

A Simple Whole-Blood Test for Detecting Antibodies to Human Immunodeficiency Virus

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We developed an immunochromatographic whole-blood test (WBT) which detects antibodies to human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) from fingerstick blood. The sensitivity and specificity of the WBT were 99.41% (1,018 confirmed positive patients) and 99.89% (941 uninfected patients), respectively (enzyme immunoassay [EIA] on serum or plasma as a reference). WBT performance was comparable to those of licensed EIAs and Western blotting, using 18 HIV-2 sera, 23 HIV-1 seroconversion panels, and a low-titer performance panel (in lieu of whole blood).

The diagnosis of human immunodeficiency virus (HIV) infection can occur in two basic settings. If an immediate result is not required, specimens can be sent to a central laboratory, where they are accumulated and tested batchwise. In situations where immediate diagnosis is desirable, an on-site test is necessary. In the latter case, a rapid fingerstick test overcomes any prerequisite processing steps associated with the use of sera. Such a test (unlike dried-blood-spot tests) offers the health care provider a timely result, even in remote locations. Although many serum- or plasma-based rapid diagnostic tests have been described (1–6, 8–11), there have been few reports on whole-blood-based tests. What we describe here is a whole-blood method for the expeditious detection of antibodies to HIV, comparable in simplicity of operation to contemporary tests used by diabetics to measure blood glucose levels.

Specimens. Specimens were collected from patients visiting the Clinical Laboratory Hospital de Infectología “Dr. Daniel Mendez Hernandez,” Centro Medico Nacional la Raza, Instituto Mexicano del Seguro Social, Mexico City, Mexico. All participants gave informed consent, and epidemiological and demographic data were collected; pre- and posttest counseling was offered. Patients were classified as HIV seropositive (i.e., asymptomatic or at identified AIDS stages) or HIV seronegative (i.e., either with other infectious or noninfectious diseases or certain physiological conditions or clinically healthy). Blood was collected from participants by fingerstick (medical lancet) and immediately analyzed with the whole-blood test (WBT) device under investigation. Thereafter, blood was collected by venipuncture into tubes to obtain serum or plasma. An HIV type 1 (HIV-1) low-titer performance panel and seroconversion panels (panels D, E, H, I, J, K [modified], L, M, N, P, Q, R, S, U, V, W, X, Y, Z, AB, AC, AD, and AE, comprised of serum and/or plasma specimens) were purchased from Boston Biomedica, Inc. (BBI; West Bridgewater, Mass.). Enzyme immunoassay (EIA) and Western blot test results were provided along with each panel. A total of 18 HIV-2 serum specimens (13 from the Ivory Coast and 5 from Serologicals, Clarkston, Ga.) were analyzed by approved strategies by using EIA and/or

Western blotting (kit from Cambridge Biotech Corp., Worcester, Mass.).

Test device and protocol. The WBT device (HemaStrip HIV-1/2; Saliva Diagnostic Systems, Inc., Vancouver, Wash.) consists of a pen-like transparent cylinder having a capillary tip. A test strip resides inside the cylinder. A few microliters of blood is taken up by capillary action into the distal tip of the cylinder when a blood droplet contacts it. The distal end is then pressed down through the foil barrier of a provided buffer vial. The force of this action propels buffer into the tip of the cylinder; the blood specimen is thereby mixed with and diluted by the buffer and deposited at the base of the test strip. The WBT device can then be placed upright (for instance, in a rack) or laid down on a flat surface. Within 15 min, via lateral-flow deposition of a chromophore on a membrane, either a single line (control line, indicating an HIV nonreactive specimen) or two distinct lines (a control line and a test line, indicating an HIV reactive specimen) will develop.

The antigens utilized in the WBT are synthetic peptides and represent determinants of HIV-1 (gp41 and gp120) and HIV-2 (gp36); the immunochemistry components are essentially those of a previously described serum test (3). For the majority of reactive specimens, the test line can be recognized visually within 5 to 10 min, although weakly reactive specimens may require 15 min (the stipulated read time) to develop sufficiently to be discerned. The hands-on time per test for a first-time user unfamiliar with the WBT is about 1 min.

Clinical specimens were analyzed in a blinded fashion; different technicians performed the WBT and the EIA (the reference EIA was Abbott HIV-1/2 [Abbott Laboratories, Abbott Park, Ill.]), and the EIA technician had no prior knowledge of the WBT results. The code was broken by the supervisor after the assays were completed, and specimens with discordant results were retested with the reference (EIA) test whenever possible. Specimens reactive in the EIA and/or WBT and specimens with discordant results were analyzed, whenever possible, by Western blotting (kit from Organon Teknika Co., Durham, N.C.) as the confirmatory method. If indeterminate results were obtained by Western blotting, attempts were made to obtain an additional serum specimen from the patients for reanalysis at a later time (>8 weeks after the first collection).

Whole-blood specimens were not available for seroconver-

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TABLE 1. Comparative performance of the WBT with a low-titer performance panel (BBI panel PRB105)

| Test(s) ^a | No. of samples reactive ^b |
|----------------------|--------------------------------------|
| 1, 2, 3, 4..... | 14 |
| 5, 6..... | 12 |
| 7..... | 12 (2 indeterminate) |
| WBT, 9, 10..... | 11 |
| 11..... | 10 |
| 12, 13..... | 10 (4 indeterminate) |

^a The comparison tests used were as follows: 1, Abbott HIV-1; 2, Abbott HIV-1/2; 3, Cambridge Biotech Corp. HIV-1; 4, Syva HIV-1; 5, Cellular Products Inc. HIV-1; 6, Genetic Systems HIV-1; 7, Ortho/Cambridge Western blot; 9, Organon Teknika HIV-1; 10 Genetic Systems HIV-1/2; 11, Fluorognost indirect immunofluorescence assay; 12, Bio-Rad Western blot; 13, Organon Teknika Western blot.

^b The data for all tests but the WBT were provided by BBI; the EIA data were from BBI's in-house analyses.

sion or low-titer performance panels or for HIV-2 testing; in these cases, 2.0- μ l specimens were micropipetted into the device's capillary tip in lieu of a whole-blood specimen.

Performance. The diagnostic sensitivity of the WBT in this study was 99.4% (six false negatives among 1,018 confirmed positive patients); the specificity was 99.9% (one false positive among 941 noninfected individuals). False negatives occurred in AIDS patients at stages I ($n = 3$), II ($n = 1$), III ($n = 1$), and IV ($n = 1$) (three of these six WBTs were read as faintly positive at later times). The lone false positive was from a high-risk patient (partner seropositive for HIV) with an indeterminate Western blotting result but with a negative EIA; this patient subsequently (approximately 8 months later) tested positive by both EIA and Western blotting and so may have been in the process of seroconversion. No specific cross-reactivity correlating with any (non-HIV) pathological condition was identified. The clinical conditions (suspected or confirmed) of the noninfected individuals were viral infection ($n = 521$); bacteriological, fungal, or parasitic infection ($n = 68$); hematological or renal disorders ($n = 51$); other diseases or conditions ($n = 238$), and none (healthy) ($n = 63$). The sensitivity and specificity for the EIA in this study were 100 and 97.9%, respectively. EIA false positives in this study (20 of 941) were those which gave more than one positive reading (in two or three testings).

All 18 HIV-2 serum specimens on hand tested positive by the WBT. When the WBT was used to similarly evaluate specimens from an HIV-1 low-titer performance panel (Table 1) or

TABLE 2. Identification of reactive specimens in 23 seroconversion panels by seven commercial EIAs and the WBT

| Assay ^a | No. of members identified as reactive ^b |
|--------------------|--|
| 1..... | 81 |
| 2..... | 68 |
| 3..... | 67 |
| 4..... | 61 |
| 5..... | 53 |
| WBT..... | 52 |
| 7..... | 52 |
| 8..... | 45 |

^a The comparison tests used were as follows: 1, Abbott HIV-1/2; 2, Cambridge Biotech Corp. HIV-1; 3, Sieve HIV-1; 4, Abbott HIV-1; 5, Organon Teknika HIV-1; 7, Genetic Systems HIV-1/2; 8, Genetic Systems HIV-1.

^b The total number of seroconversion panel members was 143.

HIV-1 seroconversion panels (Table 2), the results were comparable to those of standard laboratory tests.

Discussion. The sensitivity of the WBT in this study was slightly lower, and its specificity was somewhat higher, than those of the reference method employed (EIA) when used to evaluate serum or plasma specimens in a clinical setting. It fell within the performance range of several commercially available EIAs and Western blotting procedures when used to evaluate seroconversion and low-titer performance panels. This is noteworthy, since immunoglobulin G (and not immunoglobulin M) antibodies are detected by the WBT and since its signal is not enzyme amplified. In one study, the device (foil pouched as a stand-alone kit) maintained good stability and functionality for a year when stored at several constant temperatures, including 45°C (data not shown). In addition, a recent report (7), based upon a test that was otherwise immunochemically identical (3), suggests that the WBT would efficiently detect immune responses to a variety of HIV subtypes.

One disadvantage of the WBT is that the signal line is read visually. Also, no printed record is produced, and there may be interoperator variability in interpretation of a result (in such cases, users would be advised to rerun the test).

Using such a test, however, does offer certain advantages. There is no need for electricity, refrigeration, ancillary reagents, or lab equipment; the specimen does not require prior processing (as is the case for serum); and there are no sequential additions of solutions or washes, characteristic of flow-through tests (for examples, see references 1, 2, and 5). The specimen size is small (3 to 5 μ l of blood per test), and the specimen is effectively sequestered by the testing apparatus after it is collected, minimizing the chance for user contact with the patient's blood. The WBT's ease of use reduces the chance of technical error, and its performance characteristics may make it an attractive choice for use in HIV screening or epidemiological surveys in various diagnostic algorithms.

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