Technical methods

A simple, safe nitroprusside test using Ketostix reagent strips for detecting cystine and homocystine in urine

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For detecting urinary cystine or homocystine a test such as the cyanide-nitroprusside is required, as recommended in the Association of Clinical Pathologists Broadsheet no. 61. It is not safe to allow inexperienced workers to use such a toxic reagent without supervision, and for this reason an alternative way of performing this test seemed desirable. This has been achieved by using zinc and hydrochloric acid to reduce the disulphides with hydrogen, and, for convenience, Ketostix reagent strips (Ames Company) are then employed to detect the cysteine and homocysteine produced.

Method

1 Dip Ketostix in the urine and record result.
2 Pour approximately 1 ml urine into a small test tube, eg, as used with Clinitest reagent tablets.
3 Place a few small pieces of zinc granulated AnalaR BDH in the tube.
4 With a Pasteur pipette (or Ames dropper) add 50% v/v concentrated hydrochloric acid AnalaR BDH in water drop by drop to the urine, with gentle tapping of tube to mix, until the zinc is seen to give off hydrogen bubbles. (Alkaline urine may froth initially and this must not be confused with the action of the acid on the zinc on adding further acid.)
5 Leave the urine in the tube for about half an hour then repeat the Ketostix procedure and record the result again.
6 Finally repeat the Ketostix procedure once more and record the result when hydrogen evolution has ceased. If necessary leave overnight before final testing.

When cystine or homocystine are present in the urine a pink Ketostix colour develops *after* reduction. With high concentration of the disulphides the test becomes positive after only a few minutes. The colour may be seen only at the edges of the impregnated strip in slightly positive urine, and develops after the 15 seconds' observation recommended for ketones.

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Result

Cystine and homocystine were added to normal urine, which gave a negative result alone, and both produced positive findings in the new test when their concentration was 5 mg/100 ml or more. The cyanide-nitroprusside test was positive with more than 3 mg/100 ml of the disulphides in this urine. An obviously positive result for disulphides was produced by urine samples from eight patients with known cystinuria, and by urine from two children with homocystinuria and an adult with Wilson's disease and aminoaciduria. One urine from a child with cystinuria gave a positive Ketostix result initially but it became negative after reduction. The amino-acid chromatogram showed large amounts of the basic amino-acids but little cystine (cysteic acid). On further examination the urine was found to be infected and alkaline. Moistened Ketostix and lead acetate paper became positive when held over the urine which had only been acidified and hydrogen sulphide was smelt. It was concluded that the cystine had been converted to sulphide before testing.

The effect of ketones, which give an initial positive reddish-purple Ketostix colour, was studied by adding acetone and acetoacetic acid to normal urine and testing also 16 urines from patients with ketosis. After reduction, Ketostix became orange-brown. With high concentrations of ketones it seemed possible that this would mask a positive thiol colour, but with acetone 100 mg, and cystine, 50 mg/100 ml normal urine, an initial purple Ketostix became a distinctive pink after reduction.

The new test was performed on urines submitted for routine examination with the following results. In 120 cases in which two-way paper chromatography, performed as described by Smith (1960), showed a normal amino-acid pattern the test for disulphides was negative; four of these had been positive initially for ketones. In 14 further urines the amino-acids were also normal and a trace of cystine (cysteic acid) was seen. By comparison with standard spots cystine excretion was found, in these cases, to be high but within normal limits (less than 70 mg/1 g creatinine). Seven of these urines were negative for disulphides and seven slightly positive, two of the latter having also given an initial positive ketone reaction. Two of the patients with cystinuria were also discovered in this routine trial.

Urines from one patient with phenylketonuria and one with maple syrup urine disease gave an initial slight browny-pink Ketostix colour, somewhat slow to develop, as did aqueous solutions of sodium phenylpyruvate and α-ketoisovaleric and α-ketoisocaproic acids. These all became negative after reduction. Urine from one patient with alcaptonuria and an aqueous solution of homogentesic acid also gave a similar initial colour which persisted after reduction.

The tests described were performed with paper Ketostix, but a small number of additional tests later showed that the new plastic product is equally effective.

Comment

These experiments have shown that both the new test and the original cyanide-nitroprusside reagent have a similar sensitivity. The new test has proved reliable in

detecting pathological cystinuria and homocystinuria. The experience described with it, however, emphasizes that, like other screening procedures, it should only be used as a guide to consideration and further investigation of positive findings.

Reference

Smith, I. (1960). *Chromatographic and Electrophoretic Techniques*, vol. 1, p. 101. Heinemann, London.

Direct microscopical examination of tube cultures for the detection of trichomonads

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Many laboratories culture suitable material for trichomonads in addition to the microscopical examination of films. Clinical specimens, often genital swabs, are inoculated into trichomonas medium in small bottles which are incubated for several days. At intervals wet films are prepared from these cultures for microscopical examination, and this may involve a considerable amount of work. We describe a simple, quick method which allows direct microscopical examination of tube cultures for trichomonads with the low-power objective without the need to make wet preparations.

Procedure

Medium suitable for the cultivation of trichomonads is dispensed into sterile tissue culture or similar tubes (4 in. × ½ in.) to within an inch of the top. The tubes are stoppered tightly with white rubber bungs and stored in racks at + 4°C for not longer than three weeks. Alternatively, sterile plastic disposable tissue culture tubes with screw caps (Sterilin) may be used. In this laboratory, a cysteine-peptone-liver infusion-maltose medium similar to that described by Stenton (1957) is used, with the omission of penicillin and streptomycin and the addition of the following reagents to give concentrations in the final medium as indicated: chloramphenicol (100 μg/ml), nystatin (50 μg/ml), and Oxoid ion agar no. 2 (0·05%). After inoculation of routine culture media and the preparation of both wet and Gram-stained films, the swab is placed in a tube of trichomonas medium previously warmed to room temperature, and the stopper replaced. The inoculated tubes are incubated upright in racks at 36°C for up to seven days.

Each culture tube is examined daily with the low-power objective of the microscope. Before examination, the tube is inverted to ensure even distribution of any trichomonads and then held on a small cradle made from glass rods placed on the microscope stage. By sliding and rotating the tube in the cradle the whole culture may be quickly examined. With a little experience trichomonads are easily recognized by both their flagellae and their characteristic motility which is often independent of currents within the tube.

Comment

This method for the direct microscopy of tube cultures will be familiar to those with experience of routine virus tissue culture techniques. It is quick, simple, saves both time and effort, and thus encourages daily examination. It allows rapid observation of the unopened whole tube of medium in contrast to the drop needed for a wet preparation. Neither a deposit nor the swab itself obscure the recognition of motile trichomonads. This technique, which may be used with other trichomonas media, also permits early detection of trichomonads, not only in cultures which grow well, but also in cultures in which growth is poor.

Reference

Stenton, P. (1957). The isolation of *Trichomonas vaginalis*. *J. med. Lab. Technol.*, **14**, 228-230.

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