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A Field Test for the Detection of Antibodies to Human Immunodeficiency Virus Types 1 and 2 in Serum or Plasma

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In response to the need for simple and rapid tests for infectious diseases, we have devised a test for antibodies to human immunodeficiency virus type 1 (HIV-1) and HIV-2 which resembles many contemporary strip-style pregnancy tests in format and ease of use. The test was evaluated with 2,928 serum specimens (1,541 reactive and 1,387 nonreactive) collected and tested at a Mexico City hospital clinic and was compared with a laboratory assay (Abbott) performed simultaneously. The sensitivity and specificity of the test using these serum specimens were 99.68 and 99.71%, respectively (before the code of the blinded study was broken). This compares with 100% sensitivity and 97.55% specificity with the laboratory assay (specificity upon reassay after the code was broken, 99.21%). In a survey of HIV-2 specimens, reactive (positive) specimens were detected in 51 of 51 cases. The test was examined with 21 commercially available (HIV-1) seroconversion panels. The performance of the test was comparable to that of a group of Food and Drug Administration-approved (antibody-based) HIV tests.

There is an urgent and growing need to quickly and easily test for infectious diseases, including human immunodeficiency virus (HIV) infection, in parts of the world that are not served by clinical laboratories equivalent to those found in industrialized countries (3, 5, 9). It is crucial that appropriate means be found by which epidemiological surveys, screening, and diagnoses can be carried out without elaborate laboratory support. In order to appropriately advise member states, the World Health Organization coordinates the evaluation of tests for HIV according to established criteria (2, 12); these criteria emphasize minimal requirements for specific technical skills or equipment and reasonable cost.

With these criteria in mind, we have developed a test for HIV type 1 (HIV-1) and HIV-2 that resembles at-home pregnancy tests in its simplicity. It is an immunochromatographic strip test, designed to detect the presence of antibodies to HIV in serum or plasma. It does not require refrigeration, additional reagents, or any laboratory equipment. This study was designed to establish the performance characteristics of the strip test and compare it with several traditional laboratory assays, using specimens from seroconversion panels.

Specimens. Serum specimens were collected at the Clinical Laboratory, Hospital de Infectologia "Dr. Daniel Mendez Hernandez," Centro Medico Nacional la Raza, Instituto Mexicano del Seguro Social, Mexico City, Mexico. These were typically 1- to 2-ml samples; all were tested with test strips (Sero · Strip HIV-1/2; Saliva Diagnostic Systems, Pte. Ltd., Singapore, Republic of Singapore) and analyzed with an enzyme immunoassay (EIA) (Abbott 3A10; Abbott Laboratories, Abbott Park, Ill.). HIV seroconversion panels (panels D, E, H, I, J, K, L, M, N, P, Q, R, S, U, V, W, X, Y, Z, AB, and AC) were purchased from Boston Biomedica, Inc. (West Bridgewater, Mass.). EIA evaluations for each panel were provided by Boston Biomedica, Inc.

HIV-2 specimens were obtained from the Ivory Coast, Gambia, Ghana, and Europe. The specimens were analyzed by approved strategies by using immunoassays and/or Western blot (immunoblot) analysis (20 specimens were tested by Western blotting [Diagnostic Biotechnology, Singapore, Republic of Singapore], and 18 specimens were tested by Western blotting [Cambridge Biotech Corp., Worcester, Mass.]; for the remaining specimens HIV-2 was discriminated from HIV-1 by a specific HIV-2 GACPAT-2 assay [8]).

Test strips and testing protocol. The test strips (Sero · Strip HIV-1/2) are provided in kits containing buffer, specimen transfer loops, and the test strips proper. Briefly, about 200 μl of buffer is removed from a dropper bottle into a test tube (12 by 75 mm). A specimen is added to the buffer by means of a specimen transfer loop: the plastic loop is dipped into the serum or plasma specimen and placed into the test tube containing buffer. The loop is removed and the test strip is dropped into the test tube. The diluted specimen travels up the strip by capillary action, crossing a membrane and reaching a fibrous reservoir. A chromogenic conjugate becomes immobilized at a "control" line consisting of protein A, by virtue of complex formation with immunoglobulin G (IgG) in the specimen. The same conjugate becomes immobilized at the "test" line, but only if the specimen contains IgG which specifically cross-reacts with the target antigen(s), i.e., IgG specific to HIV. The antigens are synthetic peptides representing antigenic determinants of HIV-1 and -2 (for HIV-1, gp41 and gp120; for HIV-2, gp36). For the majority of reactive specimens, the test line can be recognized in about 5 min. Because up to 15 min may be required to recognize weakly reacting specimens (e.g., some seroconversion specimens), strips can be read at or any time after 15 min. The hands-on time for a novice user is on the average 45 s per specimen.

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TABLE 1. Clinical conditions of diseased individuals whose samples were nonreactive with the strip test[a]

Condition or characteristic	No. of individuals strip test negative
Infectious diseases, viral	
Hepatitis (viral, unclassified)	365
Hepatitis B (chronic or carrier)	45
Hepatitis C (or contact with hepatitis C infected individual)	324
Herpes	9
Rubella	14
Parasitic diseases (toxoplasmosis)	19
Infectious diseases, bacterial	
Brucellosis	28
Tuberculosis	24
Leprosy	12
Uveitis	3
Hematological disorders	16
Chronic renal insufficiency	7
Diverse infections	305
Other	
Adenopathy	2
Homosexual	3
Contact or probable contact with HIV-infected individual	26
Spontaneous abortion	78

[a] The four false-positive specimens in this study were from individuals with uveitis (one specimen), unclassified hepatitis (two specimens), and hepatitis C (one specimen).

All specimens were analyzed in a blind fashion for both assays (i.e., Sero · Strip and Abbott 3A10). The code was broken by the supervisor of the technicians after both assays were completed, and samples with discordant results were retested in duplicate (Abbott and test strips).

Specimens that were reactive on the EIA and/or the test strips and specimens with discordant results between the assays were analyzed by Western blotting (Organon Teknika Co., Durham, N.C.) as a confirmatory method. When indeterminate results were obtained by Western blotting (World Health Organization criteria [2]; two *env* bands, with or without *gag* or *pol*), an additional specimen was sought from the patients at a later time (>8 weeks after the first collection).

Performance. The diagnostic sensitivity of the strip test was 99.68%, with five false-negative results (n = 1,541 infected participants); the specificity was 99.71%, with four false-positive results (n = 1,387 negative specimens). The five false-negative specimens were from individuals at stage I (one patient; Western blot positive), stage III (two patients; both Western blot positive), and stage IV (two patients; one Western blot positive and one whose results were indeterminate [1]). In this study, the sensitivity and specificity of the reference EIA were 100 and 97.55%, respectively. The sensitivity and specificity for the test strips upon reassay after the code for the blind study was broken were 99.87 and 99.86%, respectively; for the Abbott test, the sensitivity and specificity were 100 and 99.21%, respectively.

Fifty-one different confirmed HIV-2-positive specimens were all positive by the strip test.

Among the nonreactive specimens were 108 serum samples from healthy individuals, as well as many samples from patients with diseases and pathological conditions other than HIV infection (Table 1). These include infectious and noninfectious diseases, bacterial and viral infections, sexually transmitted diseases, autoimmune diseases, and a number of other conditions. No cross-reactivity correlating with specific pathological conditions has been identified.

Evaluation of seroconversion panels. The test strips were investigated with 21 seroconversion panels. All seroconversion panels were evaluated by Boston Biomedica, Inc., using eight commercially available Food and Drug Administration-approved enzyme immunoassays. The performance of the test strips was compared directly with that of the EIAs. The criterion for a reactive specimen by EIA was a value of ≥1.0 for the signal/cutoff ratio. The criterion for a positive strip test was a clearly visible signal line.

The 21 panels contained a total of 132 individual specimens, and the number of reactive specimens varied according to the test method used. In order to portray the relative sensitivities of the tests (8 EIAs and the strip test), we listed the numbers of reactive specimens that the tests detected and ranked them from most to least sensitive (Table 2). The strip test detected 52 reactive specimens, while the EIAs detected from 45 to 77 reactive specimens.

In addition, we listed the mean numbers of days between each assay's first reactive result and the most sensitive assay's (Abbott HIV-1/2) first reactive result, defined as zero (Table 2). This valuation is somewhat arbitrary, because the intervals between draw dates within a given panel and among panels are not consistently spaced.

The strip test performs with a sensitivity and specificity comparable to those of traditional assays performed in the laboratory. In fact, the specificity of the reference assay used for this study (Abbott 3A10) was lower (97.55% initially and 99.21% after rerun), whereas its sensitivity was higher.

For specimens that are the most difficult to analyze, i.e., samples collected during seroconversion, the overall performance of the test strips was comparable to that of eight commercially available EIAs. For some seroconversion panels, certain EIAs were found to perform better than the test strips

TABLE 2. Identification of reactive specimens in 21 seroconversion panels by eight commercial EIAs and the strip test and the days on which the first positive panel members were identified

Assay[a]	No. of members identified as reactive[b]	No. of days to reactivity[c]
1	77	0
2	65	6
3	65	6
4	60	7
5	60	5
Strip test	52	9
6	52	9
7	50	9
8	45	10

[a] EIA was performed by Boston Biomedica, Inc. 1, Abbott HIV-1/2; 2, Cambridge Biotech Corp. HIV; 3, Syva HIV; 4, Abbott HIV; 5, Cellular Products, Inc., HIV; 6, Organon Teknika HIV; 7, Genetic Systems HIV-1/2; 8, Genetic Systems HIV.

[b] Total number of seroconversion panel members = 132.

[c] Mean number of days elapsed between identification of the first panel member as reactive and identification of the first panel member as reactive by the Abbott HIV-1/2 assay. The time intervals between draw dates were different for each panel, i.e., the value of this number depends on which panel members a given assay missed.

(Table 2). However, in several instances certain EIAs used performed worse than the strips. This is remarkable in view of the fact that the signal line in the test strips is not an amplified signal (i.e., no enzymatic reaction is involved) and that only IgG (not IgM, e.g., as in the Abbott HIV-1/2 EIA [7, 11]) is detected by the test strip. In addition, all the reagents in the test strips are stable at room temperature (20 to 25°C) for ≥18 months and for at least 22 weeks at temperatures as high as 45°C (stability data not shown). The design of the strip test as a particle immunoassay simplifies the test procedure to the extent that nonlaboratory personnel can perform it.

This strip test deviates in several respects from "rapid" tests used for detecting antibodies to HIV, but it resembles in its design contemporary pregnancy tests. Rapid tests for HIV are typically flowthrough devices, the protocols for which usually entail several steps, some of which need to be performed with timing (4, 6, 10). The strip test was developed to meet or exceed the criteria of the World Health Organization for assessment of HIV assays (2, 12), to keep the number of necessary steps to a minimum, and to make these steps as simple as possible while maintaining a robust assay. Because of its inexpensive design (the current wholesale price is estimated to be comparable to other rapid tests) and because it does not require highly trained personnel or special facilities, this type of strip test further facilitates the diagnosis of HIV infection in areas with less sophisticated medical support infrastructure or public health programs.

The disadvantage of this type of analytical method is that the signal is read visually and so no instrument-generated record is created. Clerical errors and inappropriate readings are also possible. In the case of a weak signal line, users are advised to rerun the assay in duplicate.

While the described strip test is useful for evaluating serum or plasma specimens, it cannot be used directly for other bodily fluids. We are currently developing alternate versions of this basic strip format which are especially suited to whole blood obtained from a finger prick and to saliva specimens. Also under development is the adaptation of the basic format to other infectious diseases.

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