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Performance of a Rapid, On-Site Human Immunodeficiency Virus Antibody Assay in a Public Health Setting

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Rapid, on-site human immunodeficiency virus (HIV) testing has the potential to improve the delivery of prevention services in publicly funded counseling and testing sites. The Single Use Diagnostic System (SUDS) HIV-1 is the only rapid enzyme immunoassay (EIA) approved for diagnostic use in the United States. To evaluate the feasibility of using SUDS in public clinics and to validate the test's performance in a public health laboratory, we conducted blinded SUDS testing on plasma sent for HIV testing. From 19 March through 30 June 1993, 1,923 consecutive samples from a sexually transmitted diseases clinic and an HIV counseling and testing clinic were tested on site with SUDS. Tests done in the first two weeks with a malfunctioning centrifuge ($n = 402$) and those done when there were excessively high temperatures in the laboratory ($n = 53$) were analyzed separately. Of 1,466 tests, 39 were positive by both SUDS and EIA (with Western blot [immunoblot] confirmation) and 7 were SUDS positive and EIA negative. Western blotting was used as the "gold standard" to adjudicate these discrepancies. There were no SUDS-negative and EIA-positive tests. Compared with that of EIA (with Western blot confirmation), the sensitivity of SUDS was 100% (95% confidence interval, 88.8 to 100%) and the specificity was 99.5% (95% confidence interval, 98.9 to 99.8%). The positive predictive value of SUDS was 88% in the STD clinic and 81% in the HIV counseling and testing clinic. There was a 7.7-fold increase in false positives, from 0.48 to 3.7%, when there was inadequate centrifugation and when the temperature exceeded the manufacturer's recommendations. Rapid, on-site HIV testing by the SUDS assay is feasible and practical in public health settings. The test can be performed accurately, at reasonable cost, and within the time frame of a typical clinic visit. Caution should be used, however, as two conditions adversely affected the accuracy of this test: inadequate specimen preparation and elevated temperature.

Rapid, on-site human immunodeficiency virus (HIV) testing in public clinics offers several potential advantages to the current testing strategy, chief among them that patients can receive their results and result-specific counseling on the day of their initial visit, eliminating the need for a return visit for persons who test negative. The Single Use Diagnostic System (SUDS) HIV-1 (Murex) was the first rapid enzyme immunoassay (EIA) HIV assay approved for diagnostic use in the United States by the U.S. Food and Drug Administration. In published studies, SUDS has a sensitivity of 99.9% and a specificity of 99.6% (6, 8) and is comparable to the standard EIA. Thus, a negative test does not require further confirmation, and negative SUDS results can be reported at the initial visit. A positive SUDS test requires confirmation either by the immunofluorescence antibody assay or Western blotting (immunoblotting), so clients with a positive SUDS result need to return to receive their results of their confirmatory test (1, 2).

While the use of a rapid test has the potential to improve clinical and prevention services, its use in a public health setting has not been evaluated. Because the public health setting may differ from reference laboratories generally used to support approval of new diagnostic testing, we sought to validate the test's performance in a public health setting. We also sought to assess the feasibility of using SUDS in this setting. Thus, in the Dallas County Sexually Transmitted Disease (STD) and HIV Counseling and Testing Clinics, we conducted a blinded, parallel comparison of SUDS testing on site with EIA and Western blot testing on specimens sent to the Texas Department of Health laboratory.

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MATERIALS AND METHODS

Patients and specimens. Patients in the Dallas County STD Clinic were assessed for HIV risks and were offered confidential HIV testing as a part of their routine care. Clients of the Dallas County HIV Counseling and Testing Clinic visited the clinic because they wanted an HIV test. At both sites, patients gave informed consent for routine HIV testing and received standard pretest counseling. Peripheral blood was collected in 7-ml EDTA tubes (both serum and plasma can be used for the test; we chose to use plasma to reduce the amount of clotting time). The tubes were then centrifuged, and the plasma was separated, sent to the state laboratory, and tested for antibodies to HIV type 1 by EIA with a viral lysate (Vironostika; Organon Teknika). Initially reactive tests were repeated in duplicate and, if repeatedly reactive, were confirmed by Western immunoblot analysis (Cambridge Biotech HIV-1). These results were reported to the Dallas County Health Department. In 1992, the Dallas County STD Clinic reported a 3.1% seropositivity rate among voluntary HIV tests and the HIV Counseling and Testing Clinic reported a 3.6% seropositivity rate (3). The blinded seroprevalence in the STD Clinic at that time was 2.1% (4).

From 19 March through 30 June 1993, parallel, on-site SUDS testing was performed on 1,923 consecutive specimens drawn for HIV testing in the STD Clinic and the HIV Counseling and Testing Clinic. Before a specimen was sent to the state laboratory, an aliquot of plasma was withdrawn and tested with the SUDS HIV-1 (Murex) within 60 min of collection. A log of the SUDS results that could be later linked to the HIV test results from the state laboratory was kept. Samples producing discordant SUDS and EIA results were shipped to the Centers for Disease Control and Prevention (CDC) for repeat SUDS, EIA (Genetic Systems LAV), and Western blot (Cambridge Biotech HIV-1) testing.

During and after the clinic visit, the clinical staff and HIV counselors were blinded to the SUDS results; posttest counseling was conducted at a second visit and was based on the EIA and Western blot results. The study protocol was approved by the CDC institutional review board.

To determine whether the test results could be provided within the time frame of a clinic visit, a time-motion study was conducted. The handling of all 46 specimens from 1 day—15 from the HIV Counseling and Testing Clinic and 31

TABLE 1. Comparison of SUDS results and EIA and Western blot results[a]

SUDS test result	No. of patients		% Sensitivity (95% CI)[b]	% Specificity (95% CI)[b]	% Positive predictive value (95% CI)[b]	% Negative predictive value (95% CI)[b]
	EIA and Western blot positive	EIA negative				
All						
Positive	39	7	100 (88.8–100)	99.5 (98.9–99.7)		
Negative	0	1,420				
Faulty centrifuge						
Positive	12	16	100 (70–100)	95.9 (93.3–97.9)		
Negative	0	374				
Temp over 25°C						
Positive	0	1	NA[c]	98 (88.6–99.9)		
Negative	0	52				
HIV Clinic only						
Positive	17	4			80.9 (57.4–93.7)	100 (99.3–100)
Negative	0	694				
STD Clinic only						
Positive	22	3			88 (67.7–96.8)	100 (99.3–100)
Negative	0	726				

[a] SUDS tests were done on site, and EIA and Western blot tests were done at the state laboratory. A total of 1,466 STD Clinic and HIV Counseling and Testing Clinic patients was tested.

[b] The sensitivity, specificity, and predictive values for SUDS tests were calculated with EIA and Western blot tests being used as the gold standard for the detection of HIV type 1.

[c] NA, not applicable.

from the STD Clinic—was directly observed from the time of blood drawing through to completion of the test.

Personnel and equipment. To conduct the rapid testing, two medical technicians were hired, and they underwent a half-day, on-site training session conducted by the manufacturer and observed by CDC personnel. An unused 8- by 4-ft (1 ft = 30.48 cm) darkroom was converted for use as the rapid HIV test laboratory. A refrigerator was required to store the reagents and specimens. The test kits and reagents were supplied by the manufacturer.

Analysis. We defined a priori a 2-week shakedown period to evaluate the performance of the protocols and to improve testing proficiency, and we analyzed all 402 tests done during this period separately. During these first two weeks, an improperly aligned surplus centrifuge was used, limiting the speed achieved and causing blood to spiral in the tubes. After 1 April 1993, all testing was performed with a new four-head, 3,200-rpm, 1,163-*g* tabletop centrifuge (Adams Compact II; Becton Dickinson).

There were 53 tests performed during four afternoons in June when the ambient temperature in the lab reached 28°C, exceeding the manufacturer's recommended limit of 25°C. We also analyzed these tests separately. Their exclusion did not result in a change in the overall sensitivity or specificity.

We excluded one sample from the analysis that was EIA positive and Western blot positive but SUDS negative. That sample was from an individual who was documented to have participated in a National Institutes of Health GP160 AIDS vaccine study in September 1991 and whose EIA results and Western blot reactivity to bands at 160, 120, and 41 were due to the vaccine and were not due to HIV infection. We also excluded one initially discordant sample (EIA and Western blot positive but SUDS negative) that was discovered to be mislabeled; repeat testing on this sample was positive for all three tests.

The sensitivity and specificity of SUDS were calculated by using EIA screening and Western blot confirmation of the positives as the "gold standard" for detection of HIV type 1 infection. Calculations were based on 1,466 tests. We calculated 95% confidence intervals (CI), using the technique described by Fleiss (5).

RESULTS

The SUDS test was positive for all 39 specimens that were positive by EIA and Western blot (Table 1). Thus, the sensitivity was 100% (95% CI, 88.8 to 100%). Of the 1,427 EIA-negative specimens, 7 were SUDS positive. Thus, the specificity was 99.5% (95% CI, 98.9 to 99.7%).

We separately analyzed the tests performed under adverse conditions. During the first two weeks of testing, when the centrifuge was not working properly, 16 (4%) of 402 tests performed were false positives, giving a specificity of 95.8%. During the four afternoons in June when the temperature in the laboratory exceeded the manufacturer's recommended limit of 25°C, 1 (1.9%) of 53 tests performed was false positive, giving a specificity of 98.1%. The combined false-positive rate of 3.7% represents a 7.7-fold decrease in accuracy compared with the 0.47% rate (7 false positives out of 1,466 tests) observed during the remainder of the testing period.

Because the prevalence of HIV was different in each clinic and because the positive predictive value is strongly influenced by the prevalence, we calculated the positive predictive value of SUDS for each clinic. In the STD Clinic, with a prevalence of 2.9%, the positive predictive value was 88% (95% CI, 67.7 to 96.8%). In the HIV Counseling and Testing Clinic, with a prevalence of 2.4%, the positive predictive value was 80.9% (95% CI, 57.4 to 93.7%).

We repeated SUDS, EIA, and Western blot tests of 24 samples for which the results were initially discordant (the SUDS was positive and the EIA was negative) (Table 2). Seven samples (29%) remained SUDS positive and EIA negative. Thirteen samples (54%) were SUDS, EIA, and Western blot negative. Four samples (16%) were SUDS and EIA negative but Western blot indeterminate on retesting.

The results of the time-motion study indicated that the mean time from when the blood was drawn to when the results could be returned to the clinic was 22 min. This included the transport time from the clinic to the laboratory, the time for batching specimens, and the time to completion of the test.

DISCUSSION

In this study, we compared on-site SUDS HIV-1 testing and EIA testing performed in the state laboratory, with the EIA positives being confirmed by Western blot testing. The specificity of SUDS was 99.5%, confirming the published specificity of 99.6%.

Although we found no false-negative results, we did not have sufficient power to validate published sensitivity. With our sample size, we had a power of 80% to detect a 1% difference in the published sensitivity at the 95% significance level. The sensitivity could have been as low as 88.8% in our laboratory, and we would still have expected to find all 39 positives 2.5% of the time. Given a combined prevalence in both clinics of 2.6%, we would have needed to test more than 19,000 individ-

TABLE 2. Results of initial and repeat tests for discordant samples

Test condition	Initial test characteristic[a]			Repeat test characteristic[b]				Interpretation
	No. of samples	Result of:		No. of samples	Result of:			
		EIA	SUDS		EIA	SUDS	Western blot	
Normal	7	−	+	2	−	+	−	Intrinsic false-positive SUDS
				4	−	−	−	Laboratory false-positive SUDS
				1	−	−	I[c]	Laboratory false-positive SUDS
	2	+	−	1	−	−	+	Results due to GP120 vaccine
				1	+	+	+	Mislabeled sample
Adverse	17	−	+	5	−	+	−	Intrinsic false-positive SUDS
				9	−	−	−	Laboratory false-positive SUDS
				3	−	−	I	Laboratory false-positive SUDS

[a] SUDS tests were done on site; EIAs were done at the state laboratory.
[b] Repeat SUDS tests, EIAs, and Western blot tests were done at the CDC laboratory.
[c] I, indeterminate.

uals to identify the 500 positive tests necessary to detect a 1% difference between our result and the published sensitivity of 99.9%.

With a specificity of 99.5% and a prevalence of 2.4% to 2.9%, the positive predictive value ranged from 81% in the HIV Counseling and Testing Clinic to 88% in the STD Clinic. The positive predictive value has clinical utility in determining the likelihood that a positive SUDS test represents a true positive test. Thus, between 12 and 19% of positive SUDS tests in these clinics are likely to be false positives.

The sensitivity and specificity values for SUDS would yield different positive predictive values for clinics with different prevalences. For example, in a clinic with 5% prevalence, less than 10% of positive tests would be false positive, while in a clinic with 1% prevalence, 33% of positive tests would be expected to be false positives. However, for a clinic with such a low prevalence, the test may still be considered useful, because 99% of the tests would be negative and the positive predictive value of 67% would apply only to the occasional positive result.

In our analysis of discordant test results, we interpreted the seven initially false-positive samples that on retest remained SUDS positive and EIA negative as intrinsic false positives for which an intrinsic characteristic of the test likely led to the false-positive result. We interpreted the 13 initially false-positive samples that on retest were SUDS, EIA, and Western blot negative as laboratory false positives for which initial laboratory error likely led to the false-positive result. We also interpreted the four initially false-positive samples that on retest were SUDS and EIA negative and Western blot indeterminate as laboratory false positives.

In its package insert, the manufacturer states that the test should be performed at a recommended temperature of 20 to 25°C. When the recommended temperature range was exceeded by 3°C, we found an increase in the number of false-positive tests. We also learned, because of the faulty centrifuge, that the test appears quite sensitive to centrifugation.

One sample from a vaccine trial participant was initially EIA positive, Western blot positive, and SUDS negative, and on repeat testing it was EIA negative, SUDS negative, and Western blot positive. This is consistent with the observation that the likelihood that a person who may be uninfected will have a positive test because of the vaccine will vary on the basis of the type of vaccine and the antigen used in the test. In a comparison with other HIV tests, SUDS appeared to be the least sensitive in detecting vaccine-induced antibodies and thus may have potential utility in discriminating between persons who are vaccinated and those who are HIV infected (7).

We estimated the total cost to set up a laboratory similar to ours in Dallas and to run it for a year to be $99,500. This includes the annual salaries of the technicians, the actual cost to the clinic for the purchase of the necessary equipment, and the cost to purchase laboratory supplies, test kits, and reagents for a monthly volume of 760 tests. This estimate represents the incremental cost of establishing a laboratory in an existing clinic's unused space and does not include the cost of renting additional space or the cost of additional utilities or janitorial services, which would have added $3,905. In addition, the cost estimate does not include the costs of counseling or other clinical services.

We conclude that rapid, on-site HIV testing by the SUDS assay is both feasible and practical in public health settings. SUDS can be used on site as the initial assay in an HIV test. Negative results can be reported as negative. Initially reactive tests should be retested in duplicate, and if the sample is repeatedly reactive, a more specific confirmatory test, such as a Western blot, should be done. SUDS can be performed accurately, at reasonable cost, and within the time frame of a typical clinic visit. Public health laboratories should be aware of two conditions that affect the accuracy of this test: inadequate specimen preparation and elevated temperature. The apparent temperature sensitivity of SUDS is a drawback that would likely lead to real-world problems, as such conditions could be expected to be intermittent but recurrent in public settings. This problem may result in the test being less than optimal for use in developing countries. Ideally, future tests will not be so sensitive to temperature and centrifugation conditions.

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