

# Antibodies to HIV in Urine

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AIDS is caused by a blood transmitted retrovirus HIV-1<sup>1</sup>. The virus has been found in saliva, semen, blood, blood products, tears, cerebrospinal fluid, urine, breast milk, alveolar fluid and vaginal secretions. Antibodies have been detected in urine, saliva, plasma and serum<sup>1,2,3,4</sup>. Of these, saliva and urine would be the preferred samples due to non-invasive collection.

In 1988 Cao et al<sup>2</sup> reported that 79 of 80 AIDS patients tested had HIV specific antibodies in their urine. Their method required concentration of the urine and testing of the concentrate by Western Blot. In the present study, we report data for a new rapid HIV EIA test that does not require concentration of the urine sample to detect antibody to HIV.

## *Antibodies to HIV-1 in Urine Specimens of Insurance Applicants*

Specific antibodies to human immunodeficiency virus (HIV-1) were detected with a modified commercially available HIV-EIA assay. With this test, antibodies to HIV were demonstrated in 148 of 150 (98.6%) urines from previously screened HIV positive insurance applicants. In addition, three thousand six (3,006) randomly selected urines were assayed. Of these, three thousand one (3,001) were negative while five (5) were positive. For comparison the three thousand six (3,006) companion serums were tested. The data indicated exact correspondence between urine and serum for positive and negative results. In addition, one hundred fourteen (114) urine samples from two local AIDS treatment groups were similarly tested. One hundred twelve (112) samples (98%) were positive. Quantitative analyses of BUN, creatinine, uric acid, protein and glucose from insurance applicants reveal no pattern to suggest that the urine HIV-antibody positive patients had renal disease. But, the pattern for glucose and protein for AIDS patients suggests that renal disease may develop in advanced stages of the illness. A recent modification of the HIV-EIA has resulted in an increase in sensitivity to 100% compared to serum. However, the increased sensitivity has been a trade-off, for the specificity is no longer 100% but is now 99.8%. At the current prevalence of infection, this means that two (2) to three (3) applicants per thousand would have to be retested with serum.

## **Methods**

### *Random Urine Samples*

Urines were randomly selected from insurance applicant samples. The samples, both urine and serum, had been sent to the laboratory for urinalysis and serum chemistry testing. The only selection criteria was that a companion serum sample be available for testing.

### *Urine Samples Selected from pre-screened HIV Positive Insurance Applicants*

Due to the low prevalence of HIV in the population, ninety-three thousand (93,000) serum samples were tested to identify infected individuals. The companion urine samples were then stored at -20C°. The day prior to analysis, the urine samples were removed from the freezer and stored overnight at room temperature. The samples were centrifuged at 3,000 g for 10 minutes, then assayed for urine HIV antibodies. One hundred fifty (150) samples were analyzed.

### *Samples from HIV Infected Individuals*

Urine samples were collected from HIV infected patients attending two local clinics. The clinical stage of the illness was determined by the patient's doctor. Of the one hundred fourteen patients (114), 46 have AIDS, 32 ARC, and 36 were asymptotically infected serological HIV positive.

Whole blood samples were collected in lavender top (EDTA) Becton/Dickinson Vacutainer tubes. After collection, the tubes were gently inverted ten (10) times to ensure uniform mixing of the blood and anticoagulant. Serum samples were similarly collected in a red top B/D Vacutainer tube. Following collection, the sample clot tubes were set at room temperature for one (1) hour and then centrifuged at 3000 g for ten (10) minutes to separate the serum.

Serum HIV antibody and antigen levels were determined with the Genetic Systems HIV-EIA and HIV-antigen EIA. T-cell counts were with Coulter anti-CD4, anti-CD8 on a Coulter EPICS clinical cytofluorometer. Total blood counts were performed with a Coulter S-Plus 4.

The concentrations of glucose, uric acid, creatinine, bilirubin, BUN, and protein were determined for all urine samples. Quantitative analyses for glucose, protein, creatinine, bilirubin and uric acid were run on companion serum samples. Analysis was with a Hitachi 736 or Hitachi 717 operated per the manufacturer's specifications.

## **Results**

In the initial development of the urine HIV test, the effect of urine pH on the test was evaluated. No effect was found and therefore the data are not presented in this report.

### *Glucose, h-Albumin or h-Globulin*

Urine normally contains very low concentrations of glucose,

albumin and globulin. But in certain pathological conditions like diabetes and kidney disease, high amounts of these and other metabolites may be excreted.

A study was run to test the effect of high concentrations of these three analytes on the urine HIV EIA. Two urines, one negative and one low positive for HIV antibodies, were spiked with glucose, human albumin or human globulin to 10, 50, 100, 250, 500, 750 or 1000 mg/dl. The spiked urine samples were then tested using the standard assay and their final O.D.'s recorded. Summary data are presented in Table 1.

**Table 1.**  
**Spiked Urine Samples with**  
**Glucose, Human Albumin, or Human IgG**

**The Effect of Glucose, Albumin, or Immunoglobulin on the Detection of**  
**Antibodies in HIV Positive and Negative Urine Samples**

mg/dl	Glucose		Albumin		Immunoglobulin	
	Pos	Neg	Pos	Neg	Pos	Neg
10	0.354	0.035	0.345	0.040	0.344	0.037
50	0.341	0.051	0.374	0.042	0.389	0.074
100	0.413	0.040	0.360	0.041	0.359	0.103
250	0.410	0.079	0.305	0.028	0.353	0.189
500	0.408	0.075	0.369	0.039	0.291	0.321
750	0.467	0.035	0.256	0.025	0.471	0.428
1000	0.441	0.028	0.370	0.037	0.564	0.564

For negative urine spiked with either glucose or albumin, the optical density at 450 nm varied between 0.025 to 0.075 with no apparent concentration effect by either analyte. The spiked positive urine had an optical density from 0.256 to 0.467 with no major concentration effect on the final reading. The data clearly shows that even high concentrations of albumin and glucose do not affect the result of the urine HIV EIA. While samples with values this high would be considered suitable for analysis of HIV, they would also be reported as abnormal for glucose or albumin.

The urine HIV EIA detects bound human immunoglobulin with enzyme-labeled anti-antibodies. It is, therefore, not too surprising to find that high concentrations of human globulin cause false positive reactions in the assay. When HIV negative urine was spiked with 10, 50 or 100 mg/dl h-globulin the results (0.037, 0.074, 0.103) were still negative. But at concentrations at or above 250 mg/dl the HIV-negative urine was positive (OD 0.189). As would be expected, the HIV positive sample remained positive with an increase in OD from 0.344 to 0.564 as the amount of h-globulin increased from 10 mg/dl to 1000 mg/dl. Therefore, urines that contain h-globulin in excess of 100 mg/dl would not be suitable for analysis with the urine HIV EIA. However, samples would be reported as abnormal due to presence of globulin in the urine.

#### *Random Urine Samples*

Following the optimization of the urine HIV antibody test, three thousand six (3,006) randomly selected urines were assayed. Of these, three thousand one (3,001) were HIV antibody negative while five (5) were repeat reactive. When the companion serums were assayed, the five (5) urine repeat reactives corresponded to five (5) Western Blot positive samples. The other three thousand one (3,001) serums were negative.

#### *Samples from Pre-Screened Insurance Applicants*

One hundred fifty (150) urine samples were assayed. The urine

HIV EIA was positive for 148/150 (98.6%). No proteinuria or glucosuria was noted in these one hundred fifty (150) samples.

#### *HIV Infected Patients*

The urine samples voided by the one hundred fourteen (114) HIV infected patients were assayed. One hundred twelve (98%) were positive; the other two samples were negative. Sixteen (14%) of the samples contained glucose or protein; eight (7%) contained both.

Companion whole blood was assayed for two T-cell markers, CD4 and CD8, and total blood count. The CD4/CD8 (helper to suppressor ratio) was abnormal in 108/114 (95%) of the samples.

#### *The Companion Serum Samples*

The data for the companion serum samples were 114/114 (100%) HIV EIA repeat reactive, 25/114 (20%) HIV antigen EIA positive, and 113/114 (99%) Western Blot positive; one Western Blot was indeterminate.

#### **Discussion of Results and Summary**

The sensitivity and specificity of the urine HIV EIA are 98% and 99.8%, respectively. The reported sensitivity is based on correlation of 148/150 (98.6%) insurance applicant HIV EIA

antibody positive specimens. In addition, if the data for the high risk group is included, the correlation was 260/264 (98%). Thus far the sensitivity claimed for the assay is 98%. This claim is based on two observations; (1) while some high risk individuals buy insurance, they do not reflect the true risk in the general population; therefore, the data from the HIV patient study was not included in the calculation for sensitivity; (2) a statistically large enough group (the same individuals that are currently buying insurance) was tested to establish the reliability of the assay. That population should represent the full gamut of individuals from uninfected to recently infected to those with ARC and AIDS. The reported sensitivity of 98% corresponds with that reported by Cao *et al.*

The specificity of the urine HIV EIA is 99.8%. At the current prevalence rate for HIV this would mean two (2) samples would be repeated per thousand (1000) applicants.

The presence of antibodies in the urine is considered abnormal. But, is this a pathologic condition in the kidney or are antibodies produced and discharged into the urine in response to an immunologic challenge?

Other authors have reported the presence of antibodies in urine<sup>2,5,6,7</sup>. The original report by Lerner *et al* described the appearance of antibodies to poliovirus. These were intact, functional poliovirus neutralizing antibodies. The presence of such antibodies could suggest that the immune system, under correct stimulation, produces and excretes minute amounts of antibodies into the urine. Local infection by HIV and an inflammatory response by T and B lymphocytes could result in *in situ* production of antibodies in the kidney.

Alternatively, the presence of intact antibody in the urine may be the result of glomerulonephritis. In the current study of the HIV infected patient population, 16/114 (14%) had glucosuria or proteinuria; 8/114 (7%) of these sixteen (16) had both. In

striking contrast, none of the HIV positive insurance applicants (0/150) has abnormal amounts of protein or glucose in their urine. Two possible interpretations of this are; (1) individuals in the later stages of HIV infection develop proteinuria/glucosuria as a result of the infection, (2) individuals in the later stages of HIV infection develop kidney disease with proteinuria/glucosuria as a result of massive loss of red and white cells, blood transfusions and drug therapy.

These results would suggest that the AIDS patient has a higher risk of developing kidney disease than a person in the general population (about 14 fold). These findings are consistent with published reports of focal and segmental glomerulosclerosis in patients with AIDS<sup>8,9</sup>. While the demonstration of antibodies in urine is consistent with early stages of renal disease, it provides no information about the molecular cause. One has to remember that these patients are normally under strict and intensive treatment regimens. Therefore, the associated renal disease may be secondary to the HIV infection, to renal toxicity of the numerous medications these patients are taking, or the result of other factors. In our studies we have demonstrated the presence of intact antibodies (M.W. 160,000 daltons) with no albumin (M.W. 67,000 daltons) present in some HIV positive urines 3/19 (16%); data not presented. This would argue for a local, possibly luminal site, of synthesis in response to a local HIV infection. One would predict on strong theoretical and empirical grounds that the presence of post glomerular gamma globulin without albumin could not occur in a failure in the filtration bed. In that event, albuminuria should always accompany, if not precede, globulinuria due to the relative sizes of these two families of proteins.

The urine HIV EIA appears to have the sensitivity (98.6%) and specificity (99.8%) to make it an ideal test. The ability to detect the presence of this infectious agent with a sample collected by a non-invasive procedure is a major step forward in the testing of insurance applicants.

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