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Comparison of Modified Bordet-Gengou and Modified Regan-Lowe Media for the Isolation of *Bordetella pertussis* and *Bordetella parapertussis*

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Culture and fluorescent-antibody methods for detection of *Bordetella* species were evaluated by two state public health laboratories. Field-inoculated plates of Regan-Lowe agar medium were most useful if incubation was initiated on the day of collection. Regan-Lowe and Bordet-Gengou media were comparable for subculturing nasopharyngeal specimens that were transported and enriched in half-strength Regan-Lowe agar. Maximum sensitivity was achieved when the media were used in parallel. Fluorescent-antibody-stained smears of nasopharyngeal specimens were more sensitive for detection of *Bordetella pertussis* than for detection of *Bordetella parapertussis*. The fluorescent-antibody method, however, was too insensitive for use without culture.

We reported recently on the antimicrobial susceptibilities of *Bordetella* species isolated during the Centers for Disease Control Multicenter Pertussis Surveillance Project (6). We now report on an evaluation of laboratory test methods for detection of *Bordetella* species that was done during the same project at the state public health laboratories in Colorado and Wisconsin. The primary objective of the study was to evaluate the abilities of modified Bordet-Gengou agar (BG) and Regan-Lowe agar (RL) to detect *Bordetella* species.

From 1985 to 1986, paired nasopharyngeal (NP) swabs were obtained from suspect pertussis cases at closely monitored surveillance sites in Denver, Colo., and Milwaukee, Wis. In addition, face-to-face contacts of suspect cases were cultured by nurses assigned to the project. NP specimens were collected by using two calcium alginate swabs which were both inserted through each nare. Swabs were left in contact with the nasopharynx for 15 to 30 s, until a cough was elicited. Thin smears were then prepared by rolling one swab on glass slides for fluorescent-antibody (FA) staining. The second swab was used to inoculate modified Regan-Lowe charcoal agar containing 40 μg of cephalexin per ml (9) and 20 μg of anisomycin per ml (primary RL) contained in a Marbec plate (GIBCO Laboratories, Madison, Wis.). The medium was warmed to room temperature before inoculation and then sealed in a plastic bag. The NP swabs were placed in a tube of half-strength RL (transport RL) for transport to the laboratory. Primary RL and transport RL were stored under refrigeration and were used within 8 weeks of preparation.

The primary RL plates were removed from the plastic bag and incubated at 35°C in high humidity upon receipt in each laboratory. The plates were examined daily for 7 days for colonies typical of *Bordetella* species. The direct smears were stained upon receipt or within 24 h with FA reagents specific for *B. pertussis* and *B. parapertussis* (Centers for Disease Control, Atlanta Ga.). Stained smears were observed by using incident fluorescence microscopy with a tungsten halogen or mercury vapor light source. Smears were interpreted as positive if at least five morphologically typical, highly fluorescent (≥2+) cells (with only peripheral staining) were observed. Positive and negative control slides were included in each test run.

The RL transport specimens were also incubated at 35°C in high humidity (5, 9) and subcultured. In Colorado, the transport tubes were usually delivered the day of collection and incubated 3 days before subculture. In Wisconsin, the transport tubes were received by mail (1 to 2 days) and were then incubated 24 to 48 h before subculture. Although less than ideal (5), this delay by mail is common in many states. After incubation (enrichment), subcultures were made to RL and BG contained in 100-mm round plates. The subculture plates were designated secondary RL and secondary BG cultures and were considered part of the enrichment process (9). The secondary RL and BG plates were prepared weekly, stored at 4°C, and warmed to room temperature before use. BG (Difco Laboratories, Detroit, Mich.) was prepared with 20% fresh, defibrinated sterile sheep blood; cephalexin (40 μg/ml); and anisomycin (20 μg/ml). Inoculated plates were incubated at 35°C in high humidity and observed for 7 days for growth of *Bordetella* species.

Criteria for identification were typical colony morphology on RL or BG, reaction with *B. pertussis* and *B. parapertussis* FA stains, Gram stain reaction, cell morphology, ability to grow on routine blood agar, oxidase and catalase reactions, presence or absence of motility, and other biochemical tests when necessary (7).

The sensitivities of the various test methods were determined by the method of Galen and Gambino (2). The significance of differences between sensitivities of test results obtained in the two laboratories was determined by the test of two proportions (10).

Of 3,835 NP swabs, 125 (3.3%) were positive for *B. pertussis* by FA or culture or both. Specifically, *B. pertussis* was detected in 89 (4.4%) of 2,015 specimens collected in Colorado and in 36 (2%) of 1,820 specimens collected in Wisconsin. Sensitivity values for each method were based

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TABLE 1. Sensitivities of FA test and culture methods for detection of *B. pertussis* and *B. parapertussis*

Organism	No. of positive tests	FA test			Primary culture on RL			Enrichment subcultures from transport medium					
								Secondary culture on RL			Secondary culture on BG		
		% Positive (no.)	% False-negative (no.)	% Only positive test (no.)	% Positive (no.)	% False-negative (no.)	% Only positive test (no.)	% Positive (no.)	% False-negative (no.)	% Only positive test (no.)	% Positive (no.)	% False-negative (no.)	% Only positive test (no.)
B. pertussis	125	38 (48)	62 (77)	7 (9)	66 (82)	34 (43)	14 (18)	58 (72)	42 (53)	3 (4)	61 (76)	39 (49)	9 (11)
B. parapertussis	23	26 (6)	74 (17)	9 (2)	65 (15)	35 (8)	13 (3)	65 (15)	35 (8)	9 (2)	65 (15)	35 (8)	4 (1)

upon 125 positive FA and culture results. Nine positive FA specimens which were negative by culture were included for determination of sensitivity. The FA results differed significantly between the laboratories ($P < 0.04$) (10). FA sensitivities were 33 and 53% in Colorado and Wisconsin, respectively, for a combined sensitivity of 38% (Table 1). Although the combined data showed that 7% of all positive specimens were detected exclusively by FA (Table 1), in Colorado no exclusively positive FA specimen was detected, compared with 25% in Wisconsin. The differences in FA results were not the result of variability of interpretation in reading FA smears. The two laboratories achieved nearly identical results when they exchanged positive and negative FA-stained slides in a blinded study (unpublished data). Presumably, the rapid incubation of specimens in Colorado was responsible for the absence of exclusively positive FA specimens.

The sensitivities of the primary RL culture also differed significantly between the laboratories. *B. pertussis* was isolated from 73% of cultures in Colorado and 47% of cultures in Wisconsin ($P < 0.01$) (10). The combined data showed that 14% of the positive specimens were detected exclusively by the primary RL. The primary RL was the only positive test in 19% of cases in Colorado (17 specimens) and 3% of cases in Wisconsin (1 specimen). Variation in primary culture sensitivities may have been the result of differences in rapidity of incubation after collection of specimens in the laboratories. In addition, specimens were not incubated before mailing (U.S. Postal Service) and were subject to adverse temperatures in transit. These results suggest that the primary RL plate should be incubated on the day of collection. The adverse effect of transportation was previously reported for NP swabs in RL transport medium (5). The use of the primary RL plate is probably best reserved for intensive surveillance studies, such as in outbreak investigations or in the event of high incidence rates. For the 0- to 4-year age group, the incidence rates of pertussis for 1985 were 16.1 and 14.5 cases per 100,000 in Colorado and Wisconsin, respectively.

Regan and Lowe (9) reported excellent recovery of *B. pertussis* and *B. parapertussis* with a cephalexin-containing charcoal agar (Oxoid, Columbia, Md.). They did not, however, concomitantly compare RL and BG. Regan and Lowe (9) also reported a significant increase in the recovery rate of *Bordetella* species by using an enrichment technique. Using seeded saliva specimens, Hoppe et al. (5) confirmed the report of Regan and Lowe (9). By contrast, a recent study reported decreased recovery of *Bordetella* species and increased overgrowth by other organisms with RL enrichment (S. A. Young, G. L. Anderson, and P. D. Mitchell, Editorial, Clin. Microbiol. Newsl. **9**:176–179, 1987). In our study, the NP swabs from the RL transport medium were subcultured to secondary RL and BG at various times after incubation (from 1 to 3 days). The sensitivities of the secondary RL and BG were 58 and 61%, respectively (Table 1). We found that only 3% of the positive specimens were detected exclusively on secondary RL, compared with 9% on secondary BG. This difference was equally apparent in the data from Colorado and Wisconsin. Twenty-two percent of the 125 positive specimens were detected exclusively by the combination of secondary RL and BG cultures. The secondary RL yielded 13 strains of *B. pertussis* not recovered on secondary BG, whereas secondary BG recovered 17 strains not recovered on secondary RL. Our data support the observation of Ahmad and Calder (1) that maximum sensitivity is obtained by using BG and RL.

We also demonstrated that BG and RL media did not differ from subculturing NP swabs after enrichment in RL transport medium. This result conflicts with a recent report (F. Chan, E. Rossier, A. MacKenzie, H. Heick, A. Mohammed, and A. Camus, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C289, p. 371). We attempted to determine whether the commercial source of dehydrated BG media was a factor in this difference. However, all 56 stock strains of *B. pertussis* tested grew equally well on BGs from two manufacturers (Difco; BBL Microbiology Systems, Cockeysville, Md.). These results confirm the report of Hoppe and Vogl (3) that growths of stock cultures of *B. pertussis* were comparable on RL and BG.

An advantage of BG is that colonies of *Bordetella* species are more distinct than are those on RL. The colonies have a narrow zone of weak beta-hemolysis. Contaminants that had colony morphologies resembling those of *Bordetella* species were also frequently observed on RL. This was not observed on BG. A significant advantage of RL is the 8-week shelf life at 4°C (9), compared with 1 to 2 weeks for BG. RL was also superior at half strength for transport and enrichment. Presterilized tubes of BG base, however, can be stored for 1 year under refrigeration. The prepared base can then be melted, cooled to 50°C, and supplemented with sheep blood and cephalexin (8). These plates can be used for plating transported NP specimens before and after enrichment. A disadvantage of these media is overgrowth of miscellaneous gram-negative rods, gram-positive cocci, and fungi, despite the presence of cephalexin and anisomycin.

Twenty-three specimens (0.6% of all specimens, or 15.5% of all specimens positive for *Bordetella* species) were positive for *B. parapertussis* (Table 1). Sixteen positive specimens were from Colorado, and seven were from Wisconsin. The sensitivity of FA was 26%, and the proportion of positive specimens detected exclusively by FA was 9%. The low incidence and sensitivity values suggest that routine FA smears for *B. parapertussis* should not be performed. In this study, 1,917 *B. parapertussis* FA smears had to be processed and examined to detect 1 positive specimen that was missed by the culture methods. These results confirm a recent report on the nonvalue of FA for detection of *B. parapertussis* (Young et al., Editorial, Clin. Microbiol. Newsl. **9**: 176–179, 1987). Fortunately, *B. parapertussis* is not difficult

to culture. The sensitivities of primary RL, secondary RL, and secondary BG were 65%. The proportions of positive specimens detected exclusively by each method were as follows: primary RL, 13%; secondary RL, 9%; and secondary BG, 4%. Eight strains of *B. parapertussis* (35% of the total) were detected only on the secondary enrichment subculture plates. Of these, four grew on secondary RL and BG, three grew only on secondary RL, and one grew only on secondary BG. Although our combined culture methods detected 91% of the positive specimens, the expense of a primary RL plate is unwarranted. Enrichment RL medium with subculture to RL and BG is sufficiently sensitive (74%) for detection of low incidences of *B. parapertussis*. FA, then, probably should be used for *B. parapertussis* only in the event of a high incidence of *Bordetella* infections as detected by culture or if the culture plates are frequently overgrown by other organisms.

In summary, the primary RL plate was most useful for detection of *B. pertussis* if it was incubated on the day of collection. The FA test should not be used by itself for detection of *B. pertussis*. Enrichment subcultures to RL and BG increased the positivity rate by 22%, and BG was as effective as RL. The enrichment method alone is sufficient for detection of *B. parapertussis*.

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