A Simple Procedure for Obtaining Large Amounts of HIV Antigens for Serodiagnostic Purposes

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The established method of purifying human immunodeficiency virus (HIV) involves centrifuging the lysates of infected cells onto a sucrose gradient. This article reports an alternative methodology for purifying large amounts of HIV antigens that avoids the need for expensive equipment such as an ultracentrifuge and requires no purification gradient. These semipurified antigens have been used to perform a "homemade" confirmatory Western blot assay that produced reactivity comparable to that obtained with a commercial Western blot assay. Although the "homemade" procedure did not detect one important viral protein (env *gp160), this purification method seems to offer a suitable and economical means of obtaining antigens to confirm the presence of antibodies to HIV.*

Several assays for detection of human immunodeficiency virus (HIV) antibodies have been developed. Among these, the enzyme-linked immunosorbent assay (ELISA) (*1*) and the Western blot test (*2, 3*) are the methods of choice used respectively for screening sera and confirming seropositivity. Nearly all these tests utilize viral antigens that are either purified by sucrose density gradient centrifugation (*4, 5*) or obtained by genetic engineering (*6, 7*). The consequent need for high-cost equipment and reagents such as ultracentrifuges and approved Western blot tests precludes routine use of HIV confirmatory assay in laboratories lacking resources. In this communication we report the results of an effort to devise a simpler, cheaper, and less time-consuming way to obtain viral antigens suitable for serologic confirmation of HIV infection.

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MATERIALS AND METHODS

A lymphoblastoid H9 cell line infected with productively replicating HIV-1, kindly provided by Dr. R. C. Gallo of the U.S. National Cancer Institute in Bethesda, Maryland, USA, was cultured as described elsewhere (*8*). The culture medium (RPMI 1640 plus 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin) was changed every three or four days.

Virus Purification

A differential centrifugation protocol for virus purification was followed. The infected cells (10^6/ml) were initially spun down at low speed (200 times the acceleration of gravity [g] for 10 minutes at 4°C). The supernatant was then centrifuged at 5,000 g for 30 minutes at 4°C to remove cellular debris, and the viruses present in the supernatant were pelleted by centrifuging at 41,000 g for 2 hours at 4°C. The device used was a Beckman J2-21 centrifuge with a JA 21 rotor. The supernatant

was carefully removed, and the viral pellet was resuspended at one five-hundredth of the original volume in 0.01 M Tris, 0.15 M NaCl, and 0.25% Triton-X-100 nonionic detergent at a pH of 7.2. The protein concentration was determined by Lowry's technique (*9*), and the viral suspensions were maintained at −70°C until use.

Western Blot

A sample of semipurified HIV antigen was dissociated in sodium dodecyl sulfate (SDS) and 2-mercaptoethanol, and was subjected to SDS–gel electrophoresis in a 10% acrylamide gel. The resulting protein bands were then transferred to nitrocellulose paper (Schleicher and Schuel, Dassel, Federal Republic of Germany) in a transblot apparatus (BioRad Laboratories, USA) at 40 volts over the course of 14–16 hours. Twenty percent methanol in 0.025 M Tris and 0.192 M glycine was used as a transfer buffer. The nitrocellulose paper was blocked (that is, the remaining protein-binding sites were inactivated) in a 0.3% Tween 20 and phosphate-buffered (pH 7.2) saline solution with 5% non-fat dry milk for 60 minutes. The strips were then incubated with serum samples diluted 1:100 in the same buffer for 60 minutes. All incubations and washings (in PBS/0.3% Tween 20) were carried out at room temperature.

The strips were subsequently incubated with a goat anti-human horseradish peroxidase labeled IgG diluted 1:1,000 in blocking solution for 60 minutes. After washings, the strips were incubated with a chromogenic substrate consisting of diaminobenzidine (0.25 mg per ml), citrate-phosphate buffer (pH 5.0), and 0.001% H_2O_2. The reaction was stopped by immersion of the strips in distilled water.

For comparison, a commercially available Western blot assay (Du Pont Company, Wilmington, Delaware, USA) was carried out according to the manufacturer's instructions.

Serum Samples

Serum samples were obtained from 85 patients. Twenty-one of these patients had the acquired immunodeficiency syndrome (AIDS), 17 had the AIDS-related complex (ARC), 27 had persistent generalized lymphadenopathy (PGL), and 20 had asymptomatic HIV infections. The clinical criteria used to evaluate the clinical status of the AIDS, ARC, and PGL cases were those of the U.S. Centers for Disease Control in Atlanta, Georgia (*10*). The serum samples were found to be positive by ELISA (*1*), indirect immunofluorescence (IIF) (*11*), and Western blot assay (Du Pont Company, Wilmington, DE, USA). Sera from six healthy persons without any evidence of HIV infection were included as negative controls.

RESULTS

The usual yield of the semipurification procedure was 100–120 μg of total protein per 50 ml of infected cell supernatant. Although the viral pellets were contaminated with proteins of culture medium and/or cellular origin, as evidenced by Red Ponceau staining of antigens transferred to nitrocellulose paper (data not presented), these contaminants did not interfere with immunostaining of the viral proteins. However, because of their presence we did not determine the actual amount of viral protein in the pellet.

In general, a good correlation was found between the results obtained with our "homemade" Western blot assay and with the commercial assay (Figure 1). However, some differences were observed in the intensity of reactivity to the *gag* p55, *env* gp120, and gp160 antigens. Some AIDS sera that did not react with

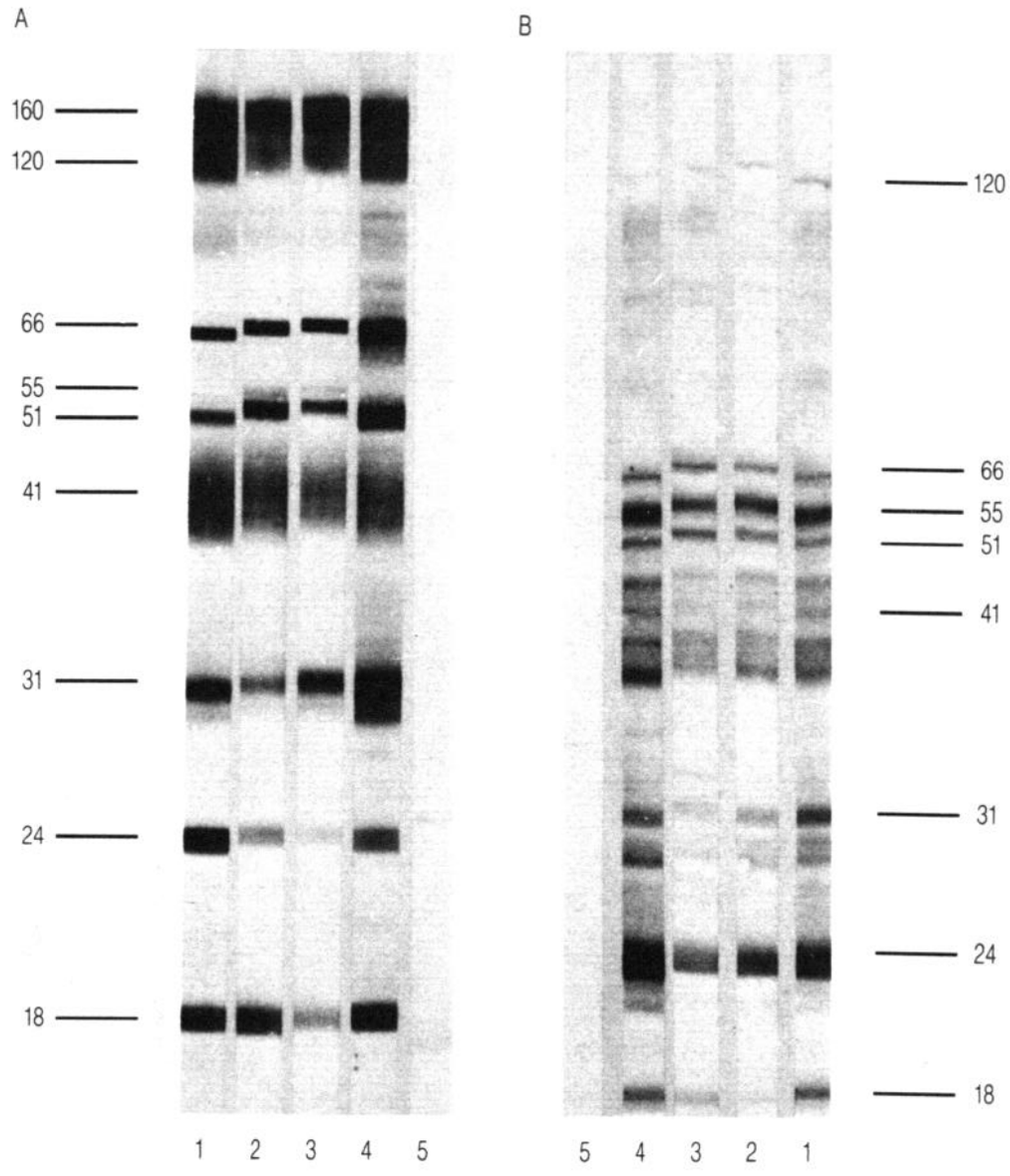

Figure 1. Photographs showing the reactivity of IgG antibodies to HIV-1 antigens electroblotted onto nitrocellulose strips from (A) the commercial Western blot assay and (B) our "homemade" Western blot assay (7 μg of protein per strip). The numbered strips were tested against sera from patients with AIDS (1), the AIDS-related complex (2), persistent generalized lymphadenopathy (3), and asymptomatic HIV infection (4), as well as against normal human sera (5). The numbers at the left and right represent molecular weights (in daltons $\times 10^{-3}$).

p55 of the commercial kit did react with the p55 of our Western blot assay (Table 1). On the other hand, the commercial Western blot assay consistently showed a reaction to gp160 in all appropriate sera, while evidence of this reaction was absent when our semipurified antigens were used.

In the course of this work, we verified that it was necessary to maintain the percentage of cells expressing the virus above 90% in order to avoid any appreciable background. At the opposite extreme, when we obtained the virus from a culture where only about 30% of the cells were expressing the virus (as indicated by IIF), the yield was so low that we were unable to perform an adequate assay (data not presented).

DISCUSSION

Over the past three years HIV antibody tests have transformed our understanding of the epidemiology of AIDS and HIV infection. The starting point was the find-

Table 1. Comparison of the Western blot assay results obtained with our "homemade" method and the commercial (Du Pont) assay. Aside from negative controls (not shown), the sera tested were from patients with AIDS, the AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), and asymptomatic HIV infection (HIV). "gp" = glycoprotein, "p" = protein.

		Sera testing positive from patients with:							
		AIDS (n=21)		ARC (n=17)		PGL (n=27)		HIV (n=20)	
Band	Antigen	No.	(%)	No.	(%)	No.	(%)	No.	(%)
gp160	"Homemade"	0	(0)	0	(0)	0	(0)	0	(0)
	Commercial	20	(95)	17	(100)	27	(100)	20	(100)
gp120	"Homemade"	9	(43)	16	(94)	27	(100)	13	(65)
	Commercial	20	(95)	17	(100)	27	(100)	19	(95)
p66	"Homemade"	20	(95)	17	(100)	27	(100)	20	(100)
	Commercial	21	(100)	16	(94)	27	(100)	20	(100)
p55	"Homemade"	17	(81)	15	(88)	25	(93)	20	(100)
	Commercial	4	(19)	10	(59)	11	(41)	13	(65)
p51	"Homemade"	18	(86)	17	(100)	27	(100)	20	(100)
	Commercial	17	(81)	15	(88)	27	(100)	20	(100)
gp41	"Homemade"	21	(100)	15	(88)	27	(100)	18	(90)
	Commercial	20	(95)	15	(88)	27	(100)	16	(80)
p31	"Homemade"	18	(86)	15	(88)	26	(96)	16	(80)
	Commercial	18	(86)	14	(82)	26	(96)	15	(75)
p24	"Homemade"	12	(57)	15	(88)	24	(89)	19	(95)
	Commercial	17	(81)	17	(100)	26	(96)	19	(95)
p18	"Homemade"	9	(43)	13	(76)	22	(81)	13	(65)
	Commercial	15	(71)	8	(47)	22	(81)	16	(80)

ing of cell lines suitable to serve as hosts for the growth of the virus (*8*). This permitted establishment of serologic procedures for screening and confirmatory diagnosis. However, the costs of confirmatory tests such as the Western blot are so high as to hamper their routine use in countries that have insufficient resources.

The data presented here indicate that a cheaper and simpler methodology could be employed to procure suitable amounts of viral antigens for use in a Western blot confirmatory assay. However, we have observed some differences between the results obtained with our "homemade" Western blot assay and with a commercial product. In particular, we found no evidence of one important viral protein, *env* gp160. This could be explained by a partial loss of the envelope proteins during the "homemade" antigen centrifugation procedure (*12*). On the other hand, our preparations seem to contain higher concentrations of *gag* p55, because some AIDS sera that did not react with *gag* p55 in the commercial test did so when tested with our antigen preparation.

A point to note regarding the suitability of our antigen preparation method is that the results with all of the positive sera tested, representing a broad range of clinical forms of HIV infection, met the Western blot positivity requirements of the World Health Organization; that is, each test serum's immunoglobulins reacted with at least one polypeptide from each of the virus's three structural genes (*env*, *gag*, and *pol*) (*13*). On the other hand, in order to provide a complete evaluation of the specificity of our "homemade" Western blot test, and also of the commercial test, it would be appropriate to test sera from subjects with local

endemic infections such as Chagas' disease, leishmaniasis, and malaria—because these diseases produce immunologic disorders that might confuse the immunoassays.

Overall, the results obtained with our semipurified antigens procured through simple differential centrifugation indicate that such antigens can be utilized to perform the Western blot assay. It thus appears that this simple method for obtaining HIV antigens can serve as a good alternative for laboratories with limited resources available to purchase expensive equipment and reagents.

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