collection procedure should be standardised so that samples remain at room temperature (20°C) for 2–3 hours to allow equilibrium to occur before separation. Keeping blood samples strictly at 37°C before and during separation is not practical for routine monitoring. As yet no clear correlations between the whole blood CyA concentration, immunosuppressive effect and toxicity have been found, although in the future the measurement of whole blood CyA concentrations may eliminate variation due to changes in cellular binding of CyA in vitro.

**Recognition of Haemophilus species**

It is fairly general practice, and is also stated in some texts\(^1\) that in the clinical laboratory the recognition of Haemophilus species is primarily based on the observation of satellitism on a fresh blood agar plate, either in a mixed culture or, when growing in pure culture, on such a plate streaked with a staphylococcus strain which provides V factor.

This useful procedure is inapplicable, however, when dealing with Haemophilus species that are X factor dependent only, such as *H. aphrophilus*, *H. haemoglobinophilus* (canis) or *H. ducreyi*, and with haemolytic species such as *H. haemolyticus*, *H. parahaemolyticus*, *H. parahaemolyticus* and *H. pleuro pneumoniae*. On fresh blood agar plates the haemolytic species obtain their optimum supply of V factor by virtue of their haemolytic property alone and, therefore, reach their maximum colony size without additional sources of V factor. Thus, the primary recognition of such species depends on cultural and microscopical appearances.

Using manufactured blotting paper discs impregnated with X, V and X plus V factors we have been aware for a long time that occasionally dependence on one or both of these factors could not be elicited when it was clearly expected. During checking the point discussed in the preceding paragraphs with a number of haemolytic strains we were surprised to observe that a proportion of discs were surrounded by an area of inhibition. The discs were clearly contaminated by some antibiotics inhibiting haemophili. On inquiry the manufacturers had to admit that through faulty production technique such contamination had occurred (in our experience this occasional contamination must have been taking place for years) but assured us that measures had been taken to avoid such unfortunate errors. In the experience of one of us this is not the first time that a manufactured laboratory product from a reputable firm contained a substance which was not expected to be present.

Testing for X and/or V factor requirements can be organised easily with a microbiological laboratory's own resources and is based on two simply prepared media. The scheme was first recommended in 1960\(^2\) and in its modified form is perfectly adequate for first stage testing of X and/or V factor requirements.

Medium 1 is an autoclaved blood agar which, apart from adequate nutrients, contains X factor only. Medium 2 is either Difco Proteose Peptone No 3 agar or Oxoid Isosensitest agar both of which do not contain X or V factors. More than one strain suspected of belonging to the genus *Haemophilus* can be tested on one plate each of these two media by first being streaked separately across the plates from edge to edge. Then a strain of *Staphylococcus aureus* is streaked at right angle to the previous streak(s) down the centre of the plates from edge to edge. After overnight incubation X dependent strains will grow uniformly from edge to edge on the autoclaved blood agar plate only. V dependent strains will grow on both plates for a distance of 0·5 to 1·0 cm from the staphylococcal streak whilst X plus V dependent strains will grow for a similar short distance on chocolate agar plates only.

Any further tests, if desired, such as δ-amino-laevulinic acid assimilation, sugar fermentations and tests for the presence of enzyme systems\(^3\) can be carried out as a second or third stage progression but for most practical clinical purposes the first stage redescribed here will be found to be quite adequate. As one colleague remarked in 1960 "the method does not look very scientific and is a bit messy but it works."

**References**


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**Fig. 2** Decrease of serum concentrations of cyclosporin A after storage of whole blood at room temperature (20°C) and 37°C. The initial serum cyclosporin A concentration was 235 ng/ml
References


Trimethoprim susceptibility to staphylococci

We have observed that inclusion of 1-(4-nitrophenyl)-glycerol (PNPG) as an anti-swarming agent in antibiotic susceptibility tests can give rise to anomalous results.

We performed disk tests in parallel to break point sensitivity tests in our studies. Isoensitest agar (Oxoid) with 5% added dried blood and NAD was used in both methods, but PNPG was added at a final concentration of 50 mg/l (the concentration suggested by Mast Laboratories) to plates used in the break point method. The final concentration of trimethoprim used in the break point method was 0.5 mg/l and discs containing trimethoprim 1-25 μg were used in the disc diffusion method.

Forty-five of 118 clinical isolates of *Staphylococcus aureus* and 11 of 34 coagulase-negative staphylococci were resistant by the break point method, but all were sensitive by the disc method. Subsequent isolates were tested for susceptibility to trimethoprim by the break point method using media with and without added PNPG. Thirty of 90 strains of *Staph aureus* and one of 21 coagulase-negative staphylococci were found to be resistant only in the presence of PNPG. In tests to determine the minimum inhibitory concentration (MIC) of trimethoprim, all resistant isolates were inhibited by 0.25 mg/l without PNPG, but the MIC was 1.0 mg/l in its presence.

We wish to draw attention to our findings, which we have only observed when testing the susceptibility of staphylococci to trimethoprim. Reduction of the concentration of PNPG to 15 mg/l inhibits the swarming of proteins in our hands, but does not produce anomalous results in trimethoprim susceptibility.

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Book reviews


This valuable book draws together information on the theory and practice of immunofluorescence. The fifteen chapters, on selected topics, are without exception clearly written. In the first part of the book the authors discuss the theoretical basis of immunofluorescence and ways of standardizing the methods, as well as describing the techniques and instruments for measuring it. In the second part they discuss immunostaining techniques and their applications to patholoy, in particular to identification of autoimmune sera, renal disease, and immune complexes. Throughout the book the reader is led gently through the complexities and finishes each chapter with useful insights into the problems. Details of the authors' individual ways of carrying out the techniques include the practical tips that are all too often omitted. Some bias towards the authors' preferred methods is apparent, but the bibliography is extensive, up to 1980, and references are given in full. The book is well illustrated and exceptionally clearly printed.

**Anaerobic Infections.** Public Health Laboratory Monograph Series. 3rd ed. AT Willis and KD Phillips. (Pp 53; paperback £2.75.) HMSO.

The Luton team now adds to its significant contributions to clinical anaerobic bacteriology by giving us a clear and straightforward guide that takes account of the considerable advances of the last decade, especially in relation to the non-sporing anaerobes. This new monograph is a fine example of realistic condensation based on much experience. There is obvious self discipline in restricting the text to useful simplified accounts. The practical methodology is within the compass of a busy clinical laboratory, and helpful tables and identification schemes are easy to follow. Aerotolerant and microaerophilic species are included, with guidance on campylobacter isolation and identification. There is a very helpful general section on media and methods, and an informative note on gas-liquid chromatography. This is essential reading for all clinical bacteriologists and it will be in constant demand at the bench.

**Letters to the Editor**

Some new titles

The receipt of these books is acknowledged, and this listing must be regarded as sufficient return for the courtesy of the sender. Books that appear to be of particular interest will be reviewed as space permits.


Correction

With reference to the article by Thompson et al in the March 1983 issue1 Professor Kohn's present address is now: Department of Clinical Pathology, Royal Marsden Hospital, Downs Road, Sutton, Surrey.

Reference