

Technical method

SMA 12/60 method. Its improved standardisation is of considerable clinical importance. Its economy (cost per test is less than half of that with SMA 12/60) and other advantages merit consideration for its general application to the determination of uric acid in serum and other biological fluids. Furthermore, the principles outlined here can also be adapted to any continuous-flow automated system.

Preliminary experiments on this procedure were done with the assistance of Mr R. C. King at the Department of Pathology, Upstate Medical Center, Syracuse, NY, USA. We thank Dr von Redlich for a helpful review and suggestions and appreciate the excellent editing and preparation of this manuscript by Mrs Jean P. Nelson and Miss Janice Perry.

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Letters to the Editor

A sensitive urine-test for monitoring the ingestion of isoniazid

Ellard and Greenfield (1977) described in your Journal a highly sensitive 'new isonicotinic acid method' for controlling isoniazid ingestion in urine. This new method is one of several modifications of the chemical procedure published 19 years ago by Nielsch (1958). His basic principle has also been utilised by others in the design of tests for the detection of isonicotinic acid in urine (Kasik *et al.*, 1962; Belles and Littleman, 1962; Tuberculosis Prevention Trial, 1967). All these procedures are suitable for the control of the daily intake of INH provided 300-400 mg therapeutic doses are administered. Our control test (Eidus and Hamilton, 1964a, b), to which reference is made by Ellard and Greenfield (1977), detects *acetylisoniazid*, the main metabolite of INH, in urine. The *acetylisoniazid* method is considered as sensitive as the tests introduced by Kasik *et al.* (1962) and Belles and Littleman (1962). The advantage of the Eidus-Hamilton (1964a) procedure is its great simplicity. It may be carried out by laboratory workers, without technologist qualification, trained at the job. Employing automatic pipettes, approximately 250 tests can be completed

in one hour. The sensitivity of the *acetylisoniazid* procedure could be increased; however, such modification would affect the speed and ease of the performance. Because of its simplicity, the *acetylisoniazid* method has in the last decade been extensively used by the British Medical Research Council in their tuberculosis chemotherapeutic trials undertaken in Europe, South-East Asia, and Africa.

The test detects 10-15 $\mu\text{g/ml}$ *acetylisoniazid* in urine (Eidus and Hamilton, 1964a; Venkataraman *et al.*, 1965). Hence it was most surprising to note that in the experiments of Ellard and Greenfield (1977) urine samples containing 30 and 40 $\mu\text{g/ml}$ *acetylisoniazid* produced with our method positive readings in only 82% and 91%, respectively, of the specimens.

A volunteer study was also conducted by Ellard and Greenfield in which oral doses of 50 and 100 mg isoniazid were taken by the participants and urine specimens were collected at various intervals for the performance of the *acetylisoniazid* procedure and 'the new isonicotinic acid test'. In this trial, the new test exhibited, with a dose of 100 mg INH, 96% and 34% positive readings in urine samples collected between 23-24 and 47-48 hours, respectively, after drug administration. If the authors intended to prove that their method is suitable for checking daily INH intake, at least therapeutic doses should

also be employed in the volunteer trial. The experimental design of this study does not allow conclusions to be drawn. It seems from the results obtained with 100 mg isoniazid in volunteers that the new method is not only cumbersome but far too sensitive to control the regularity of daily INH self-medication.

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The authors have commented as follows:

As we stated in our paper (Ellard and Greenfield, 1977), our urine-test method for monitoring the ingestion of isoniazid used the reaction procedure we reported previously for quantitatively determining isonicotinic acid and isonicotinylglycine (Ellard *et al.*, 1972). In this previous paper we described how the procedure had been modified from that originally described by Nielsch in order to improve its reproducibility and sensitivity. The results set out in our paper demonstrated that, employing this modification, concentrations of down to about 0.75 µg/ml of isonicotinic acid and isonicotinylglycine could be reliably detected in urine. This indicates that our isonicotinic acid urine-test method is considerably more sensitive than the Belles and Littleman, and Kasik modifications (to about 2.5 and 10 µg/ml isonicotinic acid, respectively).

Notwithstanding Dr Eidus' surprise with some of our results, there can be no doubt from the results given in our paper and the experience of others who have tried both methods that our new isonicotinic acid method is much more sensitive than the Eidus and Hamilton acetylisoniazid procedure. The acetylisoniazid method has been of great use in the past for monitoring the regularity of ingestion of isoniazid but it is now being replaced by our more sensitive isonicotinic acid method in current British Medical Research Council studies for the practical reason that it enables test urine samples to be collected at any time. The centres that have made the decision to use the new method have felt that this advantage outweighs the slightly more elaborate nature of the procedure as compared to the Eidus and Hamilton acetylisoniazid method.

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Anaerobic blood culture media

The article by Collee *et al.* (1977) raises some interesting problems. There must be better methods of obtaining an anaerobic headspace than by the method suggested, which seems to be erratic. The commercial method used by Difco, which seems to be the best so far, is to fill the vacuum in the autoclave with carbon dioxide and then screw a false lid down onto the bottles, forcing the bungs into place. As the autoclave cools still further, a vacuum is created in the bottles. After taking these elaborate procedures to produce anaerobically sterilised media, it seems a curious practice carefully to remove the bungs, thereby losing the vacuum the media had. Consequently, poor isolation of anaerobes may occur and the chances of contamination are much greater. This is due, firstly, to the shortness of the bung and, secondly, to the vacuum being released, thereby sucking air rapidly into the bottle. The bungs are invariably wet around the neck and this liquid will be contaminated fairly easily. The suggestion of using syringes and needles to subculture is a very practical method and has been practised in this department since the introduction of Thiol broth for the past four years. This difference in inoculation and sampling procedures may account for the differing results obtained by Collee *et al.* (op cit) compared with my own (Szawatkowski, 1976).

I agree with the authors that it is very difficult to compare the costs of laboratory-made media and commercially produced media. Firstly, Thiol broth is £31.00 for 100 (Difco) bottles; any media transported from London to Edinburgh (including Oxoid's Brain Heart Infusion broth) incurs a carriage fee. Southern Group Laboratories (a non-profit making laboratory) sell 50 ml of cooked meat medium at £16.80 for 100 bottles. Gibco Biocult will supply Brain Heart Infusion broth with cooked meat particles (as sug-

gested by Collee *et al.*) at £48.00 per 100 as well as Thiol at £27.00 for 100; therefore Thiol is not the most expensive.

Nowhere in my article (Szawatkowski, 1976) did I suggest that Thiol should be used on its own as a general purpose blood culture medium. Thiol has several advantages. Firstly, in this laboratory we have found that it yielded anaerobes far more rapidly than the other media tested. Secondly, it inactivated the antibiotics listed in the article; and, thirdly, it was found that Thiol grew facultative anaerobes on average 24 hours earlier than the aerobic or vented bottles.

In this laboratory, as a routine 'set' of blood cultures, three bottles are used: Thiol, tryptic soy broth, and Southern Group nutrient broth with a castenada slope. It is very difficult to find a medium which will universally support the growth of the variety of organisms that can be cultured from blood. Consequently, our set can be changed at will, depending on the type of patient who is being screened.

As Collee *et al.* (op cit) say, it is very difficult to simulate clinical microbiological problems in a carefully controlled laboratory model. I would suggest that the proof of the pudding is in the eating and the only way to compare the media in clinical cases of bacteraemia.

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Diagnosis of *Candida vulvovaginitis*

Jones and Warnock (1977) comment that Stanley and Hurley (1974) omitted to determine the incidence of candida precipitins in pregnant women harbouring yeasts as commensals in the vagina. The 200 patients whom we studied were selected because of the high probability of mycotic vulvovaginitis among them; all harboured yeasts and many had symptoms suggestive of vaginitis (Table II). In Table III we contrasted the percentage incidence