

Evaluation of locally developed ELISA for determining anti-CMV IgG antibody by comparison with two commercial ELISA kits.

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An indirect enzyme-linked immunosorbent assay for detection of anticytomegalovirus IgG antibody (anti-CMV IgG) was locally developed (ELISA-D). Evaluation of ELISA-D was done by comparison with the commercial available ELISA kit from Organon Teknika (ELISA-O), which is an antibody capture sandwich system, and Behringwerk (ELISA-B), which is an indirect detection system similar to ELISA-D. Compared to ELISA-O, ELISA-D had a sensitivity of 100%, a specificity of 94.44% with positive and negative predictive values of 98.67% and 100% respectively. In comparison to ELISA-B, ELISA-D had a sensitivity of 100%, a specificity of 89.47% with positive and negative predictive values of 97.33% and 100%, respectively. There were 3 out of 92 sera giving discrepant results, i.e., all 3 were positive by ELISA-D while 2 were positive by ELISA-O and one was positive by ELISA-B.

These results demonstrated that ELISA-D, which was developed in-house, produced results comparable to ELISA-O and ELISA-B.

Key words : anti-CMV IgG, ELISA

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ทำการพัฒนาวิธีการตรวจหาแอนติบอดีจำเพาะชนิดอิมมูโนโกลบูลินต่อไซโตเมกกาโลไวรัส (anti-CMV IgG) โดยวิธี Indirect enzyme linked immunosorbent assay (ELISA-D) ภายในห้องปฏิบัติการ และทำการเปรียบเทียบประเมินคุณภาพของวิธี ELISA-D กับชุดน้ำยาสำเร็จรูปที่ผลิตจากต่างประเทศ 2 ชุด คือ ชุดของบริษัท Organon Teknika (ELISA-O) ซึ่งมีหลักการ antibody capture sandwich และชุดของบริษัท Behringwerk (ELISA-B) โดยมีหลักการเหมือนวิธี ELISA-D ผลการทดสอบพบว่า วิธี ELISA-D เปรียบเทียบกับวิธี ELISA-O มีความไวร้อยละ 100 ความจำเพาะร้อยละ 94.44 ผลบวกที่ถูกต้องร้อยละ 98.67 และผลลบถูกต้องร้อยละ 100 เมื่อเปรียบเทียบกับวิธี ELISA-B มีความไวร้อยละ 100 ความจำเพาะร้อยละ 89.47 ผลบวกถูกต้องร้อยละ 97.33 ผลลบถูกต้องร้อยละ 100 จากตัวอย่างนำเหลืองทั้งสิ้น 92 ตัวอย่าง มี 3 ตัวอย่างที่ให้ผลไม่สอดคล้องกัน คือวิธี ELISA-D ให้ผลบวกทั้ง 3 ตัวอย่าง วิธี ELISA-O ให้ผลบวก 2 ตัวอย่าง และวิธี ELISA-B ให้ผลบวกเพียง 1 ตัวอย่าง

ผลการวิจัยนี้แสดงให้เห็นว่า วิธี ELISA-D ที่พัฒนาขึ้นสามารถใช้ในการตรวจหา anti-CMV IgG ได้ผลดีพอๆกับชุดน้ำยาสำเร็จรูปจากต่างประเทศ

Cytomegalovirus (CMV) or human herpesvirus 5 (HHV-5) produces a wide variety of clinical manifestations in infected individuals, for example salivary gland disease, cytomegalic inclusion disease in newborn infants, post-perfusion syndrome, pneumonia in immunocompromised hosts etc. (1,2) Additionally, CMV is also important in transplantation cases since CMV-seronegative organ and bone marrow transplant recipients who receive CMV-positive blood products have a significant risk of contracting a severe infection of CMV.(3,4) Determination of CMV immune status is important for reducing CMV transmission, especially in the groups at risk to acquire the infection via blood products.

Anti-CMV IgG antibody can be detected within 1 to 2 weeks after the onset of acute primary infection and persists for months to years.(5) There are many methods to identify anti-CMV IgG in human sera such as a complement fixation test,(6) an indirect immunofluorescent assay and anticomplement immunofluorescence test,(7) radioimmunoassay, and enzyme linked immunosorbent assay (ELISA).(8,9) However, ELISA is an attractive method since it is rapid and has high sensitivity and specificity.

In this study, an ELISA kit was developed (ELISA-D) and it was evaluated by comparison to 2 well-known ELISA kits (Vironostika, Organon Teknika and Enzygnostic, Behringwerk).

Materials and Methods

Cells

MRC-5 cells were grown in Eagle minimal essential medium containing 10% fetal bovine serum (Gibco, France), penicillin (100 unit/ml) and streptomycin (100 ug/ml).

CMV antigen

The CMV infected cell lysate (CIL) was prepared. Briefly, MRC-5 cells were infected with standard CMV strain AD 169 and kept incubated at 37 C until the cytopathic effect reached >75% of the monolayer, usually 48-72 hours after infection. The cells were washed twice with phosphate-buffered saline (PBS), pH 7.5, resuspended at 5×10^6 cells/ml in PBS containing 0.5 mM phenylmethylsulfonyl fluoride and disrupted by sonication for 40 seconds. The supernatant (CIL) was collected after centrifugation at 1500 xg for 15 minutes and kept aliquot in -70 C. The amount of protein was quantitated by Lowry's method.(10)

MRC-5 antigen

The uninfected MRC-5 cell lysate (UIL) was also prepared similar to CIL, but the cells were not inoculated

with CMV (AD 169).

Specimens

A number of 92 sera sending to the Viral Laboratory Unit, Chulalongkorn Hospital were randomly selected. All of them were requested for anti-CMV IgG antibody.

ELISA-O

The Vironostika kit assay for anti-CMV IgG purchased from Organon Teknika, Germany is an antibody capture sandwich system. In brief, the CMV antigen was captured by an anti-CMV antibody already coated on well. Diluted serum (1:100) was added to react with the bound CMV antigen. Then the immunocomplexes were detected by anti-human IgG labelled with alkaline phosphatase and substrate (tetramethylbenzidine hydrochloride; TMB). The enzymatic reaction of the test was determined by use of a photometer of Organon Teknika.

ELISA-B

The Enzygnostic kit produced by Behringwerk, Germany is an indirect system. The diluted serum (1:100) reacted to CMV antigen which directly coated on well, and the bound materials were detected by enzymatic reaction similar to those described in ELISA-O. The tested serum also reacted to normal cell antigen which acted as a control well and run parallelly. This kit was kindly provided by Hoechst Thai Ltd.

ELISA-D

The system was developed to be similar to ELISA-B. CMV antigen as CIL was produced in-house and used as the coating substance. Checkerboard titration, as described by Voller et al,(11) was used to optimize the amount of coating antigen and detecting antibody (Rabbit anti-human IgG labelled horseradish peroxidase, Dakopatts, Denmark).

The ELISA-D was placed in a 96-Well Microtiter plate (Nunc Immuno Maxisorp F16, Denmark). The wells were coated with 2.5 ug per 100 ul of CIL or UIL in carbonate buffer, pH 9.6 and stored at 4 C overnight (16-18 hours) and washed off with PBS. The unbound sites were saturated with 150 ul per well of 2% bovine albumin (BSA fraction IV, Sigma, USA) for 1 hour at 37 C. After washing with PBS containing 0.1% Tween 20 (Sigma), 100 ul per well of diluted serum (1:800) was added and kept for 1 hour at 37 C. The bound antibodies were detected by 100 ul per well of rabbit anti-human IgG labelled horseradish peroxidase (1:1000) at 37 C for 1 hour and 100 ul of 0.67% 1,2-phenylenediamine dihydrochloride (Dakopatts) with 0.014% H₂O₂ as substrate for 30 minutes at room temperature. The enzymatic reaction was terminated by the addition of

* IgG = Immunoglobulin G.

100 μ l of 4 N H₂SO₄ and determined by photometer. The serum was considered positive when the difference between the optical density (OD) of CIL reacted well, and that of UIL reacted well, was greater than 0.2.

Results

A total of 92 sera were tested for anti-CMV IgG using the 3 different systems. One was ELISA-D which was developed in our laboratory while the other 2 were commercial ELISA kits, i.e., Vironostika anti-CMV IgG purchased from Organon Teknika, Germany (ELISA-O) and Enzygnost anti-CMV IgG provided by Behringwerk, Germany (ELISA-B). The results are shown in Table 1. Of

the 92 sera, 75 possessed anti-CMV IgG as determined by ELISA-D. Only 74 and 73 sera could be detected by use of ELISA-O and ELISA-B, respectively. The two negative sera determined by ELISA-B were both positive by ELISA-D and ELISA-O while one negative serum of ELISA-O was positive by both ELISA-D and ELISA-B (Table 2). The sensitivity of ELISA-D in comparison to ELISA-O and ELISA-B was 100% but specificity was 94.44% and 89.47%, respectively (Table 3). The positive predictive value (PPV) of ELISA-D compared to ELISA-O (98.67%) was higher than that compared to ELISA-B (97.33%). Contrary to negative predictive values (NPV), they were the same (100%) (Table 3).

Table 1. Comparison of the results of ELISA-D with ELISA-O and ELISA-B.

		Numbers of sera, ELISA-D/ELISA-O				total
		+/+	+/-	-/+	-/-	
ELISA-B	+	72	1	0	0	73
	-	2	0	0	17	19

Table 2. Samples with discrepant results.

sera nos	(OD test/OD cut off)		
	ELISA-D	ELISA-O	ELISA-B
147	positive (0.62/0.2)	positive (0.3/0.28)	negative (0.17/0.2)
155	positive (0.41/0.2)	positive (0.35/0.28)	negative (0.18/0.2)
312	positive (0.61/0.2)	negative (0.23/0.28)	positive (0.65/0.2)

Table 3. Relative ELISA-D performance.

Assay	Performance of the ELISA-D relative to the indicated assay			
	sensitivity	specificity	PPV	NPV
ELISA-O	100	94.44	98.67	100
ELISA-B	100	89.47	97.33	100

PPV: positive predictive value

NPV: negative predictive value

Discussion

In this study, a locally developed indirect ELISA (ELISA-D) kit was evaluated for its ability to detect anti-CMV IgG antibody by comparison to two ELISA kits, i.e., ELISA-O and ELISA-B (see Materials and Methods). ELISA-D and ELISA-B are based on the same principle while ELISA-O is a capture sandwich system. All 92 sera were assayed using these 3 methods and the results are shown in Table 1. Three samples with discrepant results were detected and displayed in detail (Table 2). One sera (no.312) was positive when the detecting system was indirect ELISA (both ELISA-D and ELISA-B) while use of the antibody capture sandwich system was negative. This result seems to indicate that the sensitivity of the indirect method is greater than the capture sandwich method. In contrast, the other two sera (nos. 147 and 155) gave unexpected results (Table 2). Both of them were determined positive by ELISA-D as well as by ELISA-O but not by ELISA-B. When the optical density (OD) values of the tests were considered, they were border-line (near cut-off value, Table 2). Unfortunately, none of these 3 samples with discrepant results were repeated by any assay. Therefore, the resolution could not be solved in the present study. Even though the results were still slightly controversial, the sensitivity and specificity of ELISA-D was acceptable when compared to either ELISA-O or ELISA-B. The data in Table 3 indicated that the performance of ELISA-D was quite good relative to that of ELISA-O and comparable to that of ELISA-B. Thus, all results presented here clearly support that the locally developed ELISA can be used reliably to determine the anti-CMV IgG antibody in the laboratory.

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