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Comparison of the Sensitivity of Various Anti-HIV Tests in Early Seroconversion Sera

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SUMMARY **Paired sera from 4 patients with proven HIV infection whose initial specimens obtained 14-51 days earlier were indeterminate were simultaneously retested with 7 screening anti-HIV test kits and the immunoblot assay. The study aimed to evaluate the sensitivity of various new and old anti-HIV screening tests. The test kits evaluated were 4 ELISA test kits from Wellcome (Wellcozyme), Organon (Vironostika anti-HTLV-III), Pasteur (Rapid Elavia) and Diagnostic Biotechnology (DB, HIV-1 ELISA), 2 rapid tests based on microfiltration enzyme immunoassay procedure from Rapport (SUDS) and Disease Detection International (SeroCard), and 1 particle agglutination (PA) test (Serodia-HIV). Immunoblot strips from Diagnostic Biotechnology (HIV-1 Western blot) were used to confirm the HIV infection in these serum specimens. Out of the 4 initial serum specimens tested, all were positive by PA, 2 by SUDS, Wellcome and Pasteur, 1 by SeroCard and DB, and none by Organon. When tested by immunoblot, 1 was negative (i.e., completely without any bands) whereas 3 were indeterminate (i.e., 1 with very weak band for p18, 1 with weak band for p24, 1 with very weak band for gp160. All repeat specimens obtained 14-51 days later (mean 32.5 ± 16 days) were positive by all screening tests as well as immunoblot. Therefore, with these 4 early seroconversion sera, the sensitivity of the PA was 100%, that of SUDS, Wellcome and pasteur was 50%, of that SeroCard and DB was 25%, and Organon, 0%. None of these sera was considered positive by immunoblot.**

Antibodies to the human immunodeficiency virus (HIV) are considered evidence of HIV infection.[1,2] The antibodies are most frequently detected by the enzyme-linked immunosorbent assay (ELISA) test, due to its rapidity and convenience, particularly for large-scale screening such as in blood banking.[3] The particle agglutination (PA) test was subsequently developed for use in situations where the expensive ELISA reader and washer are not available.[4] Most recently, many rapid, visually read anti-HIV test have been developed. Some of these rapid tests have been evaluated against other conventional tests and found to have comparable sensitivity and specificity.[5]

In Thailand, many conventional and rapid anti-HIV testings are currently available in the market and still many more are coming in. No systematic study to compare the sensitivity and the specificity of these tests has been done. We therefore conducted a comparative study of the sensitivity of 7 screening anti-HIV test kits and the immunoblot assay on 4 early seroconversion sera collected in our laboratory.

MATERIALS AND METHODS

Blood samples

Four paired early seroconversion sera were selected from 24,485 individuals having anti-HIV screened at Chulalongkorn University Hospital, Bangkok during 1987-1990. Their initial sera were either anti-HIV negative or indeterminate (by immunoblot assay) but were all confirmed positive on subsequent sera. The age, sex and risk factors of these 4 patients are summarized in Table 1. The individuals acquired the infection by heterosexual means, homosexual means, intravenous drug use

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(IVDU) and blood transfusion (1 by each route) (Table 1). The intervals between the first and the second serum collections ranged from 14 to 51 days ($\bar{x} \pm SD = 32.5 \pm 16$ days). All sera were kept frozen in aliquots at -70°C until simultaneously retested.

Anti-HIV testing

All 8 sera were simultaneously tested with 7 screening anti-HIV tests and one confirmatory immunoblot assay as listed in Table 2.

1. Particle agglutination (PA)

PA was performed using a Serodia-HIV kit from Fujirebio (Shinjuku-ku, Tokyo, Japan, lot no. BP 0115). The test was performed according to the procedures described by the manufacturer. A positive test was determined by the agglutination of the HIV antigen-coated gelatin particles.

2. Rapid tests

(a) SUDS

This new rapid test system called "single use diagnostic system" (SUDS) was produced by Rapport (Dartmouth, Nova Scotia, Canada). The test is based on visually read, microfiltration ELISA for the qualitative detection of anti-HIV antibodies. The solid phase capture reagent is latex particles coated with a mixture of synthetic HIV-1 core and envelope antigens (Table 3). A distinct (1 + or greater) blue color in the center hole, as viewed from the bottom of the SUDS test cartridge, indicates that the specimen is anti-HIV reactive. Lot number 197 E was used in the study.

(b) SeroCard

The SeroCard HIV antibody test kit is a rapid, visually read, indirect ELISA test produced by Disease Detection International (Irvine, California, U.S.A., lot no. SN 1012). Each test card contains a chromatographic sorbent matrix which has two large ports at the bottom (application zones) and two small ports (reaction zones) at the top. The matrix on the right side of the reaction zone is coated with synthetic core and envelope HIV-1 peptides whereas the left side of the reaction zone serves as the negative control of each specimen. Upon applying a patient's specimen (serum, plasma or whole blood) onto each of the two large ports, particulate matter such as red blood cells will be retained at or near the application site whereas the serum component will migrate faster on the chromatographic matrix towards the reaction site. At the end of the procedure, after adding enzyme conjugate and substrate, there will be a dark blue spot on the reaction port (right side) if the test is positive due to the enzyme bound to the anti-HIV antibodies whereas there will be no color if anti-HIV is negative.

3. Enzyme-linked immunosorbent assay (ELISA)

Four commercial HIV-1 ELISA test kits, one competitive ELISA from Wellcome (Wellcozyme, lot no. K 273710) and three indirect ELISA from Organon (Vironostika anti-HTLV-III, lot no. 1114171), Diagnostic Pasteur (Rapid Elavia, lot no. CH.B: 01 228 Y) and Diagnostic Biotechnology (HIV-1 ELISA, lot no. 1105211) were used in this study. The characteristics of these ELISA tests were summerized in Table 3. The assay procedures and the interpretation of results of each test were followed as described in the manual of each kit.

4. Immunoblot

Immunoblot strips from Diagnostic Biotechnology (HIV-1 Western Blot, Singapore, lot no. 02 0 4586)

Table 1. Sex, age and risk factors of the four HIV patients with early seroconversion

	Patient number			
	1	2	3	4
Date of first serum	Feb 29, 1988	Mar 28, 1989	Sep 9, 1989	May 23, 1990
Sex	M	M	M	F
Age (yr)	25	18	28	70
Risk factor	Heterosexual	Homosexual	IVDU	Blood Transfusion
Interval between first and second sera (days)	45	20	51	14

N.B.: All sera were retested during March 20–30, 1991.

Table 2. Reagents used for anti–HIV tests.

Tests	Commercial names	Manufacturers
Screening Tests		
1. Particle agglutination (PA)	Serodia–HIV	Fujirebio (Shinjuku–Tokyo, Japan)
2. Rapid test – SUDS	MUREX SUDS HIV–1	Rapport (Dartmouth, Nova Scotia, Canada)
– SeroCard	SeroCard HIV antibody test	Disease Detection International (Irvine CA, U.S.A.)
3. ELISA – Wellcome	Wellcozyme	Wellcome (Dartford, England)
– Organon	Vironostika–anti HTLV III	Organon Tanika (Boxtel, Holland)
– Pasteur	Rapid Elavia	Diagnostic Pasteur (Marnes La Coquette, France)
– Diagnostic biotechnology (DB)	HIV–1 ELISA kit	Diagnostic Biotechnology (Singapore Science Park, Singapore)
Confirmatory Test		
1. Immunoblot	HIV–1 Western Blot	Diagnostic Biotechnology (Singapore Science Park, Singapore)

Table 3. Characteristics of anti–HIV screening tests.

	PA	SUDS	SeroCard	Wellcome	Organon	Pasteur	Diagnostic Biotechnology
Principle	Particle agglutination	Latex particle ELISA	Chromatographic matrix ELISA	Competitive ELISA	Indirect ELISA	Indirect ELISA	Indirect ELISA
Antigen	Cell culture (Molt–4)	Synthetic peptide (core and env)	Synthetic peptide (core and env)	Recombinant (core and env)	Viral lysate	Viral lysate	Viral lysate
Enzyme conjugate	No	Alkaline phosphatase	Alkaline phosphatase	Horse–radish peroxidase	Horse–radish peroxidase	Peroxidase	Alkaline phosphatase
Substrate	No	Para–nitrophenyl phosphate	Para–nitrophenyl phosphate	TMB	OPD	OPD	Para–nitrophenyl phosphate
Reaction time (min)	120	10	10	80	90	90	120
Cost per test (US $)	1.3	5.2	5.2	1.1	1.6	1.0	1.0

were used as a confirmatory test. An immunoblot is considered positive if 2 or more bands are present, one from the core region (p24 or p32) and at least one from the envelope region (gp41, 120 or 160). The absence of any band is considered negative. Any other patterns are regarded as indeterminate. [6]

RESULTS

1. Immunoblot Assay

Representative strips of Western blot assay against HIV-1 of paired sera from the 4 patients are illustrated in Figure 1. Of the 4 initial serum specimens, 1 was negative (panel 3a; completely without any bands), 3 were indeterminate (panel 1a with very weak band for p18, panel 2a with weak band for p24 and panel 4a with very weak band for gp160). All subsequent sera obtained 14-51 days later were positive by Western blot (Figure 1). The detailed profiles of the Western blot results for each patient are summarized in Table 4.

2. Anti-HIV Screening Tests

Table 5 summarizes the performance of 7 anti-HIV screening tests on the 4 early seroconversion sera. All 4 were positive by PA, 2 by SUDS, Wellcome and Pasteur, 1 by SeroCard and DB, and none by Organon. The degree of positivity of these early seroconversion sera was noticably less than that of the follow-up sera. For example, with indirect ELISA tests (Pasteur, DB), the ratio of patient's O.D. (optical density) to cut-off O.D. ranged from 1.65 to 2.94 for the positive initial sera as compared with 2.92 to 5.66 for the follow-up sera. However, only 1 of the 4 initial sera (patient No. 1) gave a weakly positive reaction on PA, i.e., agglutinated particles spreading over only half of the well whereas the other 3 sera gave a strongly positive reaction.

The overall assessment of the reactivity of each early seroconversion serum against the 7 screening tests revealed that serum of patient No. 2 was most reactive (i.e., positive in 5 out of 7 screening tests), followed in order by patients No. 4, 1 and 3, respectively (Table 5). Serum of patient No. 3, which was positive only PA, was the one with negative Western blot. The follow-up sera of these 4 patients were all strongly positive in all screening tests.

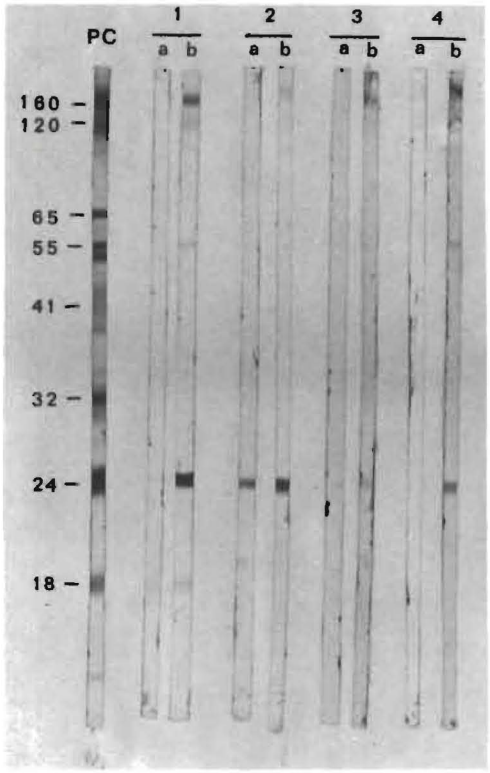

Fig. 1 Representative strips of Western blot assay against HIV-1 in 4 early seroconversion patients (panels 1, 2, 3, 4).
lanes a : first serum specimen
lanes b : second serum specimens from the same patients obtained 14-51 days later
PC : Positive control for anti-HIV core proteins (p18, p24, p55), polymerase (p32, p65) and envelope glycoproteins (gp41, gp120, gp160).
p = protein; gp = glycoprotein.
Numbers indicate the approximate molecular weights of the antigens in kilodaltons.

DISCUSSION

Four early seroconversion sera from 4 HIV-infected Thai patients were used to study the relative sensitivity of 4 ELISA tests (Wellcome, Organon, Pasteur, Diagnostic Biotechnology or DB), 2 rapid tests (SUDS, SeroCard), 1 particle agglutination (PA) test and 1 immunoblot assay (DB) in the detection of anti-HIV-1 antibodies. All sera were positive by PA (100% sensitivity), 2 by SUDS, Wellcome and Pasteur (50% sensitivity), 1 by SeroCard and DB (25% sensitivity) and none by Organon (0% sensitivity) (Table 5).

Table 4. Western blot results of initial and subsequent sera from 4 patients.

Patient No.	Sera	Western blot results	Band profile							
			p18*	p24	p32	gp41	p55	p65	gp120	gp160
1	1st	indeterminate	v	–	–	–	–	–	–	–
	2nd	positive	w	*p*	–	–	v	–	w	*p*
2	1st	indeterminate	–	w	–	–	–	–	–	–
	2nd	positive	v	*p*	–	–	v	–	–	w
3	1st	negative	–	–	–	–	–	–	–	–
	2nd	positive	–	w	–	–	–	–	–	w
4	1st	indeterminate	–	–	–	–	–	–	–	v
	2nd	positive	–	w	–	–	v	–	v	w

*Numbers indicate the approximate molecular weights of the antigens in kilodaltons, p = protein, gp = glycoprotein.
v = very weakly positive; w = weakly positive; *p* = strongly positive.

Table 5. Comparison of the sensitivity of 7 anti–HIV screening tests in early seroconversion sera from four patients.

Patient number	Anti–HIV screening tests							No. of positive tests out of 7 tests used
	PA	SUDS	SeroCard	Wellcome	Organon	Pasteur	DB	
1	P	N	N	P	N	N	N	2
2	P	P	N	P	N	P	P	5
3	P	N	N	N	N	N	N	1
4	P	P	P	N	N	P	N	4
No. Positive/Total	4/4	2/4	1/4	2/4	0/4	2/4	1/4	
Sensitivity (%)	100	50	25	50	0	50	25	

None was considered positive by immunoblot assay, i.e., 1 was completely negative and the other 3 were indeterminate. All the follow-up sera obtained 14-51 days later (mean 32.5 days) were positive by all screening tests as well as by immunoblot.

Our results indicate that PA is the most sensitive screening test for anti-HIV. This is in accordance with other studies.[4,7-9] The reason is probably due to the fact that PA can also detect IgM antibodies in addition to IgG antibodies.[4] The relative sensitivity of other screening tests cannot be judged from this study due to the limited number of specimens and the unique criteria of sample selection. A large-scale sensitivity and specificity study of these screening tests is currently being carried out.

Immunoblot is not a sensitive test for anti-HIV detection. It can be negative in early seroconversion but more commonly, it is indeterminate (Table 4). Therefore, its use is primarily to confirm true HIV infection in individuals with a positive screening test. Nevertheless it cannot really rule out false-positive reactions on certain screening tests such as PA. That is, a positive PA test with a completely negative immunoblot does not exclude true HIV infection. This raises the question of whether immunoblot should still be considered the gold-standard confirmatory test for HIV infection. Instead, PA may be better used to confirm the results of other screening tests. In addition, the cost of immunoblot is high and the interpretation of the results greatly depends on the quality

of the immunoblot strips and the subjective reading of the reactive bands. Using different batches of Western blot strips from the same manufacteurer, the same early seroconversion sera may be interpretated as either negative or indeterminate and simultaneously, the reading may vary from indeterminate to positive.

Different early seroconversion sera may have different reactivity when tested against a panel of screening tests. For example, in our study, the initial serum of patient No. 2 appeared the most reactive (Table 5). It was positive in 5 out of 7 screening tests. This serum was also most reactive on immunoblot strip, i.e., yielding a weak band with p24 (Table 4). This is in contrast to serum No. 1 which was reactive in only 2 out of 7 screening tests. This serum gave only a very weak band with p18 (Table 4). Therefore, the reactivity of a particular serum on a panel of screening tests seems to correlate well with the intensity of the reactive bands seen on immunoblot (compare Tables 4 and 5). This implies that for a reliable anti-HIV screening, a battery of screening tests is essential, including PA in particular. In addition, a strong reactive band on so called indeterminate immunoblot must be closely followed up because it is likely to have the same clinical relevance.

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