CORRESPONDENCE

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) for screening 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 without 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. This in turn suggests 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 possibility 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 leukocytic urinary exudate with the presence of four or more polymorphonuclear leucocytes per oil immersion field (× 1000 magnification).

Each urine specimen (20 ml) was collected in Boricon containers (Medical Wire and Equipment, Corsham, Wiltshire, England). The urine samples were cultured semi-quantitatively on 10% (v/v) horse blood agar and cystine lysis deficient (CLID) 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.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 growing 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) antibiogram test plates (Oxoid Ltd). All blood cultures 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 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: Enterobacter cloacae, 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 publications1 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
J R MICHELI
I B TAIT
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 urine. In our experience, patients in the antigen 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 urethra 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 received). We can find no reference in published findings which reports urinary immunoglobulin titres in urethritis.

The action of vitamin B₁₂

Dr Chananin and his colleagues review in some detail the evidence against the methyl folate trap hypothesis and that in favour of the own folate starvation hypothesis on the action of vitamin B₁₂ on folate metabolism.3 Both hypotheses are based on the methylcobalamin dependent methionine synthetase reaction.

In 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 precut of the remethylation of tetrahydrofolate to form polyglutamate synthetase. In the other, the liberated H₄ folate is made available for the synthesis of all the single carbon atom folate compounds which are final 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-methylene tetrahydrofolate (5,10-CH₂H₄folate) the folate acid coenzyme active in the thymidylate synthetase reaction, impairment of which is regarded as the biochemical basis of the macrocytosis.

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 marrow.1 In vivo, however, vitamin B₁₂ in doses of 2 µg per day produced a reticuloocyte response in pernicious anaemia, but folate acid in doses of 200 µg per day failed to do so.1 Given the weight of 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 hypomethioninemia 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 adenosylcobalamin, the former through the methionine synthetase reaction which increases the serum methionine. The latter is a coenzyme in the methylmalonyl-CoA mutase reaction, this is the last reaction in the propionyl-CoA succinyl-CoA pathway along which methionine is catabolised to the citric acid cycle. Adenosylcobalamin, therefore, aids the catabolism of methionine and depresses its concentration. The above findings suggest that adenosylcobalamin alone is active, and that the action of methylcobalamin is not expressed, and the methionine synthetase reaction therefore not activated by these very minute doses of vitamin B12. They do, however, invariably produce a reticulocytosis response, often a very brisk one, which again suggests that vitamin B12 has an erythropoietic action which is independent of the methionine synthetase reaction, and evidence, from both the methylfolate trap and the formate starvation hypothesis, in the absence of the thymidylate synthetase action as well. Unfortunately by the time the possible interpretation of these results was revealed the work could not be repeated. If, however, the above interpretation is correct it would probably take several days for these very small doses of vitamin B12 to correct the dU suppression test. This contention could therefore readily be tested by relating this interval to the daily reticulocyte count following a daily dose of 2 μg of vitamin B12 in pernicious anaemia. A clear reticulocytosis response, not necessarily the peak, occurring before the dU test is corrected would lend it support.

T E PARRY

Axillon, Pen-y-Twrchfa,
Dinas Powys,
South Glamorgan CF6 4HG


Risk of inhaling cyanide during necropsy examination

I read with interest the article by Forrest, Galloway, and Slater on the risk of inhaling cyanide during necropsy on cases of cyanide poisoning. There is, admittedly, a theoretical risk of inhaling a large amount of cyanide, as observed by Andrews et al.

The recommendation that a respirator be worn during the necropsy or that the stomach should be opened in a fume cabinet is commendable but suffers from one drawback. In one of my cases the diagnosis of cyanide poisoning was made only after opening the stomach. This case presented a sudden and obvious cyanotic death and my diagnosis of cyanide poisoning, based solely on the smell, was greeted with considerable disbelief by the investigating police officers. Subsequent biochemical investigation of a suitable sample and note and an empty container but the impression of everybody concerned, including myself, was that of sudden natural death. At least I am fortunate that I can smell cyanide (my colleague at that time has never been able to).

Theoretically, then, a pathologist who could not smell cyanide would inhale potentially dangerous amounts of cyanide during such a necropsy. Should pathologists routinely wear respirators when performing any necropsy where the circumstances of death are not clear? Or should they routinely open the stomach in a fume cabinet in all such cases? When we refer to the changing face of pathology is it because pathologists of the future will be wearing gas masks? Perhaps readers should be told.

G C A FERNANDO

Central Pathology Laboratory,
Histopathology Department,
North Staffordshire Hospital Centre,
Harthistle, Stone on Trent ST4 7PA


Glove puncture in the post mortem room

I cannot allow Drs Weston and Locker's comments on my criticism of their paper to go unchallenged. They have not correctly cited the paper of Hall et al. This study involved 664 technicians (588 anatomical pathology technicians, 76 veterinary technicians), not 76 as they claim, plus 774 consultants. It also included a control group of Coroner's officers. Two cases of hepatitis B were indeed reported as Drs Weston and Locker state. However, in the Coroner's officer and therefore unlikely to be due to unnoticed glove puncture! The incidence in the at risk and control groups was therefore equal. The 6 reported case of tuberculosis is almost certainly unrelated to glove puncture. The discussion at the end of the paper concludes that apart from the expected high rates of respiratory disorders, the digestive and infectious disease excess noted in the technicians was similar to the findings of a large scale survey of medical 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 great of value in this respect.

P J DUNN

Department of Pathology,
Royal Infirmary,
Castle Street,
Wrexham WIR1 3AS


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.
The action of vitamin B12.

T E Parry

doi: 10.1136/jcp.45.10.941-b

Updated information and services can be found at:
http://jcp.bmj.com/content/45/10/941.2.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/