Screening test for an automated protein-bound iodine technique

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An automated method for the estimation of serum protein-bound iodine following alkaline incineration has been described by Welshman, Bell, and McKee (1966). In this procedure, the serum was pretreated with an anion exchange resin which removes relatively large amounts of inorganic iodine. However, organic iodine from x-ray contrast media was not absorbed by the resin and remained a frequent source of iodine contamination during incineration. This paper describes a simple screening test for the detection of sera containing grossly raised iodine levels. Two drops of serum are used for this test which is performed without delaying the routine protein-bound iodine estimation.

PROCEDURE

The apparatus and reagents are the same as described by Welshman *et al.* (1966) except that 12 × 100 mm Pyrex test tubes M/W are used in place of the larger tubes.

The procedure can be carried out while the normal routine batch is drying in the 115°C oven.

Place two drops of serum and two drops of 2N potassium hydroxide in a Pyrex test tube and dry in an oven at 115°C for 90 minutes. Transfer the tubes to the muffle furnace and incinerate at 620°C for 30 minutes. When the tubes are cool add three drops of acid diluent, three drops of sodium arsenite solution, and three drops of ceric ammonium sulphate solution. Shake the tubes after the addition of each reagent. Place the tubes in a water bath at 37°C and after five minutes add one drop of 1% aqueous brucine sulphate solution to stop the reaction as recommended by Faulkner, Levy, and Leonards (1961).

INTERPRETATION OF RESULTS

Serum containing less than 20 µg of iodine per 100 ml will produce a definite amber coloured solution while serum with a level greater than 30 µg/100 ml will give a completely colourless solution. The test may be adjusted to give greater sensitivity by prolonging the period of incubation. Contaminated serum detected by this procedure can be removed from the main batch before incineration.

The screening technique has been used in this laboratory for almost a year and has successfully detected all serum with grossly abnormal iodine levels. As approximately 3% of all sera received for protein-bound iodine estimation were contaminated with organic iodine the screening procedure has prevented numerous batches

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Stability of papain-cysteine-anti-D mixture on freeze drying and on storage

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Papain-cysteine solution has been used at the Glasgow and West of Scotland Blood Transfusion Service since the publication by Löw (1955) on the value of activated papain solution for red cell grouping.

Gilbey and Lindars (1962) investigated the stability of freeze-dried, activated papain and found promising results. That publication prompts this report on storage of a dried papain-cysteine-anti-D mixture over a period of 10 years.

A mixture of equal parts of Löw's papain-cysteine solution and a serum containing incomplete anti-D antibody was prepared in April 1957. The serum had been tested by Löw's technique against a panel of cells of known Rh phenotype to ensure specificity, particularly the absence of anti-C and anti-E. The titre of the serum was 16 and of the mixture was 8 when measured by the albumin replacement technique with CcDE phenotyped red cells. A simple titration with saline as diluent was not suitable because only the first tube of the serial dilutions contained adequate papain for agglutination of red cells suspended in saline. The mixture was immediately distributed into ampoules in 2 ml volumes and freeze drying was carried out with an Edwards 3 P.S. centrifugal freeze drier. The ampoules were sealed off *in vacuo* and stored in a metal box at room temperature (20°C).

Periodically, over the years, an ampoule was opened and the contents were dissolved in 2 ml of sterile distilled water. The approximate time of solution was noted and the mixture was then tested against a panel of six phenotyped red cell suspensions (probable genotypes R₁r, R₂r, R₁R₂, R'r, R'r', and rr) in saline, using one drop, of red cell suspension to one drop of papain-cysteine-serum mixture. Complete agglutination resulted with D-positive cells after incubation at 37°C for one hour and cells lacking the D-antigen showed no agglutination.

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Screening test for an automatic protein-bound iodine technique—concluded.

of serum protein-bound iodine results from being rendered worthless.

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The titre of the anti-D was again measured by the albumin replacement method using CcDE phenotyped cells. No loss of avidity or decrease in solubility or titre was detected after testing at intervals throughout 10 years.

The activity of the papain-cysteine content of the mixture was investigated by using the mixture with an incomplete anti-c serum and testing against R'r, R''r, and rr phenotyped cells suspended in saline. All proved negative when equal volumes of the mixture and anti-c serum were used but with two drops of the papain-cysteine anti-D mixture ($\equiv$ one drop of Löw's solution) and one drop of anti-c serum, complete agglutination of the red cells resulted within one hour at 37°C. The anti-c serum was checked to ensure absence of any saline agglutinating anti-c.

After testing, the reconstituted material was stored frozen at -20°C except during periods of re-tests. Each ampoule was re-tested three to seven times during the following four weeks. No loss of avidity, titre, or specificity was detected.

The pH of the reconstituted material was 6.1 but that of the original mixture had not been determined. However, a similar mixture, prepared later, had a pH of 6.3.

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Use of a pancreatin-trypsin solution for the liquefaction of sputa for routine bacteriological examination

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The irregular distribution of pathogens within a single specimen of sputum from patients with chronic bronchitis was first demonstrated by May (1953), who found that a true assessment of the bacterial flora was obtained only after multiple cultures. The liquefaction of the sputum using a 1% pancreatin solution in saline buffered at pH 7.6 and at 37°C was introduced by Rawlins (1953) to overcome the need for multiple cultures, but this technique suffered from the disadvantage that some sticky specimens were slow to liquefy. Other workers (Mead and Woodhams, 1964; Woodhams and Mead, 1965) used N-acetyl-L-cysteine (N.A.C.) as a liquefying agent which they claimed gave a higher rate of isolation of *H. influenzae* and was more rapid in its action than pancreatin.

Recently it has been found in this laboratory that the addition of trypsin to pancreatin considerably reduces the time required for liquefaction, and in the present report the efficiency of the pancreatin-trypsin mixture is compared with that of N-acetyl-L-cysteine. Since from time to time it is necessary to examine sputa for the presence of eosinophils, which may also be unevenly distributed within the specimen, the effect on the detection of these cells of adding trypsin to the pancreatin has been included in the assessment.

MATERIALS AND METHODS

Five-hundred and fifty sputa were examined. The liquefying agents were each used on alternate days in order to rotate their use for the various outpatient clinics. In the analysis of the results no account was taken of the clinical diagnosis or treatment since the details supplied were often inadequate for the classification of the specimens.

Pancreatin-trypsin solutions were prepared by dissolving two tablets of Oxoid buffered pancreatin tablets in 100 ml of sterile distilled water and adding 25 mg of pure crystalline trypsin in 5 ml of sterile diluent. The trypsin used was Novo Crystalline Trypsin (Duncan Flockhart & Evans, Ltd.), in vials of 50 mg amounts, together with a vial of sterile diluent. Preliminary trials of trypsin concentration and alternative sources had shown that this product and concentration were satisfactory. N-acetyl-L-cysteine solutions were prepared as described by Woodhams and Mead (1965).

Sputa were liquefied in sterile 2 oz containers by adding

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