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 systems Ltd) 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 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. This is possible, but 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 geriatric medicine clinic. All patients had signs and symptoms of urethral irritation, and had a urethrocystic exudate with the presence of four or more polymorphonuclear leucocytes per oil-immersion field (× 1000 magnification).

Each urine specimen (20 ml) was collected in Boric containers (Medical Wire and Equipment, Corsham, Wiltshire, England). The urine samples were cultured semiquanti-tatively on 10% (v/v) horse blood agar and cysteine lactose electrolyte-deficient (CLED) 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 cultured for Neisseria gonorrhoeae. Swabs were inoculated into the clinical on to GC non-selective agar (Oxoid Ltd) and GC selective agar (Oxoid Ltd) containing LCAT (lincomycin, colistin, amphotericin B and trimethoprim) antibiotics (Oxoid Ltd); 5% (v/v) 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.

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 is dependent only on the antibodies 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 the urethral mucosa which is involved in UTI, resulting in a less 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 trimethoprim hypothesis for the action of vitamin B₁₂ on folate metabolism.1 Both hypotheses are based on the methylco-balamin dependent methionine synthase reaction.

However, this homocysteine reacts with 5-methyltetrahydrofolate to form methionine and tetrahydrofolic acid (H₄ folate). In the one hypothesis, methionine derived from this reaction is regarded as an important pre-requisite for the remethylation of homocysteine to methionine. In the later, 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 vitamin B₁₂ 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-methylenetetrahydroyfolate (5, 10-CH₂-H₄ folate) the folate acid coenzyme active in the thymidylate synthase 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 synthase reaction and the methionine synthase 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 synthase 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.3 In vivo, however, vitamin B₁₂ in doses of 2 µg per day produced a reticuloocyte response in pernickous anaemia, but folate acid in doses of 200 µg per day failed to do so.4 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 synthase reaction.

Secondly, pharmacological doses of 200 µg of vitamin B₁₂, a day promptly increased the low pretreatment serum methione 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 methionine synthetase reaction which increases the serum methionine. The latter is a coenzyme in the methylnalonyl-CoA mutase reaction, this is the last reaction in the propionyl-CoA methyl-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 inactive 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 reticulocyte response, often a very brisk one, which again suggests that vitamin B12 has an erythropoietic action which is independent of the methionine reaction, and this evidence, from both the methylfolate trap and the reticulin starvation hypothesis, in the absence of the thymidylate synthetase reaction as well.

Unfortunately by the time the possible interaction of these results was realized the work could not be repeated. If, however, the above interpretation is correct it would probably take several days for 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 reticulocyte response not necessarily the peak, occurring before the dU test is corrected would lend it support.

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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.1 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 natural death and my diagnosis of cyanide poisoning, based solely on the smell, was greeted with considerable disbelief by the investigating police officers. Subsequent examination of the stomach revealed no cyanide, just 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 a necropsy. This pathologist might not 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.

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Dr Forrest, Galloway, and Slater would be happy to answer any question on this paper.

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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.1 This study involved 664 technicans (588 anatomical pathology technicans, 24 medical assistants, 76 as 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, this is not what 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 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 technicans 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 overoggles for the left hand are of great value in this respect.

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c-erb-B-2 expression in male breast carcinoma

Fox et al recently reported a complete lack of c-erb-B-2 expression in 21 cases of male breast carcinoma, while Wright et al reported overexpression in a single case. We have so far examined 33 cases of male breast carcinoma for c-erb-B-2 expression using the monoclonal antibody NCL-CB11 (Novocastra) and a standard immunoperoxidase technique. Omission of the primary antibody and a known positive case of female breast carcinoma were used as negative and positive controls, respectively. Membrane staining was completely absent in 20 cases, but positive membrane staining was present focally within the tumour in 12 cases and throughout the tumour in one case. Thus 39% of our cases show evidence of c-erb-B-2 overexpression. This figure is similar to the 35% reported by Gattuso et al1 in their series of 26 cases.

Our results show that a proportion of male breast carcinomas are associated with c-erb-B-2 overexpression, which is usually related to gene amplification. However, it remains to be seen whether this has the same prognostic importance as that seen in female breast carcinomas.

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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.