.24	1.22	0.10	1.12
VZV	Vero	0.27	0.27	0	0.21	0.18	0.03
CMV	MRC-5	0.06	0	0.06	0	0	0
Measles	Vero	0.30	0.23	0.07	0.23	0.15	0.08
Mumps	Vero	0.29	0.23	0.06	0.21	0.15	0.06

A = Virus infected cell; B = Control cell, A - B = Specific reaction;
Anti-HSV-1 serum : Denka, 1:15 Anti-HSV-2 serum : Dako, 1:100

The data presented in Table 1 suggested applicability of the TC-ELISA system for specific detection of HSV by using Dako anti-HSV-2 immune serum, and for simple differentiation of type 1 from type 2 HSV with the use of Denka anti-HSV-1 immune serum.

2. **Comparison of HSV types 1 and 2 in TC-ELISA (Table 2)**

Reactivity of HSV with immune sera to HSV-1 and HSV-2 was assesses by TC-ELISA. The OD reading of uninfected cell controls showed some inter-assay variations. In view of this, the HSV types 2/1 reactivity index was calculated by dividing the OD reading of wells with anti-HSV-2 immune serum by that of wells with anti-HSV-1 immune serum.

Using this index as a parameter, we compared reactivity with antisera in TC-ELISA between type 1 and type 2. The type 2/1 index of OD reading of the uninfected cell controls was consistently between 0.6 and 1.0. Cultures infected with the HF strain and MacIntyre of HSV-1 showed invariable indicies of 2.1 or lower in all instances, whereas the index was usually 3.5 or higher and in no instance below 3.0 in cultures infected with HSV-2 reference strains.

Thus the experiments demon-

Table 2 Comparison of HSV index* with commercial antisera in TC-ELISA between HSV type 1 and type 2

Virus	Vero cells		MRC-5 cells		Index Range**
	Exp.1	Exp.2	Exp.1	Exp.2	
HSV-1 : HF	1.4	1.5	0.8	1.1	0.8-1.5
MacIntyre	2.1	2.1	1.7	1.8	1.7-2.1
HSV-2 : 196	4.1	3.5	3.6	4.7	3.5-8.6
UW-268	3.5	3.0	3.0	4.8	3.0-4.8
Cell control	0.8	0.6	1.0	0.8	0.6-1.0

* Index = OD with anti-HSV-2 serum/OD with anti-HSV-1 serum

** Range of values obtained for the index in 10 replicate determination.

strated that typing of the laboratory strains of HSV might be feasible by the TC-ELISA system employed the Denka and Dako immune sera.

4. **Typing of HSV clinical isolates by TC-ELISA** (Table 3,4,5)

Typical results of tests run in duplicate are presented in this slide. All HSV-1 isolates exhibited remarkably proximate indices, being invariable 2.1 or lower as was the case of the HSV-1 reference laboratory strains.

As can be seen in the next slide, the index was 3.1 or higher for all clinical isolates of HSV-2 but varied considerably among these isolates, compared to the HSV-1 isolates.

The difference in intensity of color developed between the types of the virus was so distinct that it could be readily noted with the naked eye, prior to photometric determinations.

Table 3 Typing of HSV by TC-ELISA with commercial immune sera.

Virus		Index 1	Index 2	Range
Non-infected cells		0.8	0.6	0.6-0.9
Laboratory strains				
HSV-1	HF	1.4	1.2	
	MacIntyre	2.1	1.8	0.6-2.1
HSV-2	196	4.1	8.3	
	UW-268	3.5	4.8	3.5-8.4
Clinical isolates				
	#104	1.4	1.8	
	#153	1.5	1.6	
	#212	1.4	1.6	
	#264	1.5	1.5	
	#265	1.7	2.0	
	#287	1.7	1.6	
	#289	1.6	1.5	
	#377	1.9	1.8	1.4-2.1

* All HSV type 1 was identified by restriction endonucleases.

Table 4 Typing of HSV by TC-ELISA with commercial immune sera.

Virus		Index 1	Index 2	Range
Non-infected cell		0.8	0.6	0.6-0.9
Laboratory strains				
HSV-1	HF	1.4	0.6	
	MacIntyre	2.1	1.8	0.6-2.1
HSV02	196	4.1	8.3	
	UW-268	3.5	4.8	3.5-8.4
Clinical isolates*				
	YNO	5.6	3.7	
	YMS	7.1	3.1	
	MA	3.8	3.5	
	IBI	4.7	4.0	
	MD	6.2	NT	
	NEA	5.9	NT	
	NEB	6.0	NT	
	K29	7.1	NT	
	UD5	5.2	NT	
	UD112	4.6	NT	
	MS-1325	8.3	31.5**	CPE 30%
	MS-1420	6.5	31.2**	CPE 30%
	MS-1437	7.3	9.7	
	MS-1476	7.2	8.3	3.1-31

* All these isolates were HSV type 2 as determined by viral DNA cleavage pattern with restriction enzyme and also by immunofluorescent microscopy with HSV type specific monoclonal antibody.

Table 5 Relationship between type 2/type 1 index and HSV serotype.

	Index range
Non-infected cell control	0.6-0.9
HSV-1 : 2 Laboratory strains	0.6-2.1
8 Clinical isolates	1.4-2.1
HSV-2 : 2 Laboratory strains	3.5-8.4
14 Clinical isolates	3.1-31

Table 6 Detection limit of HSV by TC-ELISA.

Virus dose		Incubation period (hours)		
PFU/ml	PFU/well	24	48	96
1000	50	+	+	+
500	25	+	+	+
100	5	-	+	+
20	1	-	+	+
10	0.5	-	-	±
2	0.1	-	-	±

5. **Lower limit of sensitivity of TC-ELISA in the detection of HSV (Table 6)**

Experiments were conducted to explore the lower limit of sensitivity of TC-ELISA for detection of HSV. Results of TC-ELISA performed on the plates are summarized in Table 6.

Cultures of cells infected with 500 PFU/ml (equivalent to 25 PFU/well) of HSV and incubated for 24 hours showed positive reactions. When incubated for 90 hours after inoculation with 20 PFU/ml (equivalent to 1 PFU/well), cultures also showed positive tests.

These findings indicate that

Table 7 Characteristic advantages of TC-ELISA

1.	Rapid	: Virus antigen amplification for 24 hours, and identification & typing for 3 hours
2.	Simple	: Commercial immune serum, no blocking treatment, one-stage ELISA
3.	Sensitive	: 20-400 PFU/ml for 24-96 hour incubation periods
4.	Specific	: HSV specific, no cross reaction to VZV or CMV
5.	Reproducible	: Remarkably good

detection and typing of HSV can be accomplished by TC-ELISA in so far as cells are inoculated with a minimum of 1 PFU of HSV per well.

Summary and Conclusion

This new TC-ELISA system employing P*-C1q and commercially available antisera to HSV has the advantages of facilitating detection of an extremely small amount HSV, for example, 20 PFU/ml and of rapid measurement of neutralizing permitting both identification and typing if HSV simultaneously with commercial immune sera by a procedure that can be complete within 3 hours. (Table 7)

The TC-ELISA system we have developed is applicable also for rapid detection and identification of non-cytopathic viruses such as rubella virus, AIDS causing virus, as well as for a rapid measurement also non-cytopathic viruses. Thus the assay system has potential usefulness as a rapid diagnostic test for viral infections.

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