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Comparison of Sensitivities and Specificities of Latex Agglutination and an Enzyme-Linked Immunosorbent Assay for Detection of Antibodies to the Human Immunodeficiency Virus in African Sera

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The sensitivities, specificities, and positive and negative predictive values of the Cambridge BioScience Corp. (Worcester, Mass.) human immunodeficiency virus latex agglutination assay were compared by using three different blood preparations. By using the manufacturer's standard test method with diluted sera, the sensitivity of latex agglutination was 100%, the specificity was 99.58%, and the positive and negative predictive values were 99.26 and 100%, respectively. Use of diluted whole blood or undiluted whole blood did not significantly affect the sensitivity (mean, 99.72%), specificity (mean, 99.47%), positive predictive value (mean, 99.07%), or negative predictive value (mean, 99.89%). The latex agglutination assay is a simple, rapid assay for the detection of human immunodeficiency virus that would be useful in Third World countries or other areas where enzyme-linked immunosorbent assays are not available or cannot be used.

Since the appearance of the human immunodeficiency virus (HIV) in 1983 (1, 4), it has become a major international health problem affecting 5 million to 10 million people worldwide (7). To quantify the populations at risk, public health officials have used the enzyme-linked immunosorbent assay (ELISA) to identify infected individuals (6). The ELISA, which is very sensitive and specific when used with the Western blot (immunoblot) for confirmation, is unfortunately too expensive for Third World countries to implement or, in some cases, too slow to give results for their needs, i.e., at blood transfusion centers where HIV results are required in 15 min or less. Recognizing these problems, biotechnology companies are developing rapid HIV diagnostic tests which give results in less than 1 h and which are cheap, easy to use, and as sensitive and specific as the ELISA (2, 8, 10, 11).

We used a latex agglutination assay (LA; Cambridge BioScience Corp., Worcester, Mass.) in the Mama Yemo Hospital blood bank, Kinshasa, Zaire, to evaluate efficacy under conditions in which transfusions are normally given within 15 min of receipt of blood from a donor and in which approximately 6 to 8% of the donors are asymptomatic carriers of HIV (5; J. M. Mann, Int. Conf. AIDS, 1986).

Serum samples from 1,482 patients from the internal medicine wards, outpatient clinics, and blood bank of the Mama Yemo Hospital that were sent to the Project SIDA laboratories (Kinshasa) for HIV ELISA were analyzed. The patients were 771 children ranging in age from 1 month to 14 years and 711 adults between the ages of 15 and 65 years.

Whole blood was collected from each patient and tested by LA within 4 h of receipt. The plasma was stored at −20°F until ELISAs and Western blot assays could be done.

All sera were then analyzed for the presence of HIV antibody with the Wellcozyme assay (Wellcome Diagnostics, Dartford, England). Positive sera were repeatedly reactive in two separate ELISAs, and the results were confirmed by Western blot. The sera were confirmed positive by Centers for Disease Control criteria for Western blots (3).

The LA was performed with recombinant gp120 antigen (CBre3) attached to 0.5-μm polystyrene beads and suspended in 1% bovine serum albumin in phosphate-buffered saline (pH 7.6). The recombinant antigen (CBre3) was previously described (8, 12). The recombinant DNA derived from portions of the HIV envelope gene (*env*) was expressed in *Escherichia coli* by using a lambda pL expression vector. The polypeptide contains 17 N-terminal amino acids derived from phage gamma and nucleotide linker sequences followed by HIV *env* amino acids 350 to 518 and 542 to 669, by the number system of Rattner et al. (9). The expressed polypeptide was purified by standard chromatography procedures and was shown to be free of contamination with *E. coli* antigens.

The patient sample (25 μl) (described below) was spread over a 15-mm circle on a Cambridge BioScience test kit slide and mixed with 15 μl of 0.6% latex beads coated with recombinant gp120 antigen and gently rotated for 5 to 8 min. A marked flocculent agglutination reaction appeared with HIV-positive sera under high-intensity fluorescent light, whereas HIV-nonreactive sera retained a smooth, creamy homogeneous texture.

Patient samples were prepared in three ways: by the manufacturer's method of using serum diluted 1:10 with phosphate-buffered saline with 1% bovine albumin, by using whole blood diluted 1:10 with phosphate-buffered saline with 1% bovine serum albumin, and by using undiluted whole

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TABLE 1. Comparison of Western blot and LA results for 1,482 Zairian patients

Blood prepn	No. of results that were Western blot/LA:			
	+/+	+/−	−/+	−/−
Serum (diluted 1:10)	537	0	4	941
Whole blood				
Diluted 1:10	537	0	4	941
Undiluted	534	3	6	939

blood. The test results from serum, whole blood, and diluted whole blood were compared to assess the effects of using different blood preparations in the assay.

Of 1,482 patients, 537 were confirmed to be HIV positive by repeated reactivity in ELISA and a positive Western blot. Comparison of reactivities of patient samples with either the standard serum preparation or diluted whole blood demonstrated identical results (Table 1). Utilization of whole blood slightly lowered the reliability of the test, but the results were comparable to those in the ELISA (Tables 1 and 2). The Western blots of three undiluted whole blood samples that were false-negative by LA (Table 2) all showed antibodies to gp41 and gp120/160 antigens. The six samples that were false-positive by LA (four with all three blood preparations and two with whole blood preparation alone) had no identifiable antibodies to any of the HIV antigens. The sensitivity, specificity, and positive and negative predictive values of the LA compared with those of the ELISA and Western blot are shown in Table 2.

The LA is highly sensitive and specific when diluted serum, whole blood, or diluted whole blood is used. Comparison of LA results with those of the ELISA and Western blot demonstrates that the test will correctly detect HIV-infected sera in an African blood bank or outpatient clinic with the same accuracy as that of commercial ELISAs. The advantages for African and other Third World countries are test rapidity (5 to 8 min) and few technical maneuvers. The speed of the test was especially critical in a situation such as the Mama Yemo Hospital blood bank, where there are inadequate supplies of stored blood and no refrigeration and where transfusion must be done quickly. The use of a rapid test in the Mama Yemo Hospital blood bank, where approximately 8% of the donor group is infected with HIV, would prevent the transfusion of 80 to 160 HIV-contaminated blood units per month.

The performance of the LA in laboratories in Third World countries will be very dependent on the training of the technicians using the test, the lighting conditions, and the reagents used in the assay. Although it is an accurate test, the LA requires more training than other HIV rapid assays because the technicians must learn to read microagglutination patterns on the specialized kit slides. We have found that a 1- to 2-week training period was necessary for a technician who had never used agglutination assays before, to give consistent results. Since the positive sera give a microagglutination pattern, a strong light source, such as a fluorescent lamp or direct sunlight, is necessary to facilitate accurate reading of results. The reagents were found to be stable for several months at room temperature (8), which is an advantage in areas where refrigeration is not available.

The Cambridge BioScience LA offers one possible solution to the problem of Third World countries which need to select a simple, rapid test to screen their populations for HIV infections. It is a particularly desirable test for areas where complex equipment, refrigeration, or electricity are not available or reliable. The test, however, requires a training period for technician proficiency, and stringent quality controls must be followed. With the availability of such a test, blood bank screening could be implemented immediately in Third World countries which lack facilities for routine ELISAs.

TABLE 2. Comparison of sensitivities and specificities of ELISA, LA, and Western blot

Sample	% of results that were Western blot/LA:			
	+/+	+/−	−/+	−/−
Serum (1:10 dilution)	100	99.58	99.26	100
Whole blood				
1:10 Dilution	100	99.58	99.26	100
Undiluted	99.44	99.36	98.89	99.79
ELISA (Wellcome)	99.63	99.79	99.44	99.79

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