

Technical methods

Isolation of *Bordetella pertussis*: benefits of using both Bordet-Gengou and charcoal agar media

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Clinical confirmation of whooping cough depends largely on the isolation of the causative organisms within the genus *bordetella*, of which *Bordetella pertussis* is the most common.¹ Isolation of this micro-organism is difficult. It is influenced by various factors, including the method and time of specimen collection, prior antibiotic treatment, transport to the laboratory, and handling thereafter.^{2,3} Most laboratories with experience in handling such cultures take care to avoid factors that adversely influence the rate of isolation of *B pertussis*. It is common to use either Bordet-Gengou medium^{1,4} or charcoal agar medium⁴ for this purpose; most laboratories in the UK now prefer charcoal agar medium alone, although some studies have indicated the use of both media in conjunction.^{5,6}

We present our experience over three years at the Bacteriology Laboratory, City Hospital, Edinburgh, using charcoal agar medium and Bordet-Gengou medium in parallel and monitoring the growth on each type of medium.

Material and methods

A total of 919 pernasal swabs were examined during 1980-1982; most of these (80%) were from hospital patients, although a substantial number (20%) were from general practice patients. Generally, three consecutive pernasal swabs (Medical Wire and Equipment Co Ltd) were obtained from each patient early in the disease. Hospital swabs were quickly transported to the laboratory, and those from general practice were transported in Transwab transport medium (Medical Wire and Equipment Co Ltd). On receipt, each swab was immediately inoculated on to two freshly prepared thick plates: one contained Bordet-Gengou medium (Difco Laboratories containing 20% defibrinated horse blood and 0.25

units/ml of benzyl penicillin) and the other contained charcoal agar (Oxoid Ltd, containing 10% defibrinated horse blood and 0.3 units/ml of benzyl penicillin). No fixed policy was adopted to inoculate these plates in any particular order. Both plates were incubated in a moist chamber at 35-36°C and examined after three days and five days. Any suspicious colonies on a given medium were subcultured on to the same medium and growth on both media was recorded separately. The final identification was confirmed by agglutination tests using specific antiserum (Wellcome Reagents Ltd).

Results

The results of 919 pernasal swabs examined during 1980-1982 are shown in the Table.

The increase in the total number of specimens and isolations during 1982 was partly influenced by an epidemic of whooping cough in Scotland during this year and partly because of projects undertaken locally to investigate the incidence of pertussis isolation in adult patients with persisting cough.

Discussion

Charcoal medium is generally preferred to Bordet-Gengou medium because of the ease in its preparation and its superiority in growing *B pertussis* by absorbing the toxic metabolites of the organism.^{2,4,6,7} Growth on Bordet-Gengou medium tends to autoagglutinate and may offer difficulty in typing the strain.² For these and other technical reasons, charcoal agar medium is used widely for the primary isolation of *B pertussis*,^{6,7} although some workers have noted poor isolation with the recommended charcoal agar medium.⁸ It is also documented that a correctly made Bordet-Gengou medium is quite adequate for the routine isolation of *Bordetella*.³ In our experience, while most strains were isolated from both Bordet-Gengou and charcoal agar media, some strains were only isolated either from Bordet-Gengou or from charcoal agar medium alone (see Table). Although it could be argued that our increased isolation rate might simply be the quantitative effect of inoculating two plates, it is more likely that the small but distinct advantage that each medium has over the other was more contributory to the higher isolation. Thus there would appear to be a definite advantage in using both charcoal agar and Bordet-Gengou media for routine isolation. We believe that the extra cost of using both

Number of *Bordetella pertussis* strains isolated on Bordet-Gengou medium or charcoal agar medium, or both, during 1980-1982

Year	Total no of specimens	Total no of positive cultures	Growth on Bordet-Gengou medium only	Growth on charcoal agar medium only	Growth on both Bordet-Gengou and charcoal agar medium
1980	275	59	6 (10.6%)*	14 (23.7%)	39 (66.1%)
1981	233	34	4 (11.7%)	11 (32.3%)	19 (55.8%)
1982	411	80	12 (15%)	20 (25%)	48 (60%)

*Figures in parenthesis present the percentage of the total positive culture.

media is justified. A good isolation rate of *B pertussis* depends on several factors and we consider that one important factor is the use of two media in parallel.

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Microimmunofluorescence technique for detection of antibody to *Mycoplasma genitalium*

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Mycoplasma genitalium organisms were isolated originally from the urethras of men suffering from non-gonococcal urethritis.^{1,2} They have proved to be fastidious in their growth requirements, slow to replicate, and difficult to isolate.³ The metabolism inhibition test⁴ may be used as a serological approach to determine the possible association of

this mycoplasma with disease, but slow multiplication of the organisms delays a result and antibiotics in serum may inhibit mycoplasmal growth and lead to a false result. We have developed a microimmunofluorescence technique for detecting antibodies to *M genitalium* which does not have these drawbacks and appears to be more sensitive than the metabolism inhibition test. Furthermore, this mycoplasma, like *M pneumoniae*, has the ability to adhere to glass or plastic, a property which facilitates the production of antigen.

Material and methods

ANTIGEN PREPARATION

M genitalium was used after four subcultures in SP4 liquid medium. One millilitre of a culture containing 5×10^6 colour changing units was added to 40 ml of SP4 medium in a 260 ml capacity plastic tissue culture flask (Nunc). The flask was incubated horizontally at 37°C until the colour of the medium had changed from red to yellow, a period of about eight days. By this time a sheet of mycoplasmas was

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