Measurement of urinary lipopolysaccharide antibodies by ELISA as a screen for urinary tract infection

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Abstract

Five hundred and twenty two clinical urine specimens submitted for routine microbiological examination were tested in parallel by conventional microscopy and culture and for lipopolysaccharide antibodies by an enzyme linked immunoabsorbent assay (ELISA) to assess the ELISA as a screen for urinary tract infection. When the ELISA alone was compared with routine methods the specificity sensitivity, and predictive value of positive and negative tests was 73.2%, 75.7%, 51.1% and 38.5%. For ELISA with microscopy the same variables were 71.1%, 82.2%, and 92.4% and 94.7%, respectively. The ELISA absorbency increased with increasing bacterial numbers, but results varied widely. Only 65.4% of urines which contained $\geq 10^5$ bacterial/ml were positive by ELISA; 36.8% of urines with $< 10^3$ bacteria/ml were positive by ELISA; 100% of $\geq 10^5$ bacteria/ml cultures of *Pseudomonas* sp (n = 4), *Staphylococcus aureus* (n = 3), and *Streptococcus faecalis* (n = 2) were positive by ELISA but only 71.4% of *Proteus* sp (n = 7), 61.4% coliforms (n = 70), and 25% of coagulase negative staphylococci (n = 4).

It is concluded that further development is required before the ELISA can be used for routine screening for urinary tract infection.

Urinary bacteriology is a principal part of the workload of most clinical microbiology departments, hence the efficient and effective use of staff and material is of considerable importance. Many urine specimens submitted for laboratory examination do not have clinically important abnormalities; early and cost effective screening out of these specimens may therefore result in more appropriate use of resources. Dipsticks have been advocated in this role but studies have shown that increased costs and staff time are likely if they are used.^{1,2}

Antibody-coated bacteria have been used for many years to discriminate between upper and lower urinary tract infection, but unbound antibody may also be detected in urine.³ High concentrations of unbound urinary IgG and IgA have been detected by enzyme linked immunoabsorbent assay (ELISA) in acute pyelonephritis, but in lower tract infection concentrations were low.

Secretory IgA may, however, be detected in patients with cystitis or asymptomatic bacteriuria.^{4,5} Hence it has been suggested that the detection of antibodies by the use of a polyspecific lipopolysaccharide antigen in an ELISA may provide a useful screening test for urine before culture or that it could replace microscopy.⁶

We report on the evaluation of a prototype ELISA (Melisa, Utilisa) developed by Walker Diagnostics, Cambridge, England, for the detection of urinary lipopolysaccharide antibody and the evaluation of its role as a screening test for urinary tract infection.

Methods

Conventional urine microscopy and culture on CLED agar were performed on all specimens. An inverted microscope was used on 60 μ l of urine in 96-well flat bottomed microtitre trays. Red blood and white cell counts were expressed per high power field. Culture comprised a 2 μ l standard loop on to a half plate for each specimen. Urine specimens with no growth after 18 hours of incubation were assumed to have $\leq 10^3$ bacteria/ml. In parallel, urine specimens were also tested using the manufacturer's recommendations in a solid phase enzyme linked immunoabsorbent assay (ELISA) for detection of lipopolysaccharide antibodies (Walker Diagnostics Cambridge, Cambridgeshire, England).

Briefly, 200 μ l of positive and control reagent with a defined absorbency cut-off as well as test urines were pipetted into 96-well microtitre trays and pins coated with purified lipopolysaccharide were inserted and incubated for 10 minutes at room temperature. The pins were washed and transferred to 100 μ l of enzyme conjugate in a second plate and incubated for a further 10 minutes. The pins were washed again and placed in 200 μ l of enzyme substrate (0-phenylenediamine-hydrochloride). The reaction was stopped by the addition of 50 μ l 4M sulphuric acid after 10 minutes.

The optical density was read at 492 nm on a microplate reader. For each assay the samples were compared with the cut off value (COV). Samples with absorbency lower than the COV were negative, and those greater were positive, provided the positive control was more than five times the COV optical density. To enable optical densities to be compared between different ELISA plates a "corrected absorbency" was derived by multiplying the test

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Table 1 Comparison of urine microscopy, culture, and ELISA tests individually and in combination

Source	Comparisons	Sensitivity (%)	Specificity (%)	Predictive value	
				Positive	Negative
General practice (n = 246)	ELISA v MC	75.0	78.5	54.1	90.3
	WCC + ELISA v MC	83.3	77.8	53.1	93.9
	RBC + WCC + ELISA v MC	83.3	74.7	48.8	93.9
	RBC + WCC v MC	77.9	90.3	75.0	91.6
Hospital (n = 275)	ELISA v MC	76.7	69.4	31.3	94.2
	WCC + ELISA v MC	79.3	67.6	29.5	95.0
	RBC + WCC + ELISA v MC	80.7	68.4	30.3	95.4
	RBC + WCC v MC	71.9	87.1	57.5	92.7
General practice and hospital (n = 521)	ELISA v MC	75.7	73.2	41.1	92.4
	WCC + ELISA v MC	81.5	71.8	39.4	94.5
	RBC + WCC + ELISA v MC	82.2	71.1	38.5	94.7
	RBC + WCC v MC	75.2	88.5	66.2	92.2

MC = Conventional microscopy and culture; WCC = White cell count; RBC = red blood cell count.

absorbency by a factor derived by dividing the average COV for all plates by the COV for the plate on which the test was read.

A positive urine sample by conventional methods was defined as $\geq 10^5$ bacteria/ml in a pure or predominant growth in the absence of pyuria (< 10 white cells/high power field) or $\geq 10^4$ bacterial/ml in pure or predominant growth and $> 10^5$ bacterial/ml of two species in the presence of pyuria (≥ 10 white cells/high power field).

Isolates were identified by conventional means,⁷ and test sensitivities, specificities, and predictive values of negative and positive tests calculated according to Boreland and Stoker.⁸

Results

Five hundred and twenty two urines were tested. Two hundred and forty seven came from patients attending general practitioners and 275 from inpatients or those attending hospital outpatient clinics. No specimens from antenatal clinics sent for screening for bacteriuria in pregnancy were included. Four hundred and twenty five specimens were mid-stream specimens, 51 were catheter urines, 21 bag urines, one was an endoscopic urine, and for 24 the site of sampling was unknown.

One hundred and six (20.3%) were positive by conventional methods—60 (24.3%) from general practice, and 46 (16.7%) from hospital patients. The ELISA by itself and in combination with pyuria or haematuria, or both (> 10 red blood cells/high power field) was compared with conventional microscopy and culture. For comparison, routine microscopy was also compared with microscopy and culture. Table 1 shows the sensitivities, specificities, and predictive values of these tests for urine samples from hospital and general practice patients.

ELISA and microscopy combined had a negative predictive value of 94.7% for all urine specimens while the corresponding figures for microscopy or ELISA alone were 92.2 and 92.4%, respectively. The specificity of the ELISA was less than microscopy alone, and the positive predictive value of all the tests was less in hospital than general practice patients.

There was no correlation between white cells/high power fields or red blood cells/high power fields and absorbency ($r^2 = 0.1153$ and 0.0477 , respectively, but all urine samples with ≥ 100 red blood cells/high power field were positive by ELISA (data not shown). As the numbers of bacteria increased then the ELISA absorbency increased (table 2), but there was a large range of values. The percentage of specimens that were ELISA positive increased with increasing bacterial counts from 36.8% with $\leq 10^3$ bacterial/ml (no growth on CLED) to 65.4% with $\geq 10^5$ bacterial/ml.

The sensitivity of the ELISA at detecting antibody in urine samples with $\geq 10^5$ bacteria/ml of different urinary pathogens is shown on table 3: 61.4% of urines with $\geq 10^5$ coliforms/ml were ELISA positive, while all *Pseudomonas* sp, *Staphylococcus aureus*, and *Streptococcus faecalis* were detected. Coagulase negative staphylococci and *Candida* were detected less frequently.

Table 4 shows the percentage of urine samples positive by conventional methods at different corrected absorbencies. As the absorbency increases the percentage of positive urine samples increased from 5–10% to about 40%, but even at high optical densities only 39% of urines were judged positive by conventional culture. If the average COV (0.179) is compared with the range of absorbencies then it lies near the majority of the results; hence small

Table 2 ELISA absorbencies and their relation to semiquantitative bacteria counts

Numbers of bacteria	Corrected absorbency		Percentage of urine specimens ELISA positive
	Range	Mean	
$\leq 10^3$ cfu/ml (n = 219)	0.070–1.637	0.203	36.8 (n = 81)
$10^3 < 10^4$ cfu/ml (n = 163)	0.069–2.522	0.239	35.0 (n = 57)
$10^4 \leq 10^5$ cfu/ml (n = 32)	0.081–1.955	0.423	43.8 (n = 14)
$> 10^5$ cfu/ml (n = 107)	0.075–2.983	0.469	65.4 (n = 70)

Table 3 ELISA absorbency in urines with $> 10^5$ bacteria/ml cultures

Species	Corrected absorbency		Percentage detected by ELISA
	Range	Mean	
Gram negative isolates:			
Coliforms (n = 70)	0.083-2.983	0.440	61.4
<i>Proteus</i> sp (n = 7)	0.094-1.790	0.553	71.4
<i>Pseudomonas</i> sp (n = 4)	0.191-1.790	0.853	100
Gram positive isolates:			
<i>S. aureus</i> (n = 3)	0.218-1.819	0.903	100
<i>S. epidermidis</i> (n = 3)	0.119-0.200	0.158	33
<i>S. saprophyticus</i> (n = 1)	—	0.125	0
<i>S. faecalis</i> (n = 2)	0.181-0.195	0.188	100
<i>Candida</i> sp (n = 2)	0.140-0.199	0.169	50

Table 4 Percentage of urine samples positive by conventional microscopy and culture at different corrected absorbencies by ELISA

Corrected absorbency by ELISA	Percentage of urines positive by conventional methods	Total number of urine samples
0.041–0.08	8.3	12
0.081–0.12	6.2	145
0.121–0.16	16.7	120
0.161–0.20	25.0	72
0.201–0.24	18.5	27
0.241–0.28	36.4	22
0.281–0.32	18.7	16
0.321–0.36	42.1	19
0.361–0.40	41.7	12
> 0.40	39.0	77

Average COV = 0.179.

changes in the COV will produce large changes in the numbers of specimens testing positive or negative.

Discussion

The current urinary ELISA for the detection of lipopolysaccharide antibodies is not sensitive enough to screen out all negative urines and not specific enough to enable workload savings to be made. A negative predictive value of the ELISA plus routine microscopy was 94.5%, and ELISA alone 92.4% which compares poorly with dipstick testing where values of over 98% can be achieved.¹² The percentage of urine samples ELISA positive with $\geq 10^5$ bacteria/ml was only 65.4% while 36.8% of urines with no growth ($< 10^3$ bacteria/ml) were also positive. Using a more selected group of urine samples from general practice patients with frequency/dyuria syndrome and excluding those without a pure growth of lactose fermenting coliforms, McKenzie and Young detected 100% of positive urines by lipopolysaccharide ELISA.⁶ Here we were only able to detect antibody in 61.4% of specimens with $> 10^5$ coliforms/ml but we have shown that it is also possible to detect antibody in urine samples with $\geq 10^5$ bacteria/ml of *Proteus* sp, *Pseudomonas* sp, and some Gram positive cocci as well. As the numbers of urine samples with non-coliform isolates were small, however, no valid conclusion about the percentage which could be detected can be drawn.

Changing the cut off value in the ELISA will change the specificity and sensitivity of the test, but as only 40% of urine samples with high absorbencies are positive by conventional tests, and 5–10% of those with low absorbencies, a greater difference in these percentages would be needed to make this worthwhile.

At present the prototype ELISA under test as a screening method for urinary tract infection lacks the technical characteristics which would make it suitable in this role, mainly because of the high number of false positive and negative results when compared with conventional microscopy and culture. This may be because of problems with conventional methods such as the presence of antibiotic or a significant number of bacteria at less than 10^3 cfu/ml in the urine, both of which would produce false positive results by ELISA. False negative results may occur because of the inability of the test to detect the full range of antibody classes or specificities produced in response to a urinary tract infection, or by failures of a patient to mount an antibody response—for example, immunocompromised hospital patients. If some of these problems can be overcome studies of workloads and costs could be performed.

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