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Anti-HIV Antibody Titer: An Alternative Supplementary Test for Diagnosis of HIV-1 Infection

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SUMMARY **The diagnosis of HIV infection is based on screening of HIV antibodies and confirmed by a more specific supplementary test. The most common confirmation test is Western blot, which is expensive, time consuming and subject to technical skill. The present study was carried out to evaluate whether the anti-HIV-1 antibody titer is valid as a supplementary test for diagnosis of HIV-1 infection. Anti-HIV-1 antibody titers of 2,414 anti-HIV-1 positive sera determined by the particle agglutination (PA) method were analysed in comparison with the Western blot analysis. The Western blot negative result was found in 11 of 2,414 (0.46%) anti-HIV-1 positive sera, these sera also gave negative anti-HIV by ELISA. The PA titers of these sera were found in the range of 16 to 64. Seventeen samples (0.70%) with anti-HIV-1 in the titer range of 16 to 256 showed indeterminate Western blot analysis. The rest, 2,386 of these 2,414 sera (98.84%), were shown to be positive by Western blot. However, all of the 2,356 sera with antibody titers ≥ 512 (97.6%) demonstrated positive Western blot results. Five cases among the 17 (29.4%) indeterminate sera were examples of early seroconversion of HIV infection, which were confirmed in follow up specimens. The results suggest that only the samples with antibody titers < 512 are required to be confirmed for HIV infection by Western blot. It is possible that early seroconversion may be inferred from anti-HIV titers. Therefore, in order to reduce time and cost, the PA anti-HIV titer can be used as an alternative supplementary test for diagnosis of HIV-1 infection in most positive screened anti-HIV samples. Western blot is needed for testing in only a few cases.**

The serological diagnosis of human immunodeficiency virus infection is based on the detection of antibodies to HIV (anti-HIV) in serum. The serum samples which are reactive for anti-HIV antibodies by screening tests should be retested with more specific supplementary tests for confirmation. The most widely used screening tests are enzyme linked immunosorbent assay (ELISA) and particle agglutination (PA) while the most common confirmation test is Western blot (WB).[1] ELISA is a highly sensitive screening assay but it is machine-dependent and technically it is a little difficult to perform. It is appropriate to test a number of specimens in each run but inappropriate for small sample size testing and difficult to operate for a series of tests at short intervals. Particle agglutination is available in most countries.[2] It is a simple assay and can be performed in most laboratories without sophisticated equipment. It involves a one step antigen-antibody reaction by indirect agglutination principle in which the sensitized particle is coated with whole viral lysate. The PA can be carried out at any time for a small number of samples or for mass screening. The result can be

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achieved within two hours by the naked eye. Studies have shown that PA demonstrated a comparable sensitivity and specificity to most of ELISA assays,[1,3-6] but was more sensitive than most of the rapid screening tests.[1,7] It can detect both IgM and IgG anti-HIV antibodies.[3,8] Western blot is an expensive and time consuming technique. It is also subject to technical performance and reading skill,[9,10] and lacks sensitivity during early seroconversion.[7,11] Indeterminate WB results can occur in uninfected and infected HIV cases.[10] Recently a number of alternative strategies to WB have been recommended for diagnosis of HIV infection by using second and/or third different screening tests.[1,12,13] The first test should have the highest sensitivity, whereas the second and third tests should have higher specificity than the first,[1] or a different principle of assay.[13] The sample with an equivocal result should be further tested by WB.[1,13]

Anti-HIV screening has been conducted in our laboratory since late 1987. The competitive ELISA anti-HIV (Wellcozyme HIV recombinant, Wellcome, England) was used during late 1987 to 1990. The particle agglutination assay (Serodia-HIV) has replaced the ELISA for screening of anti-HIV-1 in patients and blood donors since 1991. The PA allows us to determine the anti-HIV status continuously and efficiently to meet the continuous demand of blood supply and the requirements for patient testing in a limited time frame without using a rapid screening test which has a lower sensitivity,[1,7,14] and is more expensive.[1,2] The positive anti-HIV-1 cases, positive by PA, were further confirmed by WB and the anti-HIV-1 titer was also determined by PA in those samples. In this report, we analyse anti-HIV-1 titers in comparison with the results of WB profiles in order to evaluate whether anti-HIV-1 PA titers would be an alternative supplementary test for diagnosis of HIV-1 infection.

MATERIALS AND METHODS

Serum samples

2,414 samples of anti-HIV-1 positive sera from routine screening for anti-HIV during January 1991 to October 1996, were included in this study. The samples were from 410 blood donors and 2,004 patients from Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, northeast Thailand.

Laboratory testing

Anti-HIV-1 antibody titer

Sera were screened by PA assay (Serodia-HIV, Fujirebio, Japan). The screening test was performed as follows: twenty five microliters of 1:16 dilution of test serum was tested against 25 µl of HIV-1 whole viral lysate coated gelatin particles (sensitized particles) and 1:8 dilution of test serum was also tested with 25 µl of unsensitized gelatin particles on a V type microplate. The plate was agitated and left at room temperature for 1.5 - 2 hours. The result was read with the naked eye according to the standard positive patterns of the passive agglutination test. The results are valid only when the unsensitized particle control well gives a negative result. The anti-HIV-1 titer of a reactive sample was determined by a serial two-fold dilution starting from 1:250 to 1:2,000. The sera which were positive at a dilution of < 2,000 were rediluted from 1:16 to 1:2,048 and retested to obtain the actual titer.

Western blot

All screening reactive anti-HIV-1 sera were confirmed by Western blot (Diagnostic Biotechnology, Singapore). The interpretation of the Western blot was based on USA-CDC criteria.[14]

Enzyme linked immunosorbent assay

ELISA anti-HIV 1/2 testing using Detect-HIV™ (Biochem Immuno Systems Inc, Canada) was performed in the PA anti-HIV positive samples which showed negative or indeterminate WB results.

HIV p24 antigen

Qualitative HIV antigen assay by ELISA (Coulter, England) was routinely carried out in all blood donor samples as it is mandatory for blood transfusion safety and tested only in the patients samples which were positive anti-HIV-1 with either indeterminate or negative WB results. The positive screening HIV p24-antigen samples were confirmed by neutralization test.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at different cut off anti-HIV-1 PA titers with reference to WB results were calculated according to standard methods.[15] Confidence inter-

vals (95% CI) were calculated using the formula for one proportion.[16]

RESULTS

Particle agglutination titers of 2,414 sera were compared to the WB analyses as shown in Table 1. A total of 2,386 (98.84%) samples were WB reactive by CDC/USA criteria.[14] Seventeen samples with titers of 16 to 256 demonstrated indeterminate (ID) WBs. The eleven samples with titers of 16 to 64 were non reactive by WB and ELISA. Based on the titer ≥ 256, a total of 2,365 (99.96%) samples were WB positive, only one sample was indeterminate and none were negative. In addition, it was observed that none of the sera with PA titers ≥ 512 yielded indeterminate or negative WB results.

The sensitivity, specificity, PPV and NPV of the different cut off anti-HIV-1 PA titers with reference to WB results are presented in Table 2. The samples with an indeterminate WB pattern were included in the WB negative group for calculation. The Receiver Operator Characteristic (ROC),[15] plot of the sensitivity against false positive (1-specificity) rate of the different PA titers is also shown in Fig 1. At a titer of ≥ 512, the specificity was 100%, the sensitivity was 98.74%, PPV was 100% and the NPV was 48.28%. Therefore, the cut off point of PA titer ≥ 512 is appropriate for diagnosis of HIV infection since the false positivity is 0% and no indeterminate WB result

Table 1. The comparison of particle agglutination (PA) anti-HIV-1 titers with Western blot analyses.

PA results		Western blot results		
Titer value	No. cases	No. positive cases (%)	No. indeterminate cases (%)	No. negative cases (%)
16	16	2 (12.50)	8 (50)	6 (37.50)
32	9	1 (11.11)	5 (55.55)	3 (33.33)
64	9	4 (44.44)	3 (33.33)	2 (22.22)
128	14	14 (100)	0 (0)	0 (0)
256	10	9 (90)	1 (10)	0 (0)
512	18	18 (100)	0 (0)	0 (0)
1,024	1	1 (100)	0 (0)	0 (0)
≥ 2,000	2,337	2,337 (100)	0 (0)	0 (0)
Total	2,414	2,386 (98.84)	17 (0.70)	11*(0.46)

*These samples demonstrated negative anti-HIV by ELISA

Table 2. Sensitivity, specificity, PPV, and NPV of different cut off anti-HIV-1 PA titers with reference to WB

PA titers	Sensitivity		Specificity		PPV		NPV	
	%	95 CI	%	95 CI	%	95 CI	%	95 CI
≥ 32	99.92	99.8-100.0	50.00	48.0-52.0	99.42	99.1-99.7	87.50	86.2-88.8
≥ 64	99.87	99.7-100.0	78.57	76.9-80.2	99.75	99.5-99.9	88.00	86.7-89.3
≥ 128	99.71	99.5-99.9	96.43	95.7-97.2	99.96	99.9-100.0	79.41	77.8-81.0
≥ 256	99.12	98.7-99.5	96.43	95.7-97.2	99.96	99.9-100.0	56.25	54.3-58.2
≥ 512	98.74	98.3-99.2	100.00	100.0-100.0	100.00	100.0-100.0	48.28	46.3-50.3
≥ 1,024	97.99	97.4-98.5	100.00	100.0-100.0	100.00	100.0-100.0	36.84	34.9-38.8
≥ 2,000	97.95	97.4-98.5	100.00	100.0-100.0	100.00	100.0-100.0	36.36	34.4-38.3

Table 3. The correlation between the PA titers, Western blot protein band, HIV p24 antigens and ELISA anti-HIV of the 17 indeterminate cases

Case No.	PA titer	HIV Ag	Western blot protein bands						Anti-HIV ELISA
			p17	p24	p39	p55	p64	gp160	
1[a], 2[a]	16	-	+	-	-	-	-	-	-
3[a]	16	-	-	+		-	-	-	-
4[a]	16	-	-	-	-	-	+	-	-
5[b], 6[b]	32	-	+	-	-	+	-	-	-
7[b]	64	-	+	-	-	-	-	-	-
8, 9	16	-	+	-	-	-	-	-	-
10	16	-	-	-	+	-	-	-	-
11	32	-	+	-	-	-	-	-	-
12	32	-	-	+	-	-	-	-	-
13	16	+	-	+	-	-	-	-	±
13(+13wks)	≥2,000	-	all protein bands						+
14	32	+	-	+	-	-	-	-	+
14(+17day)	512	-	-	+	-	-	-	+	+
15	64	-	-	+	-	-	-	-	+
15+(+1yr)	≥2,000	-	all protein bands						+
16	64	+	-	-	-	-	-	+	+
16(+7wks)	1,024	-	all protein bands						+
17	256	-	-	+	-	-	-	-	+
17(+3wks)	1,024	-	-	+	-	-	-	+	+

[a] The second sample which was collected from cases No. 1, 2, 3, 4 was available at 2, 6, 2, 12 weeks, respectively, showed negative anti-HIV by PA and ELISA.
[b] The follow up blood sample from cases No. 5, 6, 7 was available at 2, 3, 12 weeks, respectively, gave the same PA titer and Western blot protein bands but negative anti-HIV by ELISA.

was found at this titer (Table 2 and Fig.1).

Details of the WB analyses including HIV p24 antigen, ELISA anti-HIV of the 17 indeterminate sera are shown in Table 3. The follow up blood samples were available from 12 of these 17 indeterminate cases. Second specimens collected from cases Nos. 1, 2, 3, 4 at 2, 6, 2, 12 weeks, respectively, were anti-HIV negative by PA and ELISA. The follow up specimens collected from cases Nos. 5, 6, 7 at 2, 3, 12 weeks, respectively, gave the same PA titer and WB protein bands with negative anti-HIV by ELISA. There were 5 cases (Nos. 13, 14, 15, 16, 17) of early seroconversion which were confirmed to be positive HIV infections in second specimens. The remaining 5 cases (Nos. 8, 9, 10, 11 and 12) with negative anti-HIV by ELISA, were lost to follow up.

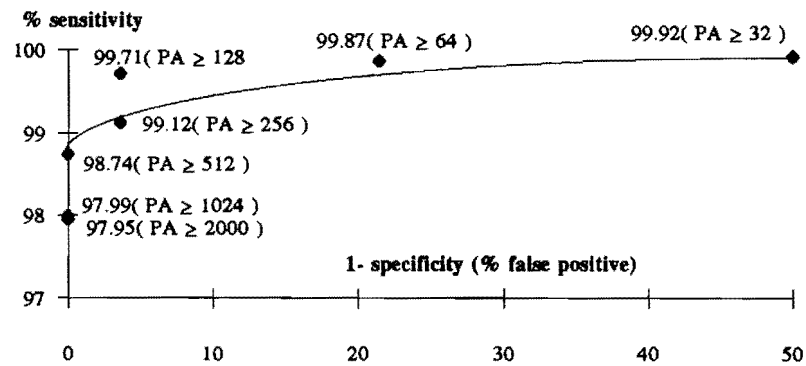

Fig. 1 Receiver Operator Characteristic (ROC) curve of sensitivity and false positive rate at different anti-HIV PA titers with reference to WB

DISCUSSION

The PA titer can be assessed easily at any time without

the requirement of expensive equipment, or electricity with only one step antigen-antibody reaction. The result can be achieved within 1.5 hours. In addition, PA can detect some early seroconversion cases. Constantine, *et al.*[17] determined the sensitivity of eight HIV-antibody assays (2 rapid dot blot, 5 ELISA and PA) in seroconversion compared to the Abbott HIV-1/2 third generation ELISA. The PA exhibited the best sensitivity of the eight tests, with a mean detection delay of 0.5 days, and the PA detected anti-HIV 7 days prior to the Abbott ELISA reference test in one panel. WB is more specific but not as sensitive as a screening test for anti-HIV antibodies. It may yield either indeterminate or negative results in the early period of seroconversion.[5] Studies have shown that currently available supplementary antibody assays are not as sensitive as the screening test for the detection of antibodies to HIV-1 in early seroconversion cases.[11,16] We were able to detect early seroconversion samples that gave an indeterminate WB result in the primary specimen which was consistent with that reported by others in suggesting that the PA assay is among the most sensitive tests for anti-HIV.[5,8,17,18]

We demonstrated that 98.84% (2,386 of 2,414) of the PA positive sera were confirmed by WB, 17 samples (0.7%) were indeterminate and 11 samples (0.46%) were negative by WB (Table 1). The negative WB results were found in sera with titers of ≤ 64. All samples with titers of ≥ 512 (97.6%) were WB reactive. The results of this study demonstrated that 96.8% (2,337 of 2,414) of positive anti-HIV sera contained a high titer (≥ 2,000) of anti-HIV antibodies, only 3.2% (77 in 2,414) had a titer of 16 to 1,024. Among the 58 sera which demonstrated anti-HIV titers < 512 (2.4%), 30 samples with titers of ≤ 256 were WB positive, 17 samples gave indeterminate WB and 11 samples were negative by WB and ELISA.

For implementing PA anti-HIV-1 titers as a supplementary test for diagnosis of HIV infection, the cut-off titer should give high specificity without false positivity. Indeterminate WB can be found in uninfected and in early seroconvertion,[2,7,10,11] as was also found in this study. Therefore, cases of inconclusive HIV status (indeterminate WB) were included in the WB negative group for calculation of sensitivity, specificity, PPV and NPV (Table 2). Those sera with titer ≥ 512 (2,356 or 97.6%) gave 100% specificity with no false positivity or an indeterminate WB result (Fig. 1 and Table 1) can be considered as definitely positive. Therefore, the cut-off for anti-HIV-1 PA titer for diagnosis of HIV-1 infection is ≥ 512. Practically the serum with a PA titer ≥ 512 needs no further confirmation by WB.

Among the 17 indeterminate samples, the follow up specimens were available from 12 cases. Five cases (Nos. 13, 14, 15, 16, 17 in Table 3) were examples of early seroconversion which could not be confirmed by WB in the primary samples but they were confirmed in the follow up specimens demonstrating increased PA titers. The second specimen from cases Nos. 1, 2, 3, 4 gave negative anti-HIV testing by PA and ELISA, indicating false positive anti-HIV by PA in the primary samples of these cases. The results of anti-HIV by PA and WB obtained from the follow up specimens from cases Nos. 5, 6, 7 remained unchanged and they were negative by anti-HIV ELISA in both primary and follow up samples, may therefore also be considered to be negative for HIV-1 infection. Unfortunately, we were unable to follow up the rest of the 5 cases with PA titers of 16 to 32 (Nos. 8, 9, 10, 11, 12) which gave indeterminate WB results but were negative by ELISA. This study also demonstrated that the presence of antibodies to p24 or gp160 is a predictive marker of early seroconversion for HIV infection.[19] On the other hand, we demonstrated that the early seroconversion of HIV infection gave low titers of anti-HIV by PA. Thus, we propose that the determination of anti-HIV antibody titer can be applied as an alternative supplementary test for confirmation of HIV infection and probably the occurrence of the early seroconversion in HIV infected persons can be presumed by the low anti-HIV PA titer. By this strategy, the confirmation of HIV testing can be performed in any laboratory or health center. The cost of confirmation per test by titration is also reduced to about one-fifth of the WB costing. If the anti-HIV PA titer determination is applied, only 2.4% (58 of 2,414) of positive samples by screening tests from this study needed WB confirmation.

Based on our finding, we propose the procedure for the serodiagnosis of HIV samples as follows: 1) PA titration can be implemented as a supplementary test for diagnosis of HIV infection, 2) serum samples with PA titers ≥ 512 are considered as anti-HIV positive

and 3) samples with PA titers < 512 should be interpreted with care, confirmed by WB and followed up in the instance of indeterminate cases. This recommendation could reduce the cost by reducing use of WB, as well as the work load and time consumption. Furthermore, a laboratory report of the HIV tests in most HIV positive cases, *ie* screening and confirmation tests, can be concluded within 4 hours.

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