the National Blood Transfusion Service for blood group serology. The method for tanning and sensitizing the cells was that of Kessel, Lewis, Pasquel, and Turner (1965) with one modification, viz, the stabilization of the sensitized cells was effected with phosphate buffer containing 3% of bovine albumin. The test was performed in the following way. Doubling dilutions of sera were made from 1/8 upwards in standard plastic plates. One standard drop (0.035 ml) of the serum dilution being tested was placed on a glass well slide. One standard drop of a 2% suspension of tanned sensitized cells was added. These were mixed gently with a glass rod, the slide was put on a mechanical horizontal rotator for 10 minutes, at about 70 rpm, and the result read immediately.

Positive reactions showed distinct clumping of the cells as seen in the classical ABO grouping tile technique. Agglutination could be graded from 1+ to 3+ on the size of the clumps. To standardize the system it was important to rotate for a fixed time as longer rotating times resulted in higher titres, which would have necessitated an adjustment of the borderline titre indicative of positivity.

Result

We studied this method of reading indirect haemagglutination using filaria antigen to sensitize the cells. Seventy sera from individuals who had never left Britain were all negative at a dilution of 1:32. Eight hundred sera from patients who had resided in areas of endemic filariasis showed 65% of microfilaria carriers and 13% of non-microfilaria carriers to be positive at dilutions of 1:64 or higher. Occasional sera gave high titres of the order of 1:1,000 or higher. We could not detect any particular cross-reactions with any specific helminths, and in contrast to the complement-fixation test for filariasis cross reactions in *Strongyloides* infections were a rarity; but there were some unexplained positives. After treatment for filariasis the haemagglutination test remained positive for much longer than the complement-fixation test. These results are in line with those obtained by other workers using filaria haemagglutination (see Kagan and Norman, 1970, for a review).

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Present day practice

Antibiotics in mycoplasma media and the temporary storage of specimens containing mycoplasmas of the genital tract

C. S. GOODWIN, M. C. COWAN, AND D. J. LAVIS *From the Public Health Laboratory, Portsmouth*

Colour changes in liquid media are used to detect the presence of mycoplasmas, including *Mycoplasma pneumoniae* (Smith, Chanock, Friedewalde, and Alford, 1967) and T-mycoplasmas (Taylor-Robinson, Addey, and Goodwin, 1969). However, these colour changes can also be produced by contaminating bacteria and yeasts, and the growth of these organisms may not be inhibited by the antibacterial agents, penicillin and thallium acetate, commonly used in selective media (Andrews, 1969). Ampicillin, 1 mg/ml, was reported by Hutchinson (1969) to suppress contaminating bacteria, and is recommended by Fallon (1969). Nystatin, 50 units/ml, is included in selective media for the isolation of some animal mycoplasmas (Whittlestone, 1969). Polymyxin B, 50 µg/ml, and amphotericin B, 5 µg/ml, are preferred by some workers (Braun, Klein, Lee, and Kass, 1970).

In our laboratory in 1967, in all selective media, penicillin was replaced by ampicillin (reported by Taylor-Robinson, 1968); in media for the isolation of *M. pneumoniae* we also added methicillin, 1 mg/ml, and nystatin, 57 µg/ml (200 units/ml). When sputum was used as the inoculum, the proportion of false colour changes due to contaminating bacteria was reduced from 53 out of 117 (45%) to two out of 54 (4%) by these additions. The medium still fully supported the growth of *M. pneumoniae*. In media for the isolation of T-mycoplasmas when penicillin was replaced by ampicillin a few false colour changes due to bacteria continued to occur; these were found to be due to *Pseudomonas aeruginosa* and ampicillin-resistant *Proteus* organisms. To inhibit these organisms we have now replaced ampicillin with carbenicillin, 1 mg/ml, and among 150 subsequent specimens from the genital tract no false colour changes have occurred. Nystatin, 57 µg/ml, is also included in the selective medium.

The effect of different temperatures for overnight

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storage on large-colony mycoplasmas isolated from throat swabs was reported by Andrews (1969). The swabs were transported in 0.2% bovine albumin in Hanks' solution, and after storage at 4°C, of 36 swabs yielding large-colony mycoplasmas, 34 (94%) remained positive. However, after 24 hours at -30°C, of 44 positive swabs, mycoplasmas could not be isolated from 25 (57%). When Grover's transport medium (Grover, Spoerk, and Evans, 1965) was used for swabs from the genital tract the isolation rate of T-mycoplasmas was the same before and after storage at 4°C for 24 hours. In this laboratory a basic growth medium including yeast extract and 20% horse serum is used for transport of swabs (Taylor-Robinson *et al*, 1969) and is recommended by Fallon (1969). T-mycoplasmas and *M. hominis* were isolated from 18 fresh specimens in growth medium and from 16 of these after storage at -30°C.

Storage for longer periods than 24 hours seems to present more difficulties. A temperature of -30°C is adequate for stock cultures containing a high initial count of organisms (Addey, Taylor-Robinson, and Dimic, 1970). We stored 22 specimens from the genital tract which contained T-mycoplasmas at -20°C for five days, and 14 (64%) remained positive. Braun *et al* (1970) reported that storage of urine at -80°C for one week did not reduce the isolation rate of T-mycoplasmas. However, most laboratories do not have space available at such low temperatures for temporary storage of specimens. We have stored aliquots of growth medium which had contained swabs from the genital tract in a wide-mouth vacuum flask in which was a care-

fully sealed plastic bag containing solid carbon dioxide, and the vacuum flask was then placed in the -20°C cabinet. After five days, some solid carbon dioxide remained, suggesting that the temperature had remained at -65°C. T-mycoplasmas were isolated from eight fresh specimens and from seven of these after storage for five days in this way.

With increasing interest in human mycoplasmas, further work on the ideal transport medium and storage temperature for clinical specimens would seem to be indicated.

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