tutes a misrepresentation of the tests and, therefore, their performance. For these reasons, we believe the authors’ data and interpretation do not adequately address these issues and should not be construed to do so.

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Author’s Reply

Dr. Creager and Mr. Mach are apparently unaware of the fact that in the early stages of this project, we worked with Dr. Howard Soule of Kallestad to address many of the issues they raise. Any modification of their recommended procedure was evaluated before being used in our study. For example, we found that using Syva mounting medium instead of the Kallestad mounting medium did not alter our results. Similarly, we compared Formalin-fixed antigen, fresh antigen, and antigens actually provided to us by Dr. Soule of Kallestad and saw no appreciable differences in the outcome when using the Kallestad staining reagent. We used 10 μl to stain the slides rather than 30 μl because the area of the wells we stained (19.6 mm²) was about one-third of the area of the wells in kits used for patient specimens. As clearly stated in the paper, the results presented in Table 1 are all with undiluted reagents. Dilution of staining reagent was not advocated for clinical use but was used to ascertain the relative intensities of the stains tested.

In summary, we believe that the procedures we used provide an objective comparison of staining reagents and should prove useful in comparing new products or modifications of other products.

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Recovery of Bordetella pertussis from Nasopharyngeal Swabs

Morrill et al. (2) present very interesting results, but words of caution seem necessary with regard to generalizing their findings. Their experiments were conducted with only one strain of Bordetella pertussis, which may not necessarily be representative. Their simulated nasopharyngeal (NP) specimens did not contain NP secretions or saliva but a mixture of rather arbitrarily chosen (why Candida krusei and not C. albicans?) stock strains of only four species intended to represent normal NP flora. Other important constituents of NP flora, such as neisseriae and Haemophilus spp., were omitted. In addition, the authors used Dacron swabs, although calcium alginate swabs are recommended for B. pertussis (1, 3).

My main point of concern is the recommendation of Morrill et al. (2) to refrigerate NP swabs in Regan-Lowe transport medium during shipment. In their experiments with B. pertussis in pure culture, the number of organisms recovered was highest after preincubation of the transport medium at 35°C, while storage at 4°C led to a >75% decrease. When they used mixtures of B. pertussis and flora, B. pertussis could not be isolated at all after preincubation due to overgrowth of cephalaxin-resistant flora, while no overgrowth was observed after storage at 4°C.

Practical experience shows that growth of cephalaxin-resistant organisms after preincubation of Regan-Lowe transport medium does not occur as frequently as in the laboratory experiments of Morrill et al. (2). We saw it in 34 of 148 cases (23%) (J. E. Hoppe and S. Wörz, unpublished results). Growth of contaminants did not always preclude growth of B. pertussis. The contamination rate after preincubation has to be weighed against the >75% loss of B. pertussis organisms seen during refrigeration, which may render isolation impossible if swabs do not contain large inocula. In my opinion, preincubation is preferable to refrigeration.

LITERATURE CITED


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Author’s Reply

We agree with Dr. Hoppe that generalizations based on laboratory experience should be interpreted cautiously, and we thank him for his opinions. Our laboratory data (1), however, support observations we made during field investigations.

The bacteria included in our mixed cultures were not arbitrarily chosen, as Dr. Hoppe suggests. The organisms selected were representative of those genera we found to predominate in cultures from pertussis patients and their contacts. Saliva and nasopharyngeal (NP) secretions were not used in order to control the numbers and proportions of bacteria present in the inocula.

Dr. Hoppe expresses concern regarding the use of a single strain of Bordetella pertussis in our experiments. Although most of our experiments were conducted with strain TX-13, another strain (9031) isolated during a separate epidemic investigation was also tested. Results were similar for both strains (unpublished data).
Our laboratory initially used calcium alginate swabs to collect NP specimens. The swab head frequently detached from the aluminum shaft during transport or partially dissolved in the transport medium. The use of Dacron swabs eliminated this problem. The effectiveness of such swabs in the recovery of *B. pertussis* has been documented (2).

The rate of overgrowth after preincubation of NP swabs quoted by Dr. Hoppe (23%) is not consistent with observations made in our laboratory. During a recent investigation of a pertussis epidemic, we compared preincubation with plating upon receipt of NP swabs in Regan-Lowe transport medium. Both the transport medium and plating medium contained cephalexin and amphotericin B. Of 173 specimens, 110 (63%) were overgrown with NP flora after 2 days of preincubation (unpublished results). Of 28 specimens that were culture positive for *B. pertussis*, not one was recovered after preincubation that was not previously isolated by culture upon receipt. When the next 142 NP swabs received were transported at 4°C, the rate of overgrowth decreased to 7%, and 16 additional isolates were recovered. While we realize that host populations differ significantly worldwide, our experience indicates that refrigeration of NP specimens during transport is preferable to preincubation for the primary isolation of *B. pertussis*.

**LITERATURE CITED**


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