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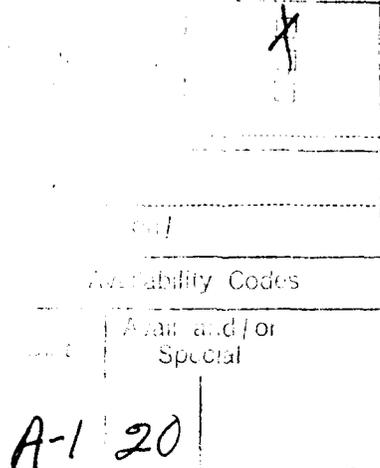


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# Application of a Rapid Assay for Detection of Antibodies to Human Immunodeficiency Virus in Urine

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The use of rapid, simple tests to detect antibodies to human immunodeficiency virus (HIV) in urine could be valuable for several testing situations, such as in private offices, for epidemiologic surveys, and in developing countries. The authors evaluated the performance of the SUDS<sup>®</sup> HIV type 1 test to detect antibody to HIV-1 peptides in urine. Test performance and applicability of the SUDS test were compared with a routine Food and Drug Administration-licensed enzyme-linked immunosorbent assay (ELISA) and Western blot using 139 serum and urine pairs collected from autopsy cases. Using a modified procedure when testing urine by the SUDS test, results indicated that a total of 15 serum/urine pairs were HIV-1 antibody positive by both the SUDS test and ELISA; all could be confirmed positive by Western blot. One sam-

ple produced discrepant results. The SUDS test produced no false-positive results when testing serum or urine, as compared with ELISA, and no false-negative results when compared with the Western blot. For optimal accuracy of detection of antibodies using urine, at least 100  $\mu$ L of sample was required. By Western blot analysis, antibody profiles in urine were generally weaker than in serum, but confirmation of positivity was not compromised when larger volumes were used. The authors concluded that this rapid HIV-1 test, when used to detect antibodies to HIV-1 in urine, is accurate, easy to perform, and appropriate for use in certain testing situations. (Key words: Evaluation; HIV-1; Rapid assay; Urine) *Am J Clin Pathol* 1994;101:157-161.

The ability to detect antibodies to human immunodeficiency virus type 1 (HIV-1) in urine offers advantages over serum testing, especially for epidemiologic studies and when sufficient volumes of blood are difficult to obtain. In addition, the collection of urine is easier, safer, and more cost-efficient, as the need for blood-drawing equipment is eliminated. Also, the use of urine for testing has been reported to result in the resolution of atypical Western blot banding patterns.<sup>1</sup> Because of these advantages, especially the difficulties in collecting blood and the high cost of medical supplies, the use of urine for testing is attractive, particularly in developing countries.

Detection of antibodies to HIV-1 in urine by enzyme-linked immunosorbent assay (ELISA) was first reported in 1988,<sup>2</sup> and several investigators have since confirmed this report.<sup>3-6</sup> Initial studies required concentrated urine samples or capture antibody techniques to achieve adequate sensitivity, but a recent

study indicated that accurate results (100% sensitivity) could be obtained using increased volumes of urine samples without concentration.<sup>3</sup> Subsequently, it was shown that antibodies to HIV-1 could be detected in urine using Western blot.<sup>3,7</sup>

Currently, more than 40 commercial companies produce more than 130 serologic tests for detecting antibodies to HIV.<sup>8</sup> Many of these are rapid assays developed to offer alternatives to ELISA and Western blot.<sup>9-11</sup> However, only two rapid assays are licensed by the Food and Drug Administration (FDA) for use in the United States: the Recombigen latex agglutination (Cambridge Biotech, Worcester, MA) and the Simple Use Diagnostic System (SUDS) HIV-1 test (Murex Corporation, Norcross, GA). The application of these rapid and simple assays can be valuable in testing situations, such as in physician's offices, emergency rooms, and autopsy rooms, where time is important and ELISA readers and washers may not be available. In addition, health-care personnel who may not be familiar with performing such techniques as ELISA and Western blot can successfully conduct rapid assays for HIV.<sup>12</sup> However, the cost of rapid assays is higher than ELISA, and their use may not be practical when testing large numbers of sera. At present, screening for HIV-1 is performed mostly using ELISA on serum samples.

The combination of the use of a rapid assay and the testing of urine samples provides many attractive features and has several applications. We evaluated the ability of a rapid peptide microfiltration assay (SUDS HIV-1 test) to detect antibody to HIV-1 in urine accurately. Our investigation compared the performance of this test with a routine FDA-licensed HIV-1/2 ELISA and an HIV-1 Western blot.

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## MATERIALS AND METHODS

## Samples

Serum and urine pairs were obtained from 139 sequential autopsy cases at an inner-city medical examiner's office. All serum samples were stored frozen at  $-20^{\circ}\text{C}$  before testing, whereas urine pairs were kept at  $4^{\circ}\text{C}$  until testing. Urine and sera appeared clear and of normal color, although several serum samples exhibited slight hemolysis. All samples were obtained without subject identifiers and tested blindly. The population consisted of persons who died of natural causes (34%), accidents (18%), homicides (17%), suicides (10%), drug abuse (17%), and other causes (4%). The average time of post mortem collection was 16 hours (range, 8–26 hours).

## Serologic Testing

All sample pairs were tested by the SUDS HIV-1 test and an FDA-licensed ELISA (HIV-AB HIV-1/HIV-2, rDNA, Abbott Laboratories, Chicago, IL) according to the procedures recommended by the manufacturers. The SUDS test is a manually performed, visually read, 10-minute immunoassay for the qualitative detection of antibodies to HIV-1 in serum or plasma. The test uses a specific gag antigen of HIV-1 and a synthetic peptide corresponding to a region of the envelope of HIV-1. It is based on a solid-phase capture of antigen-coated latex particles, microfiltration, and an antihuman conjugate and substrate system with resultant color development.

The recommended serum sample volume for the SUDS test is two drops (approximately  $30\ \mu\text{L}$ ). To compare the performance of the SUDS and ELISA when testing serum, the recommended sample volumes were used. When testing urine by the SUDS test, all samples were tested using  $300\ \mu\text{L}$  of sample. To confirm the presence of antibodies using the Western blot, urine samples were tested using a final dilution of 1:10 and 1:100.

To determine the minimum volume of sample that could be used in the SUDS test to detect antibody in confirmed positive samples, all positive samples were additionally tested by the SUDS using volumes of 30, 50, and  $100\ \mu\text{L}$ .

To compare the ability of the SUDS and the ELISA for detecting low levels of antibody in urine (artificial system of determining sensitivity), serial dilutions (1:10–1:160) of positive urine samples in phosphate-buffered saline were tested by both methods, with  $300\ \mu\text{L}$  diluted sample used in the SUDS test. For this investigation, 14 of the confirmed positive samples were chosen.

The criteria for a sample to be considered reactive by the SUDS and ELISA were those recommended by the manufacturers: reactions by the SUDS test were classified as nonreactive or reactive, and optical density readings greater than the calculated cutoff were considered reactive by ELISA. Serum and urine specimens reactive by either test were retested in duplicate before confirmation by HIV-1 Western blots (BioRad, Hercules, CA). Criteria for positivity by Western blots for HIV-1 were reactivity to any two of p24, gp41, or gp120/160, as recommended by ASTPHLD/Centers for Disease Control and Prevention (CDC).

Samples that exhibited discrepant results between either of the screening tests were retested by both methods to ensure that technical errors had not occurred and to determine if a result were initially misclassified by either assay. To evaluate the specificity of the SUDS and ELISA results, the Western blot results

TABLE 1. COMPARISON OF ASSAY PERFORMANCE OF THE SUDS TEST AND THE ELISA USING 139 SERUM/URINE PAIRS

	SUDS	ELISA HIV-1/2	Western Blot
No. reactive/ nonreactive			
Urine	15*/124	15/124	15*/ND
Serum	15/124	16/123	15/ND
Sensitivity	100%	100%	
Specificity	100%	99.2%	
Performance	Easy, no equipment required	Washer, reader incubator, required	
Time	10 min	2 h	

\*  $300\ \mu\text{L}$  used.

SUDS = Simple Use Diagnostic System; ELISA = enzyme-linked immunosorbent assay; ND = total not determined.

were used as the reference method. The sensitivity of the SUDS test was calculated in reference to the results obtained from ELISA. In addition, any specimens exhibiting discrepant results between the tests were tested for antibodies to HTLV-1/II (BioRad) and HIV-2 (Diagnostic Biotechnology, Singapore) using respective Western blots. Discrepant samples were also tested by another rapid assay designed to identify and differentiate antibodies to HIV-1 and HIV-2 (Genie, Genetic Systems, Seattle, WA).

## RESULTS

Of the 139 serum/urine pairs, HIV-1 antibodies were detected in serum and urine by both the SUDS and the ELISA in 15 pairs (Table 1), for a prevalence of 9.3%. One additional serum sample was repeatedly reactive by ELISA (mean optical density/cutoff = 1.5), but negative by the SUDS test. The Western blot profile of this serum sample showed reactivity to only the p24 antigen; the Genie assay and the HIV-2 and HTLV Western blots produced negative results. The urine pair of this serum sample was negative by both the SUDS test and ELISA. As indicated, this resulted in a higher specificity in the SUDS test than in ELISA, although the true status of this indeterminate sample must be questioned.

The 15 urine samples that were repeatedly reactive by ELISA were reactive in the SUDS test using either  $100$  or  $300\ \mu\text{L}$  (Table 2). Using  $50$  and  $30\ \mu\text{L}$  urine, only 13 of 15 (87%) and 12 of 15 (80%) were correctly identified, respectively.

When testing dilutions of urine, the ELISA could detect a greater number of the reactive samples than the SUDS test at higher dilutions (Table 3). All confirmed positive samples were detected by ELISA when using dilutions of 1:10, 1:20, and 1:40; SUDS only detected all samples using dilutions of 1:10 and 1:20. In addition, when using dilutions of 1:80 and 1:160, specimens were correctly identified by ELISA in 12 of 14 (86%) and 9 of 14 (64%), respectively, and by SUDS in 5 of 14 (36%).

Western blot analysis of the 15 repeatedly reactive pairs showed different serum and urine patterns. In 12 of 15 urine pairs, the profiles met the criteria for positivity using the 1:100 dilution recommended by the manufacturer, whereas three samples were indeterminate (strong reactivity to gp120/160, weak intensity to gp41, and no reaction to p24). By using a

Rapid HIV-1 Assay Using Urine

TABLE 2. COMPARISON OF RESULTS FROM 15 REACTIVE SAMPLES BY THE SUDS TEST USING DIFFERENT VOLUMES OF URINE

Volume	Sample Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
300 µL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
100 µL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
50 µL	N	R	R	R	N	R	R	R	R	R	R	R	R	R	R
30 µL	N	R	R	R	N	N	R	R	R	R	R	R	R	R	R

SUDS = Simple Use Diagnostic System; N = nonreactive; R = reactive.

lower dilution of urine (1:10), reactions were more closely related to the serum patterns, and all the samples met the criteria for positivity. Figure 1 indicates the Western blot profiles of the three urine samples that met the criteria for positivity only when using a 1:10 dilution. Interestingly, many urine samples showed no, or very weak, reactivity to p24. As indicated in Table 4, the percent of reactivity to most of the viral specific antigens was less when using urine, even when the volume of urine was increased to represent a 1:10 dilution. Reactivity to gp160 was clearly noted and similar in intensity in all specimens at either dilution.

DISCUSSION

The use of serum for HIV-1 antibody testing has several disadvantages. Collection requires trained personnel, sterile blood-drawing equipment, and centrifugation, and demands that all collection procedures be performed with strict adherence to safety regulations. In developing countries, there are additional disadvantages to using serum: the cost of blood-drawing equipment may compromise the attempt for conducting surveillance studies, sufficient volumes of blood may be difficult to obtain from certain patients because of religious

and cultural reasons, and blood collection devices may be reused in certain populations resulting in a significant threat to public health.<sup>13</sup>

In contrast, urine is easier to obtain than blood and it does not require an invasive method for collection or centrifugation. Urine may also be a safer sample to use, as glass containers are not required, and the risk of transmission of HIV-1 by urine has been reported to be low to nonexistent.<sup>14,15</sup> For children and newborns, in particular, urine is preferred to blood, as urine collection is painless, less traumatic, and less threatening. Finally, urine collection is rapid, simple, and inexpensive, and specimens can be self-collected, even in a nonmedical environment.

The use of rapid assays for detecting antibodies to HIV is becoming popular,<sup>8</sup> both for alternate testing strategies and for use in certain testing situations. The use of rapid assays in combination with the testing of urine specimens has not been described. The application of this combination would be valuable for such situations as physician's offices, autopsy rooms, emergency rooms, funeral homes, pediatric centers, and remote testing areas where equipment and expertise are not readily available. It is also valuable in situations where blood cannot be obtained.

TABLE 3A. COMPARISON OF ELISA RESULTS USING DIFFERENT DILUTIONS OF 14 REACTIVE URINE SAMPLES\*

Dilution	Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1:10	R	R	R	R	R	R	R	R	R	R	R	R	R	R
1:20	R	R	R	R	R	R	R	R	R	R	R	R	R	R
1:40	R	R	R	R	R	R	R	R	R	R	R	R	R	R
1:80	N	N	R	R	R	R	R	R	R	R	R	R	R	R
1:160	N	N	R	R	N	R	R	N	R	R	R	R	R	N

TABLE 3B. COMPARISON OF SUDS RESULTS USING DIFFERENT DILUTIONS OF 14 REACTIVE URINE SAMPLES

Dilution	Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1:10	R	R	R	R	R	R	R	R	R	R	R	R	R	R
1:20	R	R	R	R	R	R	R	R	R	R	R	R	R	R
1:40	N	N	R	R	N	R	R	R	R	R	R	R	R	R
1:80	N	N	N	N	N	N	R	N	R	R	R	N	R	N
1:160	N	N	N	N	N	N	R	N	R	R	R	N	R	N

SUDS = Simple Use Diagnostic System; ELISA = enzyme-linked immunosorbent assay; N = nonreactive; R = reactive  
 \* Sample 15 could not be included because of insufficient volume

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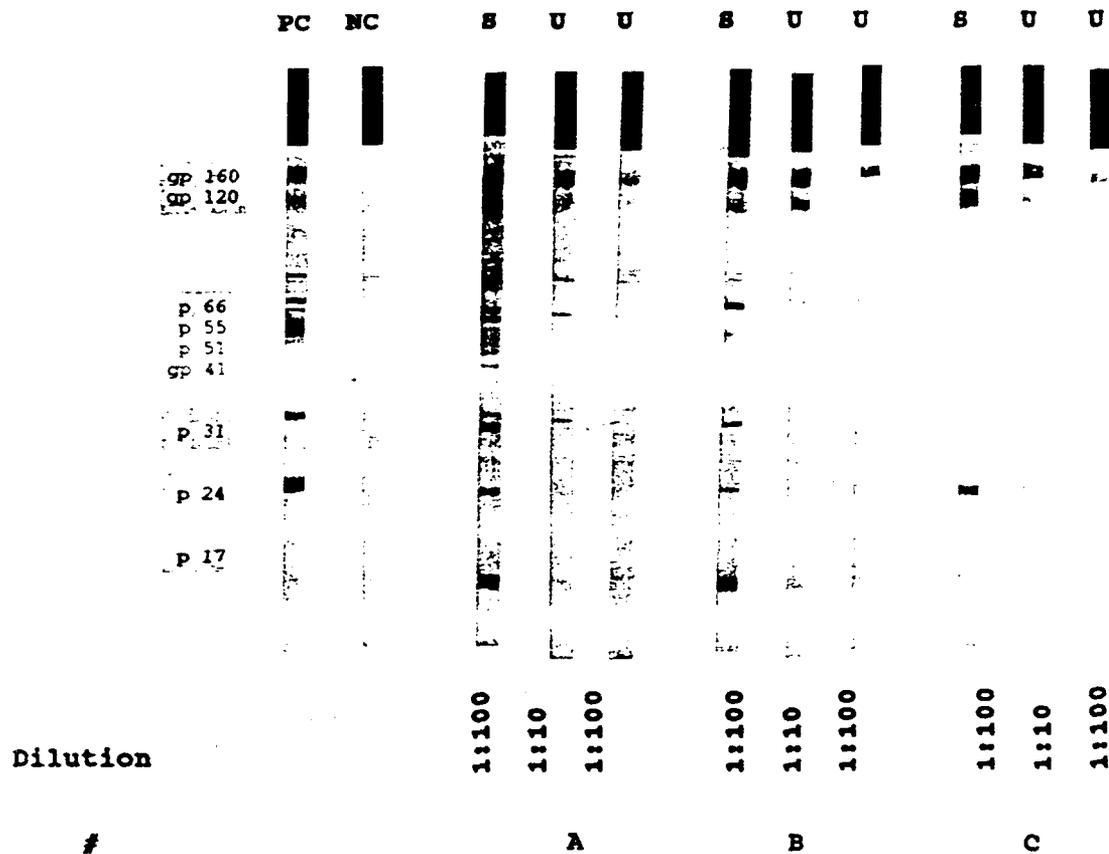


FIG. 1. Western blot profiles of three (A,B,C) representative serum (S)/urine (U) pairs at 1:10 and 1:100 dilutions. PC = positive control; NC = negative control.

In our study, the SUDS HIV-1 rapid assay exhibited successful performance when testing serum or urine, compared with routine HIV assays. However, larger volumes of urine were required to achieve the sensitivity comparable with the tests using serum, most likely because of the lower concentrations of IgG antibody in urine (10–20,000-fold less).<sup>3</sup> The SUDS test correctly identified all Western blot confirmed samples and did

not produce any false-positive reactions when urine was used as the sample. The specificity of the SUDS test may have been higher than the ELISA when testing serum, because the one sample classified as reactive by ELISA and negative by SUDS was not confirmed (indeterminate) by Western blot. The sensitivity of the modified SUDS test using urine was equivalent to the ELISA for detecting positive samples, but it did exhibit a slightly decreased sensitivity when testing reactive samples that had been diluted. Whether this artificial system of evaluating sensitivity is equivalent to the detection of truly weak-reactive samples is unknown. However, we have determined using commercially available HIV-1 seroconversion panels that the SUDS test can detect seroconversion at the same time as several FDA-licensed ELISAs (unpublished observation).

Although these results indicate that the use of a rapid assay to test urine could be used as a screening strategy in this testing scenario, attempts to confirm antibodies in these reactive urine samples by Western blot using the recommended volumes of samples were not completely successful; larger volumes of urine were required. Our results are similar to those of others,<sup>3</sup> indicating that the use of urine may not always allow for confirmation by Western blot. Interestingly, all samples consistently exhibited reactivity to gp160, which agrees with other reports<sup>3,15</sup> and suggests that these antibodies may serve as a marker for infection when testing urine by Western blot. The absence of substantial reactivity to p24 is difficult to explain,

TABLE 4. FREQUENCY (%) OF DETECTION OF HIV-1 ANTIGENS BY ANTIBODIES IN 15 SERUM/URINE PAIRS USING WESTERN BLOT

HIV-1 Antigen	Serum Samples (1:100)	Urine Samples (1:100)	Urine Samples (1:10)
gp160	100	100	100
gp120	100	93.3	100
p66	100	80.0	86.7
p55	100	60.0*	80.0
p51	93.3	53.3*	73.3
gp41	100	86.7	93.3
p31	100	60.0*	86.7
p24	100	66.7*	80.0
p17	100	40.0*	80.0

\*  $P < .05$ , Fisher's exact test.

## Rapid HIV-1 Assay Using Urine

because reactivity to p24 using serum samples was generally strong. Antibodies to p24 may be complexed with viral antigen (p24), and, therefore, may not pass through the kidney glomerulus or may be sequestered by reacting with cross-reacting antigens in the reproductive tract.<sup>16</sup> When testing semen, a recent study has also shown strong reactivity to gp160, with significantly reduced reactivity to p24 by Western blot.<sup>16</sup> Recently, Chamaret and coworkers<sup>1</sup> reported two patients with indeterminate Western blot results in serum that were positive by urine testing (p24 was present in both serum and urine, whereas only urine samples showed gp160/120). This report suggests another application for urine HIV testing.

In conclusion, our data provide preliminary evidence that the FDA-licensed SUDS HIV-1 rapid assay can be used to identify antibodies to HIV-1 in urine accurately and that these samples can be confirmed by Western blot, provided that larger volumes are used. Although the test was designed for use with serum or plasma samples, the testing of urine resulted in excellent sensitivity, without compromising specificity. Our results parallel those of others using routine and newer ELISA technologies in suggesting that urine is an adequate sample for testing.<sup>2-6</sup> In addition, we have shown the application of this rapid test when testing urine in autopsy rooms. Samples were easy to collect, did not require centrifugation, could be stored at refrigeration temperature, and could be tested rapidly and without sophisticated equipment or the need for specifically trained personnel. These advantages would also make this testing method applicable for use in developing countries. Although our results suggest the usefulness of this testing strategy for certain populations, further studies using larger numbers of both high- and low-risk living populations, and using persons at different stages of HIV infection, should be conducted.

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