

# Evaluation of enzyme linked immunosorbent assay for screening urinary tract infection in elderly people

J R Michie, B Thakker, A Bowman, A C McCartney

## Abstract

**Aims:** To evaluate the Uristat test, an indirect enzyme linked immunosorbent assay for the qualitative detection of antibodies in urine, as a screening, and in the diagnosis of urinary tract infection in the elderly.

**Methods:** Semiquantitative culture was compared with conventional microscopy, dipstick analysis and the ELISA. In the ELISA, 371 urine samples were examined for antibodies to an antigen mixture of six common urinary pathogens.

**Results:** The sensitivity was 91% and the specificity 25% for the ELISA. The negative predictive value was 81% and the positive predictive value was 43%.

**Conclusions:** In its present form the Uristat test has no clear advantages over conventional bacteriological techniques for screening urine samples for infection in an elderly population.

A considerable number of the specimens examined in a routine diagnostic microbiology laboratory are urine (in our laboratory 31%). The diagnosis of urinary tract infection is usually based on the semiquantitative culture of urine samples.<sup>1,2</sup> After overnight incubation on solid culture medium a growth in pure culture of a bacterium at a concentration of  $>10^5$  organisms/ml is the accepted criterion for urinary tract infection.<sup>3</sup> In most laboratories, however, only a small proportion of the urine samples tested are infected<sup>4</sup>; consequently many screening methods have been developed in an attempt to eliminate rapidly the negative specimen.<sup>5,6</sup>

The Uristat test (Shield Diagnostic Ltd, Dundee, Scotland) is a recently introduced indirect enzyme linked immunosorbent assay (ELISA) for the qualitative detection of antibodies in urine. The antigens used in the assay represent six common urinary pathogens: *Escherichia coli*, *Proteus mirabilis*, *Klebsiella aerogenes*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa* and *Citrobacter freundii*. We evaluated this test as a screening aid in the diagnosis of urinary tract infection in elderly patients.

## Methods

Mid-stream or catheter specimens of urine were collected from 371 patients, all over the

age of 65. There were 123 men and 248 women with an age range of 65-99 years (mean 78 years). Samples were obtained from routine admissions and therefore at random from asymptomatic and symptomatic patients. The urine specimens were collected in Boricon containers (Medical Wire and Equipment, Corsham, Wiltshire, England). Microscopy, culture, dipstick testing and tests for antimicrobial activity were performed on all specimens on the day of collection. Thereafter, samples were stored at 4°C for up to five days before being analysed using the Uristat ELISA test.

Microscopy was performed by examination of a wet film of the uncentrifuged specimen at a magnification of  $\times 400$ . The presence of epithelial cells, white cells, red blood cells and bacteria per high power field was noted and recorded semiquantitatively on a scale + to ++++; + = less than 5 white blood cells per high power field, ++ = 5-10, and +++ = more than 10.

The urine samples were cultured semiquantitatively on 10% (v/v) horse blood agar and cysteine lactose electrolyte deficient (CLED) agar and a pure growth of  $>10^5$  organisms per ml taken as an indicator of significant bacteriuria.

Each sample was tested with a multiple reagent strip (Ames Division, Miles Laboratories, Stoke Poges) for haemoglobin, nitrite, and leucocyte esterase. The results were read visually and the presence of one or more positive tests was regarded as a potential indicator of infection.

One hundred microlitres of urine was dispensed by Finn pipette on to an isosensitest (Oxoid) plate seeded with a strain of *E coli* (NCTC 10418) sensitive to all the usual urinary antimicrobial agents. Zones of growth inhibition were observed after 24 hours' incubation.

The Uristat indirect enzyme immunoassay (Shield Diagnostics) is designed to detect urinary antibodies specific for antigens representing the most common bacterial pathogens that cause urinary tract infection. The wells of the commercially prepared Uristat microtitre plates are coated with a mixture of antigens prepared from strains of *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa* and *Citrobacter freundii*. One hundred microlitres of each undiluted urine sample was pipetted in duplicate into two individual wells and incubated at room tem-

Department of  
Bacteriology, Royal  
Infirmary, Glasgow  
G4 0SF

J R Michie  
B Thakker  
A C McCartney

Department of  
Statistics, University  
of Glasgow  
A Bowman

Correspondence to:  
Dr J R Michie

Accepted for publication  
12 June 1991

perature for 15 minutes. The wells were washed three times with 200 µl borate buffer preserved with 1% (w/v) sodium azide. In the second incubation stage of 15 minutes 100 µl of a conjugate of alkaline phosphatase-labelled monoclonal antibodies to human IgG, IgA, and IgM was added to each well. After three further washes with borate buffer bound antibodies were traced by incubation with 100 µl substrate solution consisting of phenolphthalein monophosphate containing Mg<sup>2+</sup> as an enzyme cofactor. The addition of 100 µl of a stop solution of sodium hydroxide and EDTA as a chelating agent in carbonate buffer (pH > 10) terminated the reaction and provided the appropriate pH for the colour development. The manufacturer supplied both high and low controls which were included in each plate assay.

The optical density of each well was measured at 550 nm in a standard ELISA plate reader. The high control was a strong positive. Urine samples with an optical density greater than or equal to the low control were considered positive for specific antibodies to the antigen mixture coated on the wells of the plate.

The results were analysed in the statistical computing package MINITAB.<sup>7</sup> The Uristat readings were expressed as a ratio of the observed value to the low control. The distribution of these values exhibited a very high degree of skewness and a log transformation was therefore applied to the ratios. Analysis of covariance was used to assess the effect of age on the Uristat results.

**Results**

The gold standard of semiquantitative culture was compared with each of the three screening methods individually, urine microscopy, dipstick analysis, and the Uristat test. Results were expressed in terms of sensitivity, specificity, and positive and negative predictive values. These were calculated as follows:

$$\text{Sensitivity (\%)} = \frac{\text{True positives}}{\text{True positives} + \text{false negatives}} \times 100$$

$$\text{Specificity (\%)} = \frac{\text{True negatives}}{\text{True negatives} + \text{false positives}} \times 100$$

$$\text{Predictive value for positive results (\%)} = \frac{\text{True positives}}{\text{True positives} + \text{false positives}} \times 100$$

$$\text{Predictive value for negative results (\%)} = \frac{\text{True negatives}}{\text{True negatives} + \text{false negatives}} \times 100$$

The results are detailed in the table.

Sixty nine of the 371 urine samples were negative by the Uristat test, but in 13 of these pus cells were seen on microscopy, and culture of all 13 yielded significant numbers of bacteria *E coli* (n = 10), *Proteus sp* (n = 2), *Streptococcus faecalis* (n = 1), a false negative rate of 19%. Of the remaining 302 urine samples positive by the Uristat test, 172 (57%) were falsely positive when compared with culture. Overall, the sensitivity of the assay was 91%, the specificity 25%, the negative predictive value 81% and the positive predictive value 43% (table).

When the data were reanalysed to exclude the urine samples in which antibiotic activity was detected the results were very similar with a specificity of 28% and a sensitivity of 90%.

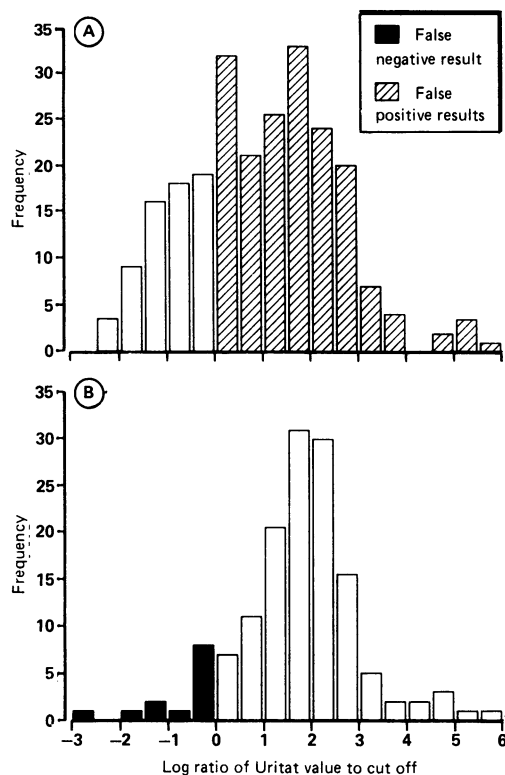
The results were compared with the two other screening methods often used to predict urinary tract infection. Microscopy in our series had a sensitivity of 96%, a specificity of 57%, a negative predictive value of 96% and a positive predictive value of 58%. False positive results were found in 42% of cases, but false negative results were found in only 4%. The dipstick method had a sensitivity of 92%, a specificity of 49%, a positive predictive value of 53%, and a negative predictive value of 90%. The false positive rate was 47% and the false negative rate was 10%.

The manufacturers recommend that a urine sample should be considered infected if the specimen was positive both by culture (> 10<sup>3</sup> cfu/ml) and either positive by the leucocyte esterase test or positive for the presence of pus cells by microscopy. Urine samples were defined as non-infected if all of these three parameters were negative. Using these criteria, the sensitivity is 90% and the specificity 26%, values essentially the same as these obtained when culture alone was used to define urinary

Comparison of results of three different screening methods for urine samples

Method	Sensitivity	Specificity	Positive predictive value	Negative predictive value	False positive (%)	False negative (%)	Variables detected
Microscopy	96%	57%	58%	96%	42%	4%	One or more of: White cells Red blood cells Bacteria
Dipstick	92%	49%	53%	90%	47%	10%	One or more of: Leucocyte esterase Haemoglobin Nitrite
Uristat Elisa test	91%	25%	43%	81%	57%	19%	Antibodies

Uristat results for the 371 urine samples analysed. Results are plotted as log ratio of OD result of Uristat to OD result of cut off value. Urine samples with results on the left-hand side of zero were considered as non-infected by the Uristat criteria, whereas urines lying on the right hand side of the zero were considered infected. Results of urine samples which were cultured negative are shown in histogram A and those which were culture positive are shown in histogram B.



tract infection (table). The figure shows that there is a substantial degree of overlap in the Uristat test results between those patients who had confirmed urinary tract infection and those who did not. Adjustment of the boundary point, to reduce the large number of uninfected urines incorrectly classified as infected (false positives) by the Uristat test, increased the number of false negative results.

### Discussion

The results of various techniques for detecting antibody in urine samples have been disappointing, despite the fact that immunoglobulins of all classes can be detected in urinary tract infections.<sup>8,9</sup> McKenzie and Young,<sup>10</sup> however, developed a method based on a simple coliform antigen mixture which would bind urinary antibody of a wide range of specificities. They were able to detect antibody in 90% of urine samples from patients with symptoms of urinary tract infection. This antibody test is the basis of the Uristat assay.

Uristat was promoted as a useful urinary tract infection screening test by the manufacturers. In our study, where the test was used to screen urines from an elderly population, it had a low specificity of 25% which was reflected in the high false positive rate of 57%. In practice this means that a large number of urine samples would have to be cultured.

The Uristat test failed to detect antibody in 13 urine samples which were culture positive. In all but one urine sample the bacteria cultured were present in the antigenic mixture included in the wells of the Uristat microtitre plate. Although *E coli* is in this mixture, the Uristat failed to detect antibody in ten urine samples from which *E coli* was cultured. It is well known that *E coli* is the commonest organism found in infected urine and it has 150

serotypes of which 1, 2, 4, 6, 18 and 75 are responsible for most urinary tract infections.<sup>11</sup>

Rigid adherence to the Kass criteria of  $>10^5$  organisms/ml or more as diagnostic of urinary tract infection has been questioned.<sup>12</sup> The presence of pyuria as an indication of host response is often used to confirm infection. If pyuria alone was used to define urinary tract infection, however, the specificity of the Uristat test was 29% with a sensitivity of 88%. These values were not dissimilar to those in the table.

In the original study by McKenzie and Young patients were diagnosed as having a urinary tract infection on the basis of the presence of urinary antibody and symptoms. It is impractical to take symptoms of urinary tract infection into account in any screening test. Furthermore, it is particularly difficult to assess symptoms in a geriatric population where asymptomatic bacteriuria is common.<sup>13</sup> Perhaps the Uristat test would be more reliable if symptoms could be scored with confidence.

The ideal screening test for urinary tract infection should be rapid, inexpensive, easy to perform and have relatively high values of sensitivity and specificity. We obtained results for urinalysis by dipstick and microscopy which were comparable with those of other studies.<sup>5,14</sup> The Uristat assay requires an expensive ELISA reader but that apart, due to the cost of Uristat kits, each test is priced at £1.30. The dipstick has no capital costs and although microscopy requires a suitable microscope this is available in all microbiology laboratories. In our study microscopy had the most favourable values in all the categories selected to express performance of screening tests for urines.

Recently MacGowan *et al*<sup>15</sup> reported an ELISA technique to measure urinary lipopolysaccharide antibodies. They concluded that further development was required before the ELISA could be used for routine screening of urinary tract infection. This is supported by our findings.

In conclusion, we have not found any evidence to suggest that the Uristat test, in its present form, has any clear advantages over conventional bacteriological techniques for screening urine samples in an elderly population.

- Haugen J, Strom O, Ostervold B. Bacterial counts in urine. 1. The reliability of the loop technique. *Acta Pathol Microbiol Scand* 1968;74:391-6.
- Leigh DA, Williams JD. Method for the detection of significant bacteriuria. *J Clin Pathol* 1964;17:498-503.
- Kass EH. Bacteriuria and the diagnosis of infection in the urinary tract. *Arch Intern Med* 1957;100:709-14.
- Curtis GDW, Johnston HH, Hack AR. Automated bacteriuria screening using the Berthold LB 950 luminescence analyser. *Eur J Clin Microbiol* 1987;6:275-80.
- Smith TK, Hudson Andrea J, Spencer RC. Evaluation of six screening methods for detecting significant bacteriuria. *J Clin Pathol* 1988;41:904-9.
- Smith TK, Eggington R, Pease AA, Harris DM, Spencer RC. Evaluation of the Malthus 128 H microbiological growth analyser for detecting significant bacteriuria. *J Clin Pathol* 1985;38:926-8.
- Ryan BF, Joiner BL, Ryan TA. *Minitab Handbook*. Second Edition. Boston: Duxbury Press, 1985.
- Jodal U, Ahlstedt S, Darlison B, Hanson LA, Lindberg U, Sohl A. Local antibodies in childhood urinary tract infection—a preliminary study. *Int Arch Allergy* 1974;47:537-46.
- Ratner JJ, Thomas VL, Sanford BA, Forhand M. Bacteria specific antibody in the urine of patients with acute pyelonephritis and cystitis. *J Infect Dis* 1981;143:404-12.

- 10 McKenzie H, Young DN. Antibody to coliform antigens in urine samples from patients with symptoms of urinary tract infection. *J Clin Pathol* 1987;**40**:787-92.
- 11 Gruneberg RN, Leigh DA, Brumfitt W. *Escherichia coli* serotypes in urinary tract infection: studies in domiciliary, antenatal and hospital practice. In: O'Grady FW, Brumfitt W, eds. *Urinary tract infections*. London: Oxford University Press, 1968:68-79.
- 12 Maskell R. A new look at the diagnosis of infection of the urinary tract and its adjacent structures. *J Infect* 1989;**19**:207-17.
- 13 Boscia J, Kobasa WD, Abrutyn E, Levison ME, Kaplan AM, Kaye D. Lack of association between bacteriuria and symptoms in the elderly. *Am J Med* 1986;**81**:979-82.
- 14 Coia JE, Wills G. Dipstick analysis for bacteriuria. *J Clin Pathol* 1989;**42**:444.
- 15 MacCowan AP, Marshall RJ, Cowling P, Reeves DS. Measurement of urinary lipopolysaccharide antibodies by ELISA as a screen for urinary tract infection. *J Clin Pathol* 1991;**44**:61-3.