Screening of urinary tract infections by ELISA

We were pleased to read the article by Gibb and Edmond. 1 Their results are in broad agreement with our evaluation of a commercially available enzyme linked immunosorbent assay (ELISA) (Uristat, Shield diagnostic Systems) for screening of urinary tract infections in an elderly population. 2 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. In the absence of these possibilities 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 leukocyturia of the urethral exudate with the presence of four or more polymorphonuclear leukocytes per oil-immersion field (× 1000 magnification).

Each urine specimen (5 ml) was collected in Boric containers (Medical Wire and Equipment, Corsham, Wiltshire, England). The urine samples were cultured semiquantitatively on 10% (v/v) horse blood agar and scanty lactococcal hydrophilic-deficient (CLIED) agar, and a pure growth of a single bacterial species of > 10 8 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. 2 Each assay plate also included high and low controls which were supplied by the manufacturers. All specimens were also tested for antibody activity by the Uristat using 100 μl of urine on to an Isosensitive (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 trimethylthrim) antibiotic supplement (Oxoid Ltd). Neisseria were supplemented with 5% (v/v) lysed horse blood (Gibco Biocult Ltd). Chlamydia trachomatis was detected by direct immunofluorescence microscopy (Microtrak, Genetic Systems Corporation, Syva UK). All positive results were confirmed by ELISA using IDEA (Boots Cellect Diagnostics Ltd).

Results of culture tests and Uristat assay are shown in the table. Eight patients were culture positive for N gonorrhoeae, two 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.

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 antigenic mixture of six common urinary pathogens: E coli (K12, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus saprophyticus, Pseudomonas aeruginosa and Citrobacter freundii. However, no details of the exact nature of the components of this antigenic mixture are provided by the manufacturer. It may well be that LPS core antigen is not a major antigenic component of this assay, and hence the lack of false positive results.

In conclusion, although recent publications 1 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.

Dr 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. The Uristat test may be relatively insensitive 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 avidity of the urothelium which is hypostatic 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 received). We can find no reference in published findings which reports urinary immunoglobulin titres in urethritis.

The action of vitamin B 12

Dr Chanarin and his colleagues review in some detail the evidence against the methyl folate trap hypothesis and that in favour of the folin folate starvation hypothesis. 4 We would like to present evidence for the action of vitamin B 12 on folate metabolism. 5 Both hypotheses are based on the methylcobalamin dependent methionine synthetase reaction.

In this, homocysteine reacts with 5-methyltetrahydrofolate to form methionine and tetrahydrofolate (H 4 folate). In the one hypothesis, methionine derived from this reaction is regarded as an important precursor of the formation of methyltetrahydrofolate for folate polyglutamate synthesis. In the other, the liberated H 4 folate is made available for the synthesis of all the single carbonation atom folate compounds which are finally polyglutamated to form the serum folate coenzymes. According to both hypotheses, therefore, all the latter are in short supply in vitamin B 12 deficiency. Of these, the most important is 5, 10-methylenetetrahydrofolate (5, 10-CH 2 =H 2 folate) the folate 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 B 12 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, folic acid is very much more effective than vitamin B 12 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 folic acid was almost as effective as 100 μg/m of vitamin B 12 in correcting the test in vitamin B 12 deficient mammals. 6 In vivo, however, vitamin B 12 in doses of 2 μg per day produced a reticulocyte response in pernicious anaemia, but folic acid in doses of 200 μg per day failed to do so. 7 Given the weight for weight approximation of such comparisons, this huge discrepancy is still strong evidence that vitamin B 12 in man has an action other than that of correcting the thymidylate synthetase reaction.

Secondly, pharmacological doses of 200 μg of vitamin B 12 a day promptly increased the low pretreatment serum methionine concentration to normal in three days in cases of pernicious anaemia. 8 Vitamin B 12 in physiological doses of 2 μg/day not only failed to do this but actually depressed it.
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