DEVELOPMENT AND ASSESSMENT OF ELISA FOR SERODIAGNOSIS OF HIV-INFECTION

P Thongkrajai and R Kaewmart

Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract. A home-made ELISA for serodiagnosis of HIV-infection was developed. It made use of the HIV viral lysate to coat on ELISA microplates. The purpose was to establish an ELISA for serodiagnosis of HIV-infection. The newly-developed ELISA, "H-ELISA", was applied to test 792 samples of HIV-postitive serum as confirmed by Western blot. All 792 samples were positive by H-ELISA. It was also applied to test 540 samples of normal sera obtained from different laboratories in Srinagarind Hospital. A number 530 normal sera was negative, 8 samples were positive and cofirmed by Western blot and 2 samples were false positive. It was concluded that the H-ELISA possessed 100% sensitivity with a false positive rate of 2/532 (0.38%). The H-ELISA, the cost/test was less than 5 bahts, appears to be promising for substitution of imported commercial kits.

INTRODUCTION

ELISA is very well recognized and widely applied for the primary serodiagnosis of HIV-infection (Cooper *et al,* 1987; Downie *et al,* 1989; Ubol *et al,* 1987; Weiblen *et al,* 1991). The method had a long history of development. It made use of different types of technics, indirect and competitive ELISA, and antigens, viral lysates, recombinant and synthetic antigens (Johnson, 1992; Pollet *et al,* 1991). All ELISA commercially available seemed to work satisfactorily in terms of sensitivity and specificity. In Thailand, all of HIV ELISA diagnostic kits are imported. However due to the economic crisis during the 1997-1998, most hospitals encountered budget restrictions. This study was to develop a home-made, enzyme-linked immunosorbent assay (H-ELISA) for primary screening test of HIV-infected blood samples. It is expected to reduce the cost per a test for approximately ten times from that of an imported commercial kit.

MATERIALS AND METHODS

Serum samples

All HIV-postitive sera were previously screened by a gel particle agglutination test (GPA : Green Cross) and confirmed by Western blot. These sera were obtained from Srinagarind Hospital, Khon Kaen University (548 samples) and Khon Keen STD clinic, Ministry of Public Health (244 samples). Normal serum consisted of 240 samples from clinical laboratories (immunology and chemistry labs), 200 samples from a community study on STD and 100 samples from blood bank. A pooled serum sample of 50 HIV-negative sera was used as a negative control. The positive control was selected from HIV-positive serum collection stock.

Development of home-made ELISA (H-ELISA)

HIV viral lysate, purchased from Gene Lab (USA) was diluted in 0.05 M carbonate buffer pH 9.6 and coated on to microplates for 100µl/well at room temperature,1 hour. Serum samples were diluted to 1: 10 in diluting buffer pH 7.2 containing 0.5 M NaCl,0.1% tween 20 and 2% milk powder in 0.2 M phosphate buffer. The diluted samples were added, 100 µl/well, to the coated wells and incubated at 37°C for 1 hour. Washing was carried out 3 times with washing buffer pH 7.2 containing 0.8 M NaCI and 0.1% tween 20 in 0.2 M phosphate buffer. After washing, a peroxidase-conjugated rabbit antihuman immunoglobulin (anti K and λ chains : DAKO), dilution 1:1,000 in diluting buffer, was added, 100 µl/well and incubated at 37°C for 1 hour. Washing 3 times was followed and the color development was done with OPD and substrate buffer (100 µl/well) pH 5.0 containing 0.1 M citric

Correspondence: Dr Pramote Thongkrajai, Faculty of Public and Environmental Health, Huachiew Chalermprakiet University,18/18 Bangna-Trad Road, Bangphli, Samut Prakan 10540, Thailand.

acid 4.9 ml, 0.2 M Na_2HPO_4 .$2H_2O$ 5.1 ml, 30% H_2O_2 0.2 ml and OPD 5 mg at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100 µl/well 6N H_2SO_4 to each well and the OD values were read by an ELISA reader at 492 nm. All assays were carried out in duplicate except the negative control was in triplicate. The optimum dilutions of HIV viral lysate used for coating microplates and that of the conjugated antiserum to human immunoglobulin were carried out by chequer board titration. The positive control serum was tested concomitantly in all experiments and coefficient variations (CV) of intra-assays and inter-assays were analysed. The cut off OD-value was calculated by 1.2x (mean negative OD value). Samples with OD values more than that of the cut off OD were considered as reactive samples. The established H-ELISA was applied to test 792 samples of positive and 540 samples of negative sera.

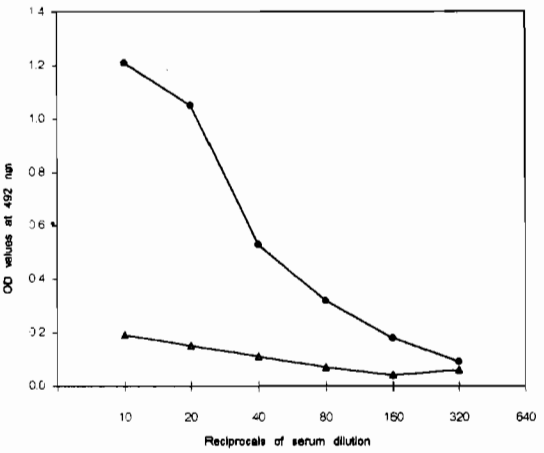

Fig 1–Titration curves of positive and negative control sera. Each dot represents mean OD value of duplicate assays.

RESULTS

Titration of positive and negative reference sera

Dilutions of positive and negative control sera were assayed against HIV viral lysate. Results are shown in Fig 1. At serum dilution 1:10 the positive and negative OD values are 1.21 and 0.19 respectively. The maximum difference between positive and negative OD-values was observed at serum dilution 1:10. The following screening tests were carried out at serum dilution 1:10. Coefficient variations of intra-assay (within the same experiment) and inter-assay (between different experiments) were 7.0% and 12.1% respectively.

H-ELISA screening test

The developed H-ELISA was applied to screen a set of previously screened HIV-positive sera and a set of tentatively HIV-negative normal sera obtained from different laboratories of Srinagarind Hospital as stated in the method. A mumber of 792 Western blot reactive sera were tested with H-ELISA and all were positive. The test was further applied to test 540 negative sera: 530 samples were negative while 10 samples were positive. Among these 10 samples, 8 were GPA-positive and Western blot reactive and 2 were GPA and Western blot non-reactive. However, the 530 samples that were H-ELISA negative had not undergone GPA and Western blot testing. Results are shown in Table 1. This indicated a false positive rate of 2/532 (0.38%)

In addition 14 samples of Western blot indeterminate sera were tested by H-ELISA Results (Table 2) showed 4 samples (no. 1-4) with GPA and H-ELISA negative tests, 4 samples (no. 5-8) with GPA and H-ELISA positive tests and 6 samples (no. 9-14) with GPA positive but H-ELISA negative tests. This means that 10 samples of Western blot indeterminate sera were not detected by H-ELISA.

DISCUSSION

All HIV-positive sera used in this study were routinely collected sera. They were screened by GPA and a commercial ELISA kit and confirmed by Western blot analysis. All of the sera were true HIV-positive (Downie *et al*, 1989). For normal sera, HIV screening test and Western blot analyses were not applied to due to the budget limitation but only samples with H-ELISA positive results were confirmed by GPA and Western blot (Table 1). In all experiments of this study the same lot of positive and negative control sera were used to reduce intra- and inter-assay variations. However, some varia-

Table 1

Screening test of HIV-positive and normal serum samples by the home-made H-ELISA.

Serum source	H-ELISA	GPA	Western blot	Number
HIV-positive serum	+	+	reactive	792
Normal serum	-	ND	ND	530
	+	+	reactive	8
	+	-	nonreactive	2

ND = not done

Table 2

Western blot profiles of 14 samples of indeterminate sera tested by GPA and developed H-ELISA.

Sample No.	Western blot Profiles	GPA	H-ELISA
1	P24, p55	-	-
2	p64	-	-
3	p55, p53	-	-
4	p55, p53	-	-
5	p17	+	+
6	p24	+	+
7	p24, p55	+	+
8	p17	+	+
9	gp160	+	-
10	p24, p55	+	-
11	p24	+	-
12	p17	+	-
13	gp160	+	-
14	p55, p53	+	-

NB. Samples No.1-4, obtained from a STD clinic, were screened by a commercial ELISA.

tions still existed (CV = 7.0% for intra-assays and 12.1% for inter-assays). At serum dilution 1:10, the normal sera yielded OD values of around 0.2 (Fig 1) which was satisfactorily low. It was similar to our previous analyses (Thongkrajai *et al,* 1989). The home-made H-ELISA could detect all 792 samples of HIV-positive sera, *ie* 100% sensitivity. Among 540 samples of normal sera from different sources in Srinagarind Hospital, H-ELISA could detect 8 samples as HIV-positive that were later confirmed by Western blot. However, it showed 2 samples to be false positive (Table 1), a false positive rate of 2/532 (0.38%).

When the H-ELISA was applied to test indeterminate sera, it could not detect 10 samples (sample no. 1-4 and no. 9-14, Table 2) while the GPA could not detect 4 samples (sample no. 1-4). These samples contained antibodies to p17, p24, p53, p64 and gp 160. Both GPA and H-ELISA could detect 4 samples (sample no. 4-8) containing antibodies to p17, p24, p55. They could not detect sample no. 1-4 containing antibodies to p24, p53, p55,p64. Thus,

it was conceivable that the quantity, quality and type of antibodies to HIV might be very much variable from one inividual to another. In some cases (no. a-14, Table 2), it appeared that the H-ELISA had a lower sensitivity (in terms of quantity of antibodies detected) than of GPA. The sensitivity of H-ELISA against gp160 was also much lower than that of GPA. This was seen in samples no. 9 and 13 (Table 2) containing antibodies to gp160 only; the H-ELISA was negative for both cases.

It could be concluded that although the H-ELISA could not pick up all sera with Western blot indeterminate (but the commercial GPA kit gave twice as many as the H-ELISA did), it possessed very high sensitivity enough to detect all HIV-positive sera. Before this home-made H-ELISA could be applied to routine screening test for HIVinfection, some improvements, *eg* using different types of HIV antigens, should be applied for a comparative titration and analysis. This is to ensure that no HIV-infected sera including Western blot reactive and indeterminate are excluded by the H-ELISA. In addition, it was estimated that the cost of all raw materials used in H-ELISA was less than 5 bahts/ test.

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