Anti-HIV-1 Antibody Testing Using Modified Gelatin Particle Agglutination : A Large Field Study

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Abstract

Anti-HIV testing using gelatin particle agglutination (GPA) assay was investigated in parallel with ELISAs from routine service at Siriraj Hospital. In the first strategy, 174,032 sera from a patient population with an HIV-1 seroprevalence of 13.72 per cent were assayed using reduced volumes of GPA reagents, giving a cost reduction of 40 per cent. In the second strategy, 90,560 pregnant women and 48,936 emigrant workers with an HIV-1 seroprevalence of 2.2 per cent and 0.3 per cent, respectively, were tested in pools of 4 sera using the manufacturer's recommended volumes, giving a cost saving of 67 per cent. Overall, the sensitivity and specificity were almost identical with standard methods. Thus, parallel use of either modified GPA might be considered appropriate when testing large numbers of samples. However, both modified versions of GPA are not recommended as the first assay for diagnostic or blood bank screening especially in high prevalence of HIV infection.

Key word : Anti-HIV, Gelatin Particle Assay, Reduced Volume, Pooled Sera

LOUISIRIROTCHANAKUL S, KANOKSINSOMBAT C, THONGPUT A, PUTHAVATHANA P, WASI C
J Med Assoc Thai 2001; 84: 1708-1713

According to the World Health Organization's algorithm for testing of anti-HIV antibodies (1992, revised 1997), two or three screening assays can be used to confirm their presence in initially reactive sera, depending on the prevalence of HIV infection in the local population and on the purpose of the test[1,2]. Several assays are available for anti-HIV antibody detection[3]. These include gelatin

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particle agglutination (GPA) which because of its comparable sensitivity and specificity to enzyme-linked immunosorbance assays (ELISA), ease of use and competitive cost has been widely used, either as the initial screen or as part of the supplementary testing[4-6].

In Thailand, HIV-1 GPA was first introduced for serosurveillance among injecting drug users (IDUs) in 1988[7] and, after the early period of the HIV-1 epidemic and was found to be more sensitive than the first generation ELISAs. Thereafter, GPA became, and remains, widely used especially in regional hospitals and central laboratories. For the last eight years the authors have routinely tested all diagnostic samples in parallel by both an ELISA and by GPA to reduce laboratory error and to detect discrepant results. The possibility of reducing the costs of HIV testing without loosing sensitivity or specificity has been explored both by reducing the volume of GPA reagents and by pooling sera [8,9]. It was, therefore, decided to evaluate these strategies in two populations. In the first population, with a seroprevalence of greater than 10 per cent, the reduced volume GPA was assessed whilst in the second population, with a seroprevalence of less than 10 per cent, pools of 4 sera were screened by an otherwise unmodified GPA assay. All samples were also tested individually by ELISA.

MATERIAL AND METHOD

Reduced Volume GPA (rvGPA)

From 1992 to 2000, 174,032 sera were obtained from patients attending Siriraj Hospital for routine testing of HIV (HIV-1/HIV-2) antibodies using second generation ELISAs (Genelavia mixt, Sanofi Pasteur, France; Innotest, Innogenetic, Belgium; Enzygnost, Behring, Germany; and Vironostika, Organon, Belgium) or third generation ELISAs (Access, Pasteur Institute, France; and AxSYM, Abbott, USA). The prevalence of HIV-1 in this population was 13.72 per cent. All individual sera were tested in parallel for anti-HIV antibodies by a modified GPA (Serodia HIV-1, Fujirebio Inc, Tokyo, Japan) in which the volumes of the reagents were reduced but kept in the same proportion as recommended by the manufacturer (Fig. 1). Briefly, serum (15 µl, at final dilution 1:8 and 1:16) was incubated with non-sensitized (15 µl, at 1:8) and sensitized particles (15 µl, at 1:16) in separate wells for at least 2-24 hours at room temperature. Any sera giving a visible agglutination of gelatin particle with the sensitized particles which did not agglutinate non-sensitized particles were considered as 'reactive' result. All reactive sera were tested with a second ELISA as a supplementary assay as per WHO recommendations for anti-HIV-1 antibody testing[2]. Any sera giving ELISA/rvGPA concor-

Standard GPA	well #1	well #2	well #3	
Serum diluent (µl)	75	25	25	
Serum specimen (µl)	25	25	25	discard
Serum dilution	1:4	1:8	1:16	
Unsensitized particles (µl)		25		
Sensitized particles (µl)			25	
Final dilution		1:16	1:32	
Modified GPA with reduced volume	**well #1**	**well #2**	**well #3**	
Serum diluent (µl)	45	15	15	
Serum specimen (µl)	15	15	15	discard
Serum dilution	1:4	1:8	1:16	
Unsensitized particles (µl)		15		
Sensitized particles (µl)			15	
Final dilution		1:16	1:32	

Fig. 1. Volume of sample and reagents in standard GPA and modified GPA with reduced volume (rvGPA).

dant results were confirmed by either western blot (Diag. Biotechnology, Singapore) or by retesting with other screening assays as an alternative confirmation strategy. The second blood was requested for reconfirmed true positive. All sera giving ELISA/rvGPA discordant results were investigated by western blot and interpreted in accordance with the manufacturer's recommendation. Follow-up sera were collected 3-6 months later from patients with indeterminate results.

Pooling of Sera (pooled GPA)

A total of 139,496 sera (90,560 pregnant women with the HIV-1 seroprevalence of 2.2 per cent and 48,936 emigrant workers with the prevalence of 0.3%) were tested individually for anti-HIV antibodies using ELISA as described above. The same sera were tested in pools of four sera by GPA using the method recommended for testing individual serum i.e., 25 µl of gelatin particles were incubated with 25 µl of diluted serum made up of equal volumes from four samples (Fig. 1). Each sera from any pool which gave a reactive or suspicious agglutination pattern were further tested individually. All reactive results and discordant results were further investigated as described above.

Cost-effectiveness of Anti-HIV Antibody Testing with the Two Modified Assays

Both the rvGPA and the pooled GPA methods were compared to standard GPA in a cost effectiveness analysis based on testing 2000 sera in a population with an HIV seroprevalence of 2 per cent.

RESULTS

Reduced Volume GPA (rvGPA)

Of the 174,032 sera assayed in parallel by both ELISA and rvGPA, 23,876 were reactive in both assays and confirmed anti-HIV-1 antibody positive by supplementary tests and/or western blot. A total of 56 sera (0.32%) were reactive in only one screening assay; 18 sera were monoreactive by rvGPA and 38 by ELISA. These discordant results were further confirmed by western blot assay. The presence of anti-HIV antibodies were confirmed in five out of 18 (27%) sera monoreactive by GPA but only in 3 of 38 (8%) sera monoreactive by ELISA. Thus, rvGPA detected 23,881 and ELISA 23,879 of 23,884 sera confirmed HIV-1 seropositive samples. The overall sensitivity (GPA 99.99%, ELISA 99.98%) and specificity (GPA 99.99%, ELISA 99.98%) were almost identical in these populations with an overall HIV-1 seroprevalence of 13.72 per cent (Table 1).

Table 1. Results of anti-HIV tested by reduced volume of GPA (rvGPA) and ELISA in 174,032 sera from hospitalized patients with seroprevalence of HIV 13.72%*.

	Number tested by	
	rvGPA	ELISA
True positive	23,881	23,879
False positive	13	35
True negative	150,135	150,113
False negative	3	5
Sensitivity (%)	99.99	99.98
Specificity (%)	99.99	99.98

* 56 sera with discordant results, 18 sera monoreactive by rvGPA and 38 by ELISA, were further confirmed by western blot

Pooling of Sera (pooled GPA)

Preliminary investigation of pools of 4, 8 and 10 sera (3, 7 or 9 negative sera mixed with one serum selected from a panel of 26 Thai HIV-1 positive sera suggested that as many as 10 sera could be pooled without loss of sensitivity (data not shown). The optical density of the pools of 10 by third generation ELISA and the agglutination pattern by GPA were still strongly reactive. In addition, anti-HIV from pools of 10, 8 and 4 sera (9, 7 or 3 negative sera mixed with reference sera with a GPA titer of 1/128) were still detected by GPA. However, to avoid missing sera with low titres of HIV-1 antibodies, pools of 4 sera were chosen for the study. All 2138 HIV-1 samples reactive by ELISA were also detected by screening pools of 4 sera. No discrepant result was found in the 22,640 and 12,334 pools of sera from pregnant women and emigrant workers, respectively.

Cost-effectiveness of Anti-HIV Antibody Testing with the Two Modified Assays

Cost savings of reducing the volume of reagents was 40 per cent, whereas, using pools of 4 sera reduced costs by 67 per cent as shown in Table 2.

Table 2. The cost-effectiveness of GPA in reduced volume (rvGPA) and pooling system with the prevalence of HIV in a population of 2% in Thailand.

	Individual serum		Pools of 4 sera
	Standard GPA	rvGPA	Standard GPA
(A) Number of initial tests	2,000	2,000	500
(B) Total cost of tests performed* (Ax100 baht/standard GPA or Ax60 baht/ rvGPA)	200,000	120,000	50,000
(C) Expected number of anti-HIV-1 positive samples	40	40	40
(D) Expected number of confirmation from reactive result	40	40	160
(E) Total cost of tests performed (Dx100 baht/standard GPA or Dx60 baht/ rvGPA)	4,000	2,400	16,000
(F) Total number of tests performed (A+D)	2,040	2,040	660
(G) Total cost of tests performed (B+E)	204,000	122,400	66,000
Relative cost saving		40%	67%

* Cost saving of using rvGPA (40%) (46 baht =1US dollar)

DISCUSSION

Previous studies have shown GPA and ELISA to have the same sensitivity and specificity for the detection of anti-HIV-1 antibodies[4,5,7]. However, during the initial period of the epidemic, when HIV-1 spread rapidly amongst Thai IDUs with the seroprevalence increasing from <1 per cent in 1987 to 44 per cent in 1988[10] HIV seroprevalence rate by GPA (and confirmed western blot) was higher (25.08%) than that detected by ELISA (20.68%)[7]. Moreover, HIV-1 Ag could be detected in 15 per cent of those reactive by GPA but not by ELISA suggesting the inability of the ELISA to detect anti-HIV IgM in early infection (unpublished data). Consequently, third generation ELISAs using the double sandwich principle, in which chemiluminescence appears with both IgM and IgG antibody isotypes reactive to an HIV Ag coated plate, were developed which will detect anti-HIV antibodies in early HIV infection[11].

Urwijiteroon et al reported high sensitivity (100%) and specificity (99.5%) in 1,569 sera, compared with ELISA and WB using a method in which the reagents used in the standard GPA assay were reduced by 40 per cent to reduce the cost of the assay[8]. The authors found that the titers of anti-HIV-1 antibodies in clinical samples were the same as both the standard and the reduced volume assay (data not shown). This reduced volume GPA assay was compared with the ELISAs routinely used in the laboratory and found their sensitivity and specificity to be comparable after testing 174,032 sera.

Introduced in 1988-89 the testing of pools of sera for seroprevalence studies has been shown to be cost effective[12-15] and both sensitive and specific with pools of 5-15 sera[13-15] for screening large numbers of samples. Subsequently, the accuracy and cost-efficiency of using pools of up to 20 sera screened by GPA have also been demonstrated[14]. However, limiting the size of the pool to 4 sera has been recommended for diagnostic tests in order to detect sera with low titres of anti-HIV-1 antibodies. The authors have confirmed the validity of using pools of 4 in a study of 139,493 sera from a population with an overall seroprevalence of 1.53 per cent showing equivalent detection rates with this and standard ELISAs confirmed according to WHO recommendations.

The present findings suggest that the cost savings of using the reduced volume assay (40%) and pools of 4 sera with the unmodified assay (67%) can be obtained without loss of sensitivity or specificity. Nevertheless, it is not recommended to apply either the modified version of the GPA assay as the first assay for diagnostic or blood bank testing in populations with a high prevalence of HIV infection. It has also been demonstrated that up to 26 per cent of HIV-2 positive sera were missed by the HIV-1 GPA assay[16] and, therefore, it is not recommended to use either modification in popula-

tions where HIV-1 and HIV-2 infections coexist. However, false negatives are reported for synthetic oligopeptide ELISAs[17] and the parallel use of either modified GPA might be considered appropriate when testing large numbers of samples such as in donated blood and pregnant women to avoid false negative results.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Graham Taylor, Imperial College, London, UK, for the manuscript preparation. We also wish to thank the staff of the HIV serology laboratory, Division of Virology, Department of Microbiology for their excellent work throughout this long study.

(Received for publication on September 4, 2001)

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แอนติบอดีย์ต่อเชื้อเอ็ชไอวีในงานบริการด้วยวิธีดัดแปลงการเกาะกลุ่มของอนุภาคเจลาติน : การศึกษาในภาคสนาม

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ได้ทำการประเมินผลการศึกษาแอนติบอดีย์ต่อเชื้อเอ็ชไอวี (anti-HIV) ด้วยวิธี gelatin particle agglutination (GPA) ควบคู่กับวิธี ELISA ในงานบริการของโรงพยาบาลศิริราช โดยใช้แนวทางปฏิบัติ 2 แบบ แบบแรกทำการทดสอบด้วยการลดปริมาณน้ำยาของ GPA ในกลุ่มผู้ป่วยซึ่งมีอุบัติการณ์การติดเชื้อเอ็ชไอวีร้อยละ 13.72 จำนวนทั้งหมด 174,032 ราย ผลการประเมินได้ความไวและความจำเพาะเกือบเทียบเท่ากับวิธี ELISA ซึ่งสามารถลดต้นทุนได้ร้อยละ 40 แบบที่สองทำการทดสอบด้วยวิธีมาตรฐานของ GPA ในทุกซีรัมซึ่งรวม 4 รายด้วยปริมาณเท่า ๆ กัน ในกลุ่มหญิงฝากครรภ์จำนวน 90,560 ราย และผู้ใช้แรงงานที่ไปทำงานต่างประเทศจำนวน 48,936 ราย ซึ่งมีอุบัติการณ์การติดเชื้อเอ็ชไอวีร้อยละ 2.2 และร้อยละ 0.3 ตามลำดับ ผลการประเมินได้ความไวและความจำเพาะใกล้เคียงกับวิธี ELISA และสามารถลดต้นทุนได้ ร้อยละ 67 ดังนั้นแนวทางปฏิบัติด้วยวิธีดัดแปลง GPA ดังกล่าวอาจนำไปพิจารณาทดสอบควบคู่ในเลือดผู้บริจาคได้ อย่างไรก็ตามไม่แนะนำให้นำไปใช้เป็นทางเลือกวิธีแรกในการวินิจฉัยการติดเชื้อหรือตรวจเลือดบริจาค โดยเฉพาะในบริเวณที่มีความชุกของการติดเชื้อเอ็ชไอวีสูง

คำสำคัญ : แอนติบอดีย์ต่อเชื้อเอ็ชไอวี, การเกาะกลุ่มของอนุภาคของเจลาติน, ปริมาณน้ำยาลดส่วน, ซีรัมรวมหลายตัวอย่างตรวจ

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