Selective medium for the isolation of *Bordetella pertussis* and *parapertussis*

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Benzyl penicillin is widely used as a selective agent in media for the isolation of *Bordetella pertussis* and *parapertussis*. We report the use of cephalexin (Muggleton, O'Callaghan, Foord, Kirkby, and Ryan, 1968) as a selective agent with a wider spectrum than penicillin.

Preliminary experiments were carried out with a freeze-dried strain of *Bord. pertussis* originally isolated by Dr E. C. Armstrong on an antibiotic-free medium, and a number of freeze-dried strains of *Bord. parapertussis*. Surface viable counts were made on Oxoid charcoal agar (CM119) with 10% horse blood and various concentrations of cephalexin (93% potency). The strain of *Bord. pertussis* was not inhibited by 10 to 80 μg/ml of cephalexin, but there was some inhibition at 100 μg/ml. The strains of *Bord. parapertussis* were more sensitive to cephalexin: there was little or no inhibition by 40 μg/ml, slight inhibition by 60 μg/ml, and greater inhibition by levels of 80 to 100 μg/ml. At 60 μg/ml of cephalexin some strains of *Bord. parapertussis* showed variation of colony size after five days' incubation but this had disappeared after seven days. From these preliminary experiments we chose a level of 40 μg/ml of cephalexin for use as a selective agent in the charcoal-blood-agar base. During these experiments we also found that some strains of *Bord. parapertussis* showed marked variation of colony size on certain batches of charcoal-blood-agar containing no antibiotic; other strains were unaffected. This inhibitory effect of some batches of charcoal-blood-agar was removed by the addition of a final concentration of 1% Difco proteose peptone no. 3.

Comparison of Isolation of *Bord. pertussis* on Two Media

MEDIUM A (ROUTINE)

Charcoal agar plates with a final concentration of 10% horse blood, 1% Difco proteose peptone no. 3, and 0·25 units benzyl penicillin per ml.

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MEDIUM B (EXPERIMENTAL)

As above with the substitution of 40 μg/ml cephalexin for the benzyl penicillin.

Plates were incubated for seven days at 36°C before final reading. Fresh batches were poured each week and stored at 4°C before use. Twenty-three routine pernasal swabs were inoculated directly on medium A, then placed in stabs of charcoal agar without blood or antibiotic and later the same day inoculated onto medium B. For the next 23 swabs the procedure was reversed and a further 29 swabs were inoculated onto both media from the charcoal agar stabs. The results of isolation of *Bord. pertussis* are shown in Table I and the growth of commensals in Table II.

Medium	*Number of Isolations of* B. pertussis
A only	2
B only	3
A & B	18
Total number of swabs	75

Table I *Isolation of* Bord. pertussis *on routine (A) and experimental (B) media*

Medium	*No. of Swabs According to Growth of Commensals*				*Total No. of Swabs*
	< 20 Colonies	*20-50 Colonies*	*> 50 Colonies*	*Overgrown by Moulds*	
A	38	5	31	1	75
B	64	2	9	0	75

Table II *Growth of commensals on routine (A) and experimental (B) media*

Although the isolation rate on each medium was similar, the experimental medium gave a significant reduction in growth of commensals, which facilitated the examination of the plates. The inhibition included penicillin-resistant staphylococci, some coliforms, and many, but not all, strains of *Haemophilus influenzae*. In a previous series of tests the results of direct inoculation of the routine medium was compared with a lower concentration of cephalexin (30 μg/ml) in charcoal-blood-agar inoculated from charcoal agar stabs. Of 146 swabs tested, *Bord. pertussis* was grown from 25 swabs on both media, from two swabs on the routine penicillin medium only, and from six swabs on the cephalexin medium only.

If serotyping is to be carried out it may be necessary to subculture strains of *Bord. pertussis* isolated on the cephalexin medium onto penicillin charcoal-blood-agar or antibiotic-free charcoal-blood-agar,

as there may be poor development of type-specific antigen on the cephalexin medium (N. W. Preston, personal communication).

Conclusions

A medium containing Oxoid charcoal agar (CM119) with 10% horse blood, 1% Difco proteose peptone no. 3, and 40 μg/ml of cephalexin has proved satisfactory for the isolation of *Bord. pertussis*. The isolation rate was as good on this medium as on the routine medium containing penicillin, but plates were easier to read due to the reduction in numbers of commensals. The cephalexin plates should be incubated for seven days at 36°C before being reported as negative, since some strains of *Bord. parapertussis* may show variation in colony size with shorter periods of incubation.

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Reference

Muggleton, P. W., O'Callaghan, Cynthia H., Foord, R. D., Kirkby, Susan M., and Ryan, D. M. (1968). In *Laboratory Appraisal of Cephalexin. Antimicrobial Agents and Chemotherapy*, edited by Gladys L. Hobby, pp. 353-360.

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