CORRESPONDENCE

Screening of urinary tract infections by ELISA

We were pleased to read the article by Gibb and Edmond. Their results are in broad agreement with our evaluation of a commercially available enzyme linked immunosorbent assay (ELISA) (Uristat, Shield diagnostic systems, UK) for screening urinary tract infections in an elderly population. We note with interest, however, the authors' comment regarding the theoretical possibility of false positive results in patients with gonococcal and non-specific urethritis. They postulate that this may result from inflammation of the urethral mucosa and subsequent leakage of unselected IgG antibodies from the serum into the urine. Indeed, in the study by Neumann and coworkers there is also the further possibility of cross-reaction between the lipopolysaccharide core (LPS core) antigen component of an assay and the specific antibodies produced against it. We investigated this problem using the Uristat assay. First, void specimens of urine were collected from 67 (41 men, 26 women) patients attending the genitourinary medicine clinic. All patients had signs and symptoms of urethritis, and had a leucocytic urethral exudate with the presence of four or more polymorphonuclear leucocytes per oil immersion field (x 1000 magnification).

Each urine specimen (20 ml) was collected in Biorcon containers (Medical Wire and Equipment, Corsham, Wiltshire, England). The urine specimens were cultured semiquantitatively on 10% (v/v) horse blood agar and corynebacterial, acid deficient (CLED) agar, and a pure growth of a single bacterial species of >10⁶ organisms per ml taken as an indicator of significant bacteriuria. Each undiluted urine sample (100 μl) was analysed, in duplicate, using the Uristat test, as described previously. Each assay plate also included high and low controls which were supplied by the manufacturers. All specimens were also tested for antibiotic activity by inoculating 100 μl of urine on to an Isosensitest (Oxoid Ltd) plate seeded with a fully sensitive strain of Escherichia coli (NCTC 10418).

All urethral exudates were cultured for Neisseria gonorrhoeae. Swabs were inoculated in the clinic on to GC non-selective agar (Oxoid Ltd) and GC selective agar (Oxoid Ltd) containing LCAT (lincomycin, colistin, amphotericin B and trimethoprim) antibiotic supplement (Oxoid Ltd) and media were supplemented with 5% (v/v) lysed horse blood (Gibco Biocult Ltd). Chlamydia trachomatis was detected by direct immunofluorescence microscopy (Microtrak, Genetic Systems Corporation, Sysa UK). All positive results were certified by ELISA using IDEIA (Boots Celltech Diagnostics Ltd). Results of culture tests and Uristat assay are shown in the table. Eight patients were culture positive for N gonorrhoeae, four for C trachomatis, and four patients had a mixed infection with both organisms. Using the Uristat assay, there were no false positive results in urine specimens from patients with gonococcal and non-specific urethritis.

Results of culture tests and Uristat assay

<table>
<thead>
<tr>
<th>Test</th>
<th>Number (%) of patients (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine:</td>
<td>0</td>
</tr>
<tr>
<td>Significant bacteriuria</td>
<td>0</td>
</tr>
<tr>
<td>Uristat ELISA positive</td>
<td>0</td>
</tr>
<tr>
<td>Antibacterial activity</td>
<td>0</td>
</tr>
<tr>
<td>Urethral exudate:</td>
<td></td>
</tr>
<tr>
<td>N. gonorrhoeae positive</td>
<td>8 (12)</td>
</tr>
<tr>
<td>C trachomatis positive</td>
<td>2 (3)</td>
</tr>
<tr>
<td>N. gonorrhoeae and C trachomatis positive</td>
<td>4 (6)</td>
</tr>
</tbody>
</table>

This may have been due to the fact that IgG antibodies that have leaked across the urethral mucosa are diluted out in the urine to a sufficiently low concentration, that is below the sensitivity threshold of this assay. Furthermore, the walls of the Uristat microtitre plates are coated with an antigentic mixture of six common urinary pathogens: E coli, Proteus mirabilis, Klebsiella aerogenes, Staphylococcus saprophyticus, Pseudomonas aeruginosa and Citrobacter freundii. However, no details of the exact nature of the components of this antigentic mixture are provided by the manufacturer. It may well be that LPS core antigen is not a major antigentic component of this assay, and hence the lack of false positive results.

In conclusion, although recent publications have reported that measurement of urinary antibodies by ELISA is not a useful method of screening urine samples before culture, there do not seem to be any false positive reactions in patients with urethritis when using the Uristat assay.

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Dri Gibb and Edmond comment:

We appreciate the response to our article from Thakker and colleagues. Their findings make a positive and interesting contribution to the debate.

They leave the anomaly that Gram positive urinary tract infections (UTI) result in the presence of antibodies to Gram negative bacteria in the urine, while gonococcal and chlamydial urethritis does not result in the presence of antibody to the mixture of Gram negative and Gram positive bacteria in the urine. In the Uristat test, the presence of antibody in the antigens involved may be important, but this seems unlikely as LPS core epitopes are probably exposed in the Uristat test just as they were in our mixed heat-killed coliform antigen. The difference may be due to the much greater area of urethral mucosa which is involved in UTI, resulting in a non-specific leakage of more antibody into the urine.

Assays of total IgG in the urine in UTI and in urethritis are required to clarify this point. We are in the process of measuring IgG in urine in suspected UTI, but unfortunately no samples from patients with urethritis are currently available (the samples reported by Thakker et al have not been made available). We can find no reference in published findings which reports urinary immunoglobulin titres in urethritis.

The action of vitamin B12

Dr Chanarin and his colleagues review in some detail the evidence against the methyl folate trap hypothesis and that in favour of the carbon folate trap hypothesis. We express the hope that this will lead to further work on the action of vitamin B12 on folate metabolism. Both hypotheses are based on the methylenobalamin dependent methionine synthetase reaction.

However, this, homocysteine reacts with 5-methyltetrahydrofolate to form methionine and tetrahydrofolate (H₄ folate). In the one hypothesis, methionine derived from this reaction is regarded as an important prelude to the remethylation of homocysteine to methionine and the conversion of methyltetrahydrofolate to folate polyglutamate synthetase. In the other, the liberated H₂ folate is made available for the synthesis of all the single carbon atom folate compounds which are finally polyglutamated to form the tetrahydrofolate coenzymes. According to both hypotheses, therefore, all the latter are in short supply in vitamin B₁₂ deficiency. Of these, the most important is 5, 10-methylenetetrahydrofolate (5, 10-CH₂-H₄ folate) the folate acid coenzyme active in the thymidylate synthetase reaction, impairment of which is regarded as the biochemical basis of megaloblastic anaemia.

I would like to suggest that vitamin B₁₂ has an additional action which is independent of both the thymidylate synthetase and the methionine synthetase reactions. Two independent lines of investigation support this view.

First, folate acid is very much more effective than vitamin B₁₂ in correcting the deoxyuridine suppression test (dU test)—a test specifically designed to measure the activity of the thymidylate synthetase reaction. In one series 5 μg/ml of folate acid was almost as effective as 100 μg/m of vitamin B₁₂ in correcting the test in vitamin B₁₂ deficient patients. In vivo, however, vitamin B₁₂ in doses of 2 μg per day produced a reticulocyte response in pernicious anaemia, but folate acid in doses of 200 μg per day failed to do so. Given the weight for weight comparison of such findings, this huge discrepancy is still strong evidence that vitamin B₁₂, in man has an action other than that of correcting the thymidylate synthetase reaction.

Secondly, pharmacological doses of 200 μg of vitamin B₁₂ a day promptly increased the low pretreatment serum methionine concentration to normal in three days in cases of pernicious anaemia. Vitamin B₁₂, in physiological doses of 2 μg/day not only failed to do this but actually depressed it