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 Diagnostics Ltd). We also noted that 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 phenomenon using the Uristat assay. First, void specimens of urine were collected from 67 (41 men, 26 women) patients attending the geriatric medicine clinic. All patients had signs and symptoms of urethritis, and had a leucocyte urinary exudate with the presence of four or more polymorphonuclear leucocytes per oil-immersion field (×1000 magnification).

Each urine sample (20 ml) was collected in Boricon containers (Medical Wire and Equipment, Corsham, Wiltshire, England). The urine samples were cultured semiquantitatively on 10% (v/v) horse blood agar and columbia coliform agar and a pure growth of a single bacterial species of >105 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 antibacterial 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 collected 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, Syva UK). All positive results were confirmed 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, 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 urinary 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, Proteus mirabilis, Klebsiella aerogenes, 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 publications1,4 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.

B THAKKER
R J MICHEL
I B TAFT
A C McCARTNEY
Department of Microbiology, Royal Infirmary, Glasgow G4 0SF


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 Uristat test. We agree with the authors that the Uristat result 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 urothelium which is hypoxic in UTI, resulting in a the 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 the 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 tested). We can find no reference in published findings which reports urinary immunoglobulin titres in urethritis.

The action of vitamin B12

Dr Chanaarin and his colleagues review in some detail the evidence against the methyl folate trap hypothesis and that in favour of the carbon folate trapping hypothesis on the action of vitamin B12 on folate metabolism.1 Both hypotheses are based on the methylcobalamin 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 pre-requisite for remethylation of 5-methyltetrahydrofolate for folate polyglutamate synthesis. In the other, the liberated H4 folate is made available for the synthesis of all the single carbon atom folate compounds which are finally polyglutamated to form the folate 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-Ch3-Ch5 folate) the folate acid coenzyme active in the thymidylate synthetase reaction, impairment of which is regarded as the biochemical basis of megaloblastosis. 2

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, folic 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 folic acid was almost as effective as 100 µg/m of vitamin B12 in correcting the test in vitamin B12 deficient marrow.3 In vivo, however, vitamin B12 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.4 Given the weight of the available evidence for such comparisons, 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
to extremely low concentrations and the hypomethioninaemia persisted for several days (unpublished observation). A possible explanation for this unexpected result lies in the fact that methionine metabolism is influenced, and in opposite directions, by both methylcobalamin and by adenosylcobalamin, the former through the methylcobalamin-synthetase reaction which increases the serum methionine. The latter is a coenzyme in the methylenyl-CoA mutase reaction, this is the last reaction in the propionyl-CoA cycle. During a cyanide poisoning, 'by Galloway, examination


the risk of blood born infection in the post mortem room would be better directed towards reducing that hazard. I have found that the available chain mail protective overgloves for the left hand are of great value in this respect.

P J Dunn
Department of Pathology, Queen's Hospital, Castle Street, Warrington WA1 5AS

Laboratory workers. I would therefore reiterate my conclusion that unnoticed glove puncture is not in itself a health hazard. Laceration of the skin is undoubtedly a health hazard but is not likely to be affected by more frequent glove changes. I agree with the other correspondents that the efforts to minimise the risk of blood born infection in the post mortem room would be better directed towards reducing that hazard. I have found that the available chain mail protective overgloves for the left hand are of great value in this respect.

PG Dunn
Department of Pathology, Queen's Hospital, Castle Street, Warrington WA1 5AS

The teaching of death certification

Death certificates are usually issued by pre-registration house officers, often badly, and sometimes with only a mode of death as opposed to the disease producing death.