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ORIGINAL ARTICLES

Anti-HIV Positivity in Thailand : The Usefulness of Another ELISA Test Kit and Western Blot as Confirmatory Tests

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SUMMARY **Sera from 47 individuals repeatedly reactive in one screening ELISA system (designated as ELISA-A) for antibodies against human immunodeficiency virus (HIV) were evaluated by a second ELISA system (designated as ELISA-B) as well as by the Western blot technique. Both ELISA systems and the Western blot were positive in all of the 14 patients with clinical diagnoses of AIDS and AIDS-related persistent generalized lymphadenopathy (PGL). Of the 7 asymptomatic gays whose sera were repeatedly reactive in ELISA-A, 5 were also reactive in ELISA-B and these were the ones with positive Western blot tests. Eight and 17 ELISA-A reactive individuals were uncovered during a survey of 2,699 female prostitutes and 15,210 potential workers for Saudi Arabia respectively. All of these 25 individuals were ELISA-B and Western blot negative, an indication of false-positive reactivity with ELISA-A. Our studies indicate that the prevalence of HIV infection among the general Thai population is still low, and that the specificity of two ELISA test kits for anti-HIV may differ considerably. We concluded that evaluation of test kits should include studies in tropical countries where ecological conditions, climate and background endemic disease patterns are different than in the countries producing the diagnostic systems. Such studies are needed to identify the most sensitive and specific kits for worldwide application. We did discover that concordant positivity of two different ELISA test kits served as a reliable and inexpensive confirmatory test for anti-HIV.**

The etiologic agent of acquired immune deficiency syndrome (AIDS) has just recently been identified. It is most widely known as human T-lymphotropic virus type III (HTLV-III) and lymphadenopathy associated virus (LAV).[1,2] Human immunodeficiency virus (HIV) is the common name recently assigned to this agent.[3] Infection with this virus can result in protean manifestations, ranging from no symptoms to persistent generalized lymphadenopathy (PGL), AIDS-related complex (ARC) and finally full-blown AIDS with opportunistic infection and malignancy.[4] Because of these perplexing clinical manifestations and the deadliness of the disease, a sensitive test to identify HIV infection is urgently needed. Abbott's anti-HTLV-III enzyme-linked immunosorbent assay (ELISA) was the first commercial screening test which has been available worldwide since March 1985.[5] Many other anti-HIV ELISA test systems have quickly followed.

The currently available anti-HIV screening ELISA test systems differ in the antigen source, the vehicles in which the tests are carried out and in the enzyme system used.[6] The sensitivity of these tests is quite good, ranging from 95.0% to 99.6% in classical AIDS patients.[5] However, the specificity of the test system is the major concern of most investigators at present. Specificity of the test system is generally evaluated in healthy blood donors not belonging to AIDS high-risk groups. In one such study comprising 51,309 units of donated blood, 419 (0.8%) were anti-HIV positive on the first screening test with Abbott's ELISA kit and 116 (0.2%) were still positive in a repeat test.[7] Whether these individuals were truely infected with HIV requires answering with certainty because of the dreadful nature of this disease.

There are several confirmatory tests for a positive screening ELISA test such as the radioimmunoprecipitation test, the indirect immunofluorescent test, the Western blot test and viral isolation.[6,8,9] This paper

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reports our results with confirmatory tests using a second ELISA test kit and the Western blot test in 47 seropositive individuals detected in Thailand by one ELISA test kit. Some of the subjects belonged to an AIDS high-risk group and some did not. The results clearly indicated that the prevalence of true HIV infection in the general Thai population is still very low and that one ELISA test kit may be reliably used as the confirmatory test for the other.

MATERIALS AND METHODS

Subjects

Forty-seven serum samples from 47 subjects were tested with the Western blot test for antibodies to HIV antigens. All of these sera were repeatedly anti-HIV positive by one screening ELISA test (see below) and were therefore tested with a second ELISA test as well as with the Western blot test. Nineteen of these were male homo / bisexuals ; two of these had full-blown AIDS, 10 had AIDS-related persistent generalized lymphadenopathy (PGL) and 7 were asymptomatic. Also included in the PGL group was a girl-friend of one of the AIDS patients who was positive for anti-HIV and who had persistent generalized lymphadenopathy and a male heterosexual with PGL who most likely became infected by promiscuous heterosexual activities while studying in the USA. Nine subjects were female prostitutes. Eight of these were seropositives among a total of 2,699 female prostitutes being screened by the Division of Venereal Diseases of the Ministry of Public Health. The ninth female prostitute (case 9 of Table 3) was tested because she was a steady sexual partner of a seropositive male bisexual with PGL. Seventeen subjects were potential workers for Saudi Arabia who had to have anti-HIV testing as a visa requirement. A total of 15,210 such workers were thus screened.

Serological tests

HIV ELISA

Two commercial ELISA kits for anti-HIV were used. The first was designated as ELISA-A* and used 6 mm polystyrene beads coated with HIV antigens derived from the H9 cell line as the solid phase of the assay. The other kit (designated as ELISA-B) used the 12-well microtitre strips as the antigen carrier. Appropriate dilutions of patients' sera were allowed to react with the antigens on the solid phase, followed by the addition of enzyme-linked antihuman immunoglobulins. The presence of specific antibodies in the patient's serum was reflected by the presence of color which developed after adding the enzyme substrate. The results were expressed as optical density (O.D.) values and related to a cut-off value calculated from control O.D. readings according to the manufacturers' instructions. Any O.D. values above the cut-off value in each run were considered positive. The cut-off value in ELISA-A was calculated in each run whereas ELISA-B used a fixed cut-off of 0.1 over the blank reading. ELISA-A was used first as the screening test. Once the ELISA-A was repeatedly positive, ELISA-B was used in order to see whether the seropositivity could be confirmed.

HIV Western Blot

Antibodies to structural proteins of the HIV were detected by the Western blot technique using the commercial kit from Biotech Research Labs, Rockville, Maryland, U.S.A.[10] In this system, HIV specific polypeptides were separated according to their molecular weights by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecyl sulfate (SDS). The separated HIV polypeptides were then transfered from the gel to a nitrocellulose membrane via eletrophoretic blotting. The serum specimens were reacted with the specific proteins on the nitrocellulose strips. Visualization of the specifically bound human immunoglobulins to HIV proteins was performed using a goat-anti-human immunoglobulin biotin conjugate, an avidin-horseradish peroxidase conjugate, and 4-chloro-1-naphthol substrate. The serum positivity is indicated by its reaction with a protein of MW 24,000 (p24) or a glycoprotein of MW 41,000 (gp41). Reaction with proteins of other molecular weights was also noted. A positive control serum was included in every run which would react with all the protein fractions illustrated on a reference picture used for comparing protein bands.

RESULTS

1. In AIDS and PGL patients

The Western blot was positive in 2 patients with full-blown AIDS (cases 1 & 2 of Table 1; strips 10 & 11 in Fig. 1). One was a Thai male bisexual and the other was a US male homosexual. Both of them had antibodies to gp41 but negative anti-p24. Antibodies to other protein bands were also found, such as to p160, p64, p53 and p31. The ELISA test in these 2 patients was also strongly positive by both ELISA test kits.

Western blot was also positive in all of the 12 patients with a clinical diagnosis of PGL (cases 3-14 of Table 1; strips 1 & 6 in Fig.1). Six were uncovered by mass screening 740 male prostitutes in 2 cities frequently visited by oversea tourists. Four were male nonprofessional homosexuals (*i.e.*, preferential gays) who volunteered for the blood test. One female patient (case 3 of Table 1) was screened because she was the consort of an AIDS patient. The last patient (case 4 of Table 1) was a promiscuous Thai

* ELISA-A was the HTLV-III EIA test kit from Abbott Laboratories and ELISA-B was the VIRGO HTLV-III test kit from Electro-Nucleonics, Inc. (ENI)

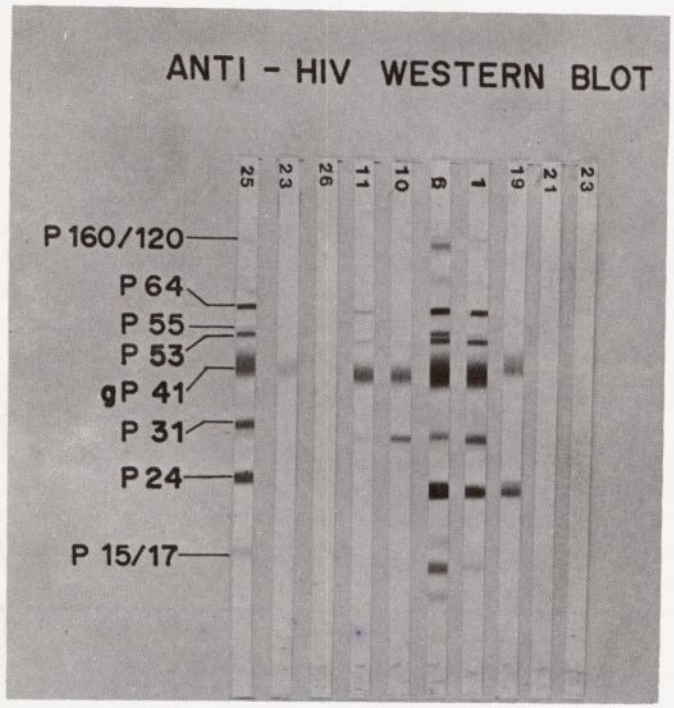

Fig. 1 Western blot strips in AIDS patients (strips 10 & 11), PGL patients (strips 1 & 6), a female prostitute who was a steady sexual partner of a bisexual man with PGL (strip 19) and Thai workers for Saudi Arabia (strips 21 & 23 far right). Strips 25,23 (2nd from left) and 26 are strongly positive, weakly positive and negative controls, respectively.

heterosexual male returning from the USA. Ten of these 12 patients (83%) had antibodies to both the p24 and gp41 as well as to other viral proteins. Two had only anti-gp41 (cases 8 and 11 of Table 1). The results of both ELISA tests correlated well in this group.

2. In asymptomatic gays

Of the 7 asymptomatic male homosexuals or bisexuals who did not have generalized lymphadenopathy or any other symptoms but were positive on screening for anti-HIV by ELISA-A, five (71%) were Western blot positive. Both anti-p24 and anti-gp41 were present in all positive Western blots (Table 2). ELISA-B was positive in all the gays with positive Western blot and negative in the 2 gays with negative Western blot (Table 2). Of these 2 Western blot-negative cases, ELISA-A was weakly positive in one (case 7) and strongly positive in another (case 6). All but one of these 7 asymptomatic seropositive gays were also uncovered during the same mass screening of male prostitutes whereas the

Table 1 Comparison between ELISA O.D. readings and Western blot test in AIDS and PGL patients

Case No	Dx	O.D. anti-HIV (ELISA)				Western blot	
		ELISA – A (cut-off)		ELISA – B (cut-off = 0.1)			
		Initial	Repeat	Initial	Repeat	p 24	gp 41
1	AIDS	1.059 (0.087)	0.568 (0.085)	0.472	0.548	–	+
2	AIDS	0.649 (0.084)	0.395 (0.087)	0.528	0.585	–	+
3	PGL*	0.975 (0.087)	0.665 (0.085)	0.543	0.911	+	+
4	PGL*	1.168 (0.152)	0.470 (0.060)	0.640	0.687	+	+
5	PGL	0.363 (0.154)	0.920 (0.167)	0.374	0.751	+	+
6	PGL	1.386 (0.152)	1.737 (0.240)	0.794	0.467	+	+
7	PGL	0.832 (0.098)	0.520 (0.058)	0.563	0.564	+	+
8	PGL	0.606 (0.072)	1.250 (0.100)	0.572	0.586	–	+
9	PGL	1.330 (0.208)	>2.000 (0.187)	0.994	0.783	+	+
10	PGL	0.446 (0.058)	0.520 (0.058)	0.825	>2.000	+	+
11	PGL	0.187 (0.047)	1.719 (0.205)	0.509	0.495	–	+
12	PGL	0.464 (0.100)	0.159 (0.017)	0.444	0.853	+	+
13	PGL	0.142 (0.047)	1.090 (0.171)	0.484	0.461	+	+
14	PGL	>2.000 (0.163)	1.692 (0.047)	1.140	0.553	+	+

*All of the PGL patients were gays with persistent generalized lymphadenopathy except case No 3 who was a girlfriend of an AIDS patient and case No 4, a heterosexual male who had promiscuous sexual activity while studying in the U.S.A.

last one (case 5) was a foreign tourist who volunteered for the blood test.

3. In female prostitutes

Western blot was negative in all of the 8 female prostitutes who were found seropositive during a mass screening of 2,699 female prostitutes with ELISA-A (cases 1-8 of Table 3). They were all asymptomatic and did not have any lymphadenopathy. Seven of these 8 prostitutes (cases 1-7) could not be confirmed by ELISA-B. The O.D. values in 3 of these ELISA-A positive individuals were quite high (cases 2,3 and 7) although the majority had low O.D. values. One prostitute had a weakly positive ELISA-A and ELISA-B (case 8) but the Western blot was negative. Included in this group but not belonging to the same survey was an asymptomatic female prostitute who was a steady sexual partner of a PGL patient (case 9 of Table 3) with positive Western blot (both anti-p24 and anti-gp41; strip 19 in Fig. 1). Both screening anti-HIV tests were strongly positive in this case.

4. In workers

Of the 15,210 workers for Saudi Arabia that were screened, 17 were repeatedly positive on ELISA-A. The O.D. values in the majority of these workers were quite high (Table 4). All were males and asymptomatic. None admitted homosexuality or drug addiction. None of these 17 seropositive workers had a positive Western blot test.

Table 5 summarizes the correlation of both ELISA test kits with the Western blot in various high-risk and low-risk groups for HIV infection. It is evident that for the high-risk groups (gays with full-blown AIDS, PGL, asymptomatic gays and female sexual partners of AIDS and PGL patients), the results of screening ELISA tests from different companies and the Western blot correlated very well. But, for the low-risk groups (Thai workers and female prostitutes), the screening ELISA-B showed more

Table 2 Comparison between ELISA O.D. readings and Western blot test in asymptomatic gays

	O.D. anti-HIV				Western blot	
	ELISA – A (cut-off)		ELISA – B (cut-off = 0.1)			
Case No	Initial	Repeat	Initial	Repeat	p 24	gp 41
1	1.190 (0.106)	1.748 (0.118)	0.739	0.779	+	+
2	1.540 (0.152)	1.318 (0.240)	1.092	0.793	+	+
3	1.369 (0.047)	>2.000 (0.138)	>2.000	>2.000	+	+
4	0.468 (0.047)	>2.000 (0.171)	0.891	0.919	+	+
5	0.726 (0.058)	0.212 (0.038)	0.570	0.575	+	+
6	0.651 (0.152)	1.013 (0.154)	0.067	0.053	–	–
7	0.255 (0.195)	0.253 (0.240)	0.054	0.052	–	–

Table 3 Comparison between ELISA O.D. reading and Western blot test in female prostitutes

	O.D. anti-HIV				Western blot	
	ELISA – A (cut-off)		ELISA – B (cut-off = 0.1)			
Case No	Initial	Repeat	Initial	Repeat	p 24	gp 41
1	0.237 (0.205)	0.047 (0.047)	0.052	0.054	–	–
2	0.441 (0.150)	0.253 (0.094)	0.049	0.054	–	–
3	1.951 (0.137)	1.347 (0.177)	0.050	0.052	–	–
4	0.225 (0.129)	0.246 (0.195)	0.051	0.053	–	–
5	0.250 (0.230)	0.042 (0.017)	0.056	0.043	–	–
6	0.271 (0.230)	0.060 (0.047)	0.047	0.053	–	–
7	0.644 (0.201)	0.387 (0.137)	0.049	0.053	–	–
8	0.194 (0.094)	0.091 (0.017)	0.189	0.194	–	–
9	0.738 (0.086)	1.229 (0.122)	0.812	0.924	+	+

specific results.

Figure 2 compares the levels of ELISA positivity expressed as the ratio of sample to cut-off O.D. to the Western blot results. It is evident that cases with weak ELISA positivity, *i.e.*, O.D. ratio below 2 in either ELISA-A or B, have not been found to have a positive Western blot test. An O.D. ratio above 2 in ELISA-B was always accompanied by a positive Western blot. When using ELISA-A, on the contrary, even with an O.D. ratio above 6, one in three may still be false-positive, namely, unconfirmable by Western blot (Fig. 2).

DISCUSSION

Although not every unit of transfused blood is tested for anti-HIV in Thailand at present, all workers for Saudi Arabia have to be tested for anti-HIV as a visa requirement. Using ELISA-A as the screening test for 15,210 workers for Saudi Arabia, 17 were found to be repeatedly reactive, a prevalence of 0.11%. The specificity of ELISA-A in this survey was calculated as 99.89%. This is not much different from the specificity of 99.83% when the same ELISA test kit was evaluated in 1,027,786 units of donated blood in the USA.[11] The only difference in our study from the US study, is that 333 of the 1,455 (23%) repeatedly positive ELISA tests in the US study were truely anti-HIV positive, *i.e.*, confirmed by Western blot,[11] whereas none in our study was truely positive. This may reflect the higher endemicity

Table 4 Comparison between ELISA O.D. reading and Western blot test in workers

Case No	O.D. anti-HIV					
	ELISA – A (cut-off)		ELISA – B (cut-off = 0.1)		Western blot	
	Initial	Repeat	Initial	Repeat	p 24	gp 41
1.	0.584 (0.152)	>2.000 (0.159)	0.060	0.054	–	–
2.	0.649 (0.159)	0.512 (0.141)	0.048	0.056	–	–
3.	0.728 (0.105)	1.038 (0.121)	0.057	0.055	–	–
4.	0.665 (0.105)	0.760 (0.121)	0.062	0.052	–	–
5.	0.631 (0.088)	0.380 (0.169)	0.045	0.050	–	–
6.	0.436 (0.116)	0.600 (0.167)	0.048	0.056	–	–
7.	0.233 (0.139)	0.202 (0.169)	0.055	0.052	–	–
8.	0.242 (0.162)	0.246 (0.157)	0.051	0.052	–	–
9.	1.274 (0.099)	1.291 (0.134)	0.056	0.059	–	–
10.	1.956 (0.149)	1.822 (0.177)	0.057	0.054	–	–
11.	0.539 (0.152)	0.263 (0.165)	0.045	0.046	–	–
12.	>2.000 (0.133)	1.046 (0.130)	0.049	0.050	–	–
13.	0.481 (0.118)	0.510 (0.117)	0.067	0.061	–	–
14.	0.700 (0.136)	0.209 (0.138)	0.074	0.063	–	–
15.	1.603 (0.159)	1.437 (0.117)	0.055	0.060	–	–
16.	0.614 (0.145)	0.690 (0.153)	0.061	0.053	–	–
17.	0.490 (0.281)	0.146 (0.068)	0.165	0.108*	–	–

* This O.D. value was less than 0.1 after subtracting the background value.

Table 5 Correlation of the two screening ELISA tests with the Western blot test in various study groups

	N	ELISA – A	ELISA – B
AIDS	2	2/2[(a)]	2/2
PGL	10	10/10	10/10
Asymptomatic gays	8	6/8	6/6
Female contacts[(b)]	2	2/2	2/2
Female prostitutes	8	0/8	0/1
Workers	17	0/17	0/0

(a) number with positive Western blot/number with positive HIV ELISA.
(b) One female was the girlfriend of an AIDS patient, the other female was the steady sexual partner of one PGL patient and she also happened to be a prostitute.

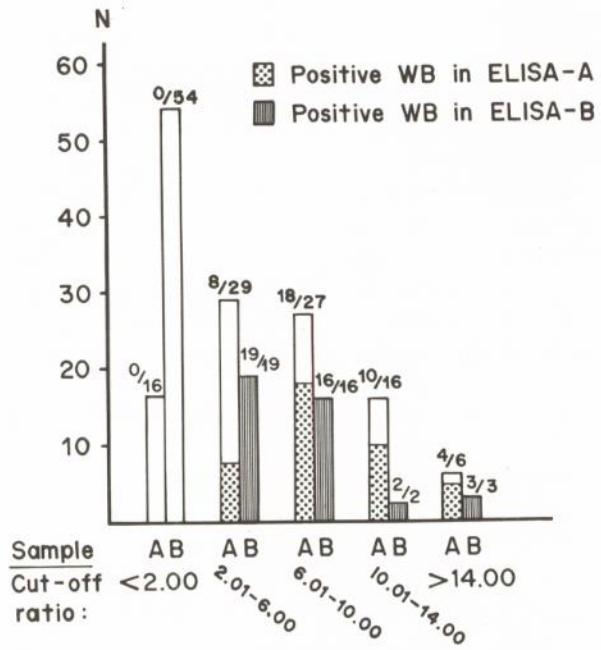

Fig. 2 Distribution of Western blot positivity at various levels of reactivity in ELISA-A and B expressed as the sample to cut-off O.D. ratios. Dotted and striped columns represent the number of positive Western blots in ELISA-A and ELISA-B positive sera respectively. Each serum specimen had two sample to cut-off O.D. ratios for each ELISA test as a result of repeat test.

of HIV infection in US. population as compared to the Thai population.

Using Western blot as the gold standard, ELISA-B correlated with Western blot much better than ELISA -A, particularly in the low-risk individuals such as the healthy young men from rural areas of Thailand who applied for a visa to work in Saudi Arabia. Our study, however, did not evaluate the specificity of the ELISA -B kit because the screening was done with ELISA-A. It is possible that if a large number of low-risk individuals is screened by ELISA-B, the specificity may not be different from that of ELISA-A.

None of the 2,699 Thai female prostitutes screened were truely positive for anti-HIV, confirming the previous smaller scale report of Wangroongsarb *et al.*[12] This is in contrast to the 31-66% prevalence of true HIV infection among African prostitutes.[13] Many reasons may be used to explain the low (or practically nil) prevalence of HIV infection among Thai female prostitutes. Firstly, the overall prevalence of HIV infection among Thai men is still very low, the heterosexual transmission risk to female prostitutes is therefore also low. Secondly, unlike the African prostitutes,[13] Thai prostitutes may use parenteral drugs less frequently, and thus have a lower risk of parenteral transmission. It is unlikely that the reason for the low seroprevalence is due to the lack of contact with North American or European clients because the prostitutes screened were from two cities of major tourist attraction. However, there is a possibility that Thai prostitutes consorting with foreign tourists have a lower number of clients (and thus contacts) than their African, American or European colleagues.

Reason for the high false positive anti-HIV rates in selected Thai populations is unknown. In USA, it was found that a false-positive anti-HIV may be due to infection with HTLV-I and viruses or due to the presence of antibodies to cytoplasmic and cell membrane antigens (including HLA antigens) of the H9 cells upon which the virus was propagated.[14,15] A confirmatory test is therefore crucial, particularly before informing a person that he/she is indeed infected with HIV. Several confirmatory tests have been proposed such as the H9 plate test, the indirect immunofluorescent test, the radioimmunoprecipitation test, the Western blot test and viral isolation.[6,8,9] The Western blot is the confirmatory test most widely accepted due to its specificity against specific viral proteins.[16] With the recent availability of commercial Western blot strips, the test is made simpler, is standardized and can be performed in any clinical laboratory. Our results with the Biotech Western blot strips were very satisfactory. The antibody bands were clearly separated and could be easily identified. The test was positive in all patients with AIDS and PGL that we encountered. It is interesting to note that both of our patients with AIDS only had antibodies to gp41 but not to p24, whereas 10 of 12 patients with PGL had antibodies to both p24 and gp41. This is in agreement with other reports that antibodies to p24 will be progressively lost once the HIV infection advances from PGL or ARC to full-blown AIDS.[16,17] The loss may be due to the increasing impairment of helper T cells in antibody production once the disease progresses. Therefore, it will be interesting to follow closely our 2 PGL patients which did not have anti-p24 to see whether this can be used as a marker for disease deterioration.

Raising the level of the cut-off O.D. of the anti-HIV ELISA test

has been proposed to increase the specificity of the test. Several studies, both in AIDS-high risk groups and in blood donors, have found a good correlation between Western blot and a strongly reactive ELISA test (sample to cut-off or to negative O.D. values of 6 or more[7,16,18]). However, we found that this was true only with ELISA-B, with O.D. ratios just above 2. One-third of the ELISA-A positive sera with O.D. ratios above 6 were still Western blot negative and thus presumably false positives.

Several anti-HIV ELISA test kits are currently available and they may differ considerably in specificity. It is therefore essential to perform a large-scale evaluation of the specificity of different test kits in order to identity the most specific one for a Thai population. It might be reasonable in the meantime to use a second ELISA test kit as low cost alternative to Western blot confirmation of HIV infection among low-risk populations in Thailand. Our preliminary results using three other commercially available anti-HIV ELISA test kits seem to support the above contention.

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