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Urine Samples as a Possible Alternative to Serum for Human Immunodeficiency Virus Antibody Screening

P. Martínez*, R. Ortiz de Lejarazu, J. M. Eiros, J. De Benito, A. Rodríguez-Torres

The detection of specific antibodies against human immunodeficiency virus (HIV) was tested by dot blot enzyme immunoassay in 95 urine samples from 72 individuals infected with HIV and 23 seronegative individuals. Western blot of paired serum samples from these same individuals was used as the gold standard. The dot blot tested had a sensitivity of 97.2% and a specificity of 100%; only two samples from HIV-infected individuals at Centers for Disease Control (CDC) stages II and IV were non-reactive. Reactive and discrepant samples (serum/urine) were confirmed by Western blot, which had a sensitivity of 98.6% and a specificity of 100%. The most commonly observed Western blot reactivity pattern in urine samples included bands against three groups of HIV structural proteins (ENV, POL, and GAG). The results indicate that urine can be used in screening for HIV antibodies in epidemiological studies of high-prevalence populations, though it is not recommended for individualized diagnostic purposes.

The increased demand for the capability to detect antibodies against human immunodeficiency virus (HIV) in different situations (diagnosis, epidemiological studies, etc.) calls for easily performed tests that enable large numbers of samples to be processed without requiring specialized equipment or personnel. It would also be desirable if such a test used a type of sample that is both easy to obtain and reliable as regards diagnostic results. Such a test could serve as a viable alternative to serological tests currently used in the diagnosis of HIV.

Microbiology Laboratory Service, University Hospital, c/ Ramón y Cajal s. n., 47011 Valladolid, Spain.
Table 1: Reactivity of urine samples by dot blot and Western blot.

| Subjects                        | Total no. of samples | HIV antibodies in urine |
|---------------------------------|----------------------|-------------------------|
|                                 |                      | Dot blot                | Western blot            |                     |
|                                 |                      | Positive | Negative | Ind. | Positive | Negative | Ind. |
| HIV seropositive                |                      |          |          |      |          |          |      |
| CDC stage II                    | 48                   | 47       | 0        |      | 48       | 0        |      |
| CDC stage III                   | 5                    | 5        | 0        |      | 5        | 0        |      |
| CDC stage IV                    | 19                   | 18       | 0        |      | 18       | 0        | 1    |
| Seronegative blood donors       | 23                   | 0        | 0        |      | 0        | 0        | 0    |
| Total                           | 95                   | 70       | 71       | 0    | 0        | 1        |      |

CDC, Centers for Disease Control; Ind., indeterminate.

HIV infection or in the context of epidemiological studies.

Among the biological fluids available, urine could be an alternative to serum if the sensitivity and specificity for HIV antibody detection were comparable to those values obtained with the techniques normally used in the laboratory for detection of HIV antibodies in serum. The aim of this study was to evaluate urine as an alternative to serum both for HIV antibody detection and for subsequent confirmation by means of Western blot.

**Materials and Methods.** Ninety-five urine samples were collected at random from 72 individuals with risk factors (i.v. drug abuse, homosexuality, prostitution) infected with HIV-1 and from 23 seronegative blood donors. The infected individuals presented different stages of infection [48 Centers for Disease Control (CDC) stage II, 5 CDC stage III, and 19 CDC stage IV], based on the 1990 CDC classification criteria without taking CD4+ lymphocyte counts into consideration. None of the individuals included in the study presented clinical evidence of renal dysfunction. The urine samples were collected by simple miction in a sterile receptacle with no additional precautions. The urine samples were stored at 4°C until processing (2 months maximum). If precipitate was observed in the sample, the urine sediment following settling at 4°C without centrifugation was discarded. HIV antibodies were detected by means of a commercially available dot blot rapid assay (Genie HIV-1/HIV-2; Genetic Systems, USA), tested previously in serum samples, which permits differential diagnosis of HIV-1 and HIV-2 (1, 2). In this assay, the synthetic proteins of the envelope (gp41 and gp36 of the HIV-1 and HIV-2 viruses, respectively) cover a series of microscopic particles that are immobilized in the reaction mem-
France). The serum samples reactive in this assay were confirmed by means of Western blot (Bioblot HIV-1 plus; Genelabs Diagnostic, USA) using World Health Organization criteria (3). The Western blot obtained with the serum of the individuals included in the study was used as the gold standard in determining the sensitivity and specificity of the dot blot assay tested with urine. Values for sensitivity and specificity were calculated using the chi-square test and Fisher’s two-tailed exact test.

**Results and Discussion.** Of the 95 urine samples processed by the dot blot test, 70 were reactive and 25 nonreactive. All of the reactive samples were obtained from individuals with HIV infection confirmed in serum. Two samples from HIV-seropositive individuals one at CDC stage II and another at CDC stage IV were nonreactive in the dot blot test. No urine sample from the group of 23 seronegative individuals was reactive.

Retesting of the initially reactive urine samples by Western blot resulted in all being confirmed as positive. Western blot of the two samples initially nonreactive and corresponding to two HIV-infected individuals was positive for the stage II individual (with reactivity to gp160 and gp120) and indeterminate for the stage IV individual (reactivity to gp41 only). The results are shown in Table 1.

The values for sensitivity and specificity of the dot blot in urine were 97.2% and 100%, respectively (positive predictive value = 1, negative predictive value = 0.92; p < 0.0001), while those of the Western blot in urine were 98.6% and 100%, respectively (PPV = 1, NPV = 0.96; p < 0.0001). These values are slightly lower than those obtained with the serum samples. Some studies have shown that the antibody concentration present in urine samples is as much as 10,000 times lower than that in serum (4, 5). This problem of low antibody concentration may be solved, as in the present study, by increasing the sample volume in the reaction (6–8), extending the incubation time (8), and using undiluted samples (8–10).

The existence of a few false-positive results among individuals at more advanced stages of infection could be due to the decrease in antibody production as a result of immunological deterioration in these patients and, therefore, to a lower concentration of antibodies in serum (11). In the case of the false-negative result from the stage IV individual, the serum Western blot did not show reactivity to proteins p66, p55, p51, p24, or p17. There are other factors that may have had an influence on the antibody concentration found in urine: administration of certain drugs (e. g. diuretics), ingestion of large amounts of liquid, or collection of urine without a sufficient interval since the last miction to permit a normal antibody concentration, for example. On the other hand, the finding of two false-negative results with the dot blot assay compared to only one false-negative result with the Western blot in serum may be due to the sample storage time and to the lower sensitivity of the dot blot technique. It seems advisable, therefore, that urine samples be analyzed as soon as possible, even when stored at 4°C.

Another important aspect to be borne in mind when considering the use of samples other than serum is specificity, which was 100% in this study. The absence of false-positive results may be due to the fact that the substances excreted in urine seldom produce alterations leading to results of this type (5). Some authors have found false-positive results in prefrozen samples for reasons not sufficiently explained (12). This reinforces the earlier recommendation of using either fresh urine samples or those stored at 4°C for a short period of time.

Confirmation of the dot blot assays with urine samples by Western blot was possible in a high percentage of HIV-infected individuals (98.6% of reactive urine samples). Among the reactivity patterns found in the urine Western blots, the most commonly observed included bands of reactivity to three groups of HIV structural proteins: ENV, POL, and GAG. The most commonly observed reactivity band corresponded to protein gp160 and the least commonly observed to p17. Our findings, and those of others (9, 13) suggest that the band corresponding to protein gp160 would be a reliable Western blot marker.

In conclusion, the sensitivity and the excellent specificity of the dot blot in urine samples permit the use of urine, a sample that is easy to obtain and store, in screening large population groups. The ease with which urine samples are obtained could facilitate periodic HIV infection studies in population groups at high risk of HIV infection. The possibility of confirming reactive urine samples by means of Western blot of the same sample according to the interpretive criteria for serum samples permits urine to be considered as an alternative to serum in epidemiological studies. On the other hand, the use of urine samples for diagnostic purposes does not seem advisable in view of the sensitivity value obtained in our study.
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PCR Detection of Mycobacteraemia in Tanzanian Patients with Extrapulmonary Tuberculosis

C. Richter1,2,*, L.F.F. Kox3,4, J. van Leeuwen3, I. Mtoni5, A.H.J. Kolk3

In 191 Tanzanian patients admitted to hospital with suspected extrapulmonary tuberculosis (TB), TB was diagnosed in 158 patients; the remaining 33 patients had neither microbiological nor clinical evidence of TB. Mycobacterium tuberculosis was detected in the blood of 25 patients, in 92% by a polymerase chain reaction (PCR) technique and in 52% by culture of buffy coat cells. The presence of mycobacterial DNA or Mycobacterium tuberculosis bacteria in peripheral blood (positive culture) was significantly associated with HIV infection; it was detected in 22 (21.4%) of 103 HIV-seropositive patients compared to only 3 (3.5%) of 55 HIV-seronegative patients (p < 0.009). In two-thirds of the patients with mycobacteraemia, TB can be detected by simple smears from other organ sites. In patients with suspected extrapulmonary tuberculosis in whom smears from the infected site are negative or not available, PCR on blood will confirm the diagnosis within 24 hours in one third of the cases.

Bacteraemia with Mycobacterium tuberculosis occurs in 26–42% of patients with tuberculosis (TB) who are coinfected with the immunodeficiency virus (HIV) (1–4), although mycobacteraemia has been reported only incidentally in African patients (5).

A polymerase chain reaction (PCR) technique exists for the detection of Mycobacterium tuber-