

patients can pay for it. In public hospitals, the LA test is used only with severe diarrhea that requires rapid and special treatment. In general, electrolyte solution and proper nutrition are prescribed for most diarrheic patients before physicians know the diagnostic results from the laboratory. Laboratory diagnosis of rotavirus is usually not an urgent need. Yet rotavirus (and other microbiological) tests are required for confirmation of clinical diagnosis and monitoring of the status of infectious diarrhea in our community. For purposes of the latter, we recommend the use of polyacrylamide gel electrophoresis (PAGE) for daily testing; it is much cheaper than the LA test and enzyme-linked immunosorbent assay (ELISA); thus, we can test most or all patients with diarrhea. Rotavirus results from PAGE can be reported in a working day, which is more rapid than several other microbiological tests. We also have a small-sized gel for running only a few specimens at a time, which takes only 1 to 1.5 h. Please note that the PAGE method that we use is different from that reported by Sanders et al. (1). We do not know how Steele et al. operated their PAGE test, because the South African journal is not available in Thailand. Maybe the use of different reagents and a different procedure yields different results.

We maintain the ELISA in our laboratory for checking equivocal results of the LA test and for confirmation of the presence of non-group A rotaviruses which appear in the PAGE test with unusual RNA patterns. We perform the direct ELISA method, which is modified from that previously described by Yolken et al. (2). More than 100 specimens must be tested at a time in the ELISA to reduce technician work load and expense. In any event, our complicated ELISA is still much cheaper than ready-made ELISA kits. Based on our experience, the ELISA is a delicate technique at all steps of performance. All reagents

used must be in good quality and must be very well controlled. That is why a careful and very well-trained technician is needed. More than that, a lot of money is needed to establish an ELISA in a laboratory. Several laboratories in Bangkok and rural areas cannot support such a test system, but recently they have been able to establish the PAGE test for rotavirus diagnosis. We found that transfer of the PAGE technique to other persons is much easier than transfer of the ELISA technique. All laboratory workers who came to learn rotavirus diagnosis methods from us preferred to use the PAGE technique because it is not too expensive, it is easy to perform, and it gives informative results.

We realize that PAGE is not the best technique in terms of rapidity and simplicity. We certainly need a better and cheaper technique for detection, as well as for differentiation of rotavirus groups, subgroups, serotypes, and species of origin, which is necessary for surveillance and management of the disease.

LITERATURE CITED

1. Sanders, R. C., A. D. Campbell, and M. F. Jenkins. 1986. Routine detection of human rotavirus by latex agglutination: comparison with enzyme-linked immunosorbent assay, electron microscopy and polyacrylamide gel electrophoresis. *J. Virol. Methods* **13**: 285-290.
2. Yolken, R. H., H. W. Kim, T. Clem, R. G. Wyatt, A. R. Kalica, R. M. Chanock, and A. Z. Kapikian. 1977. Enzyme-linked immunosorbent assay (ELISA) for detection of human reoviruslike agent of infantile gastroenteritis. *Lancet* **ii**:263-267.

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Reagents for Diagnosis of *Chlamydia trachomatis* Infections

In our opinion, the article by L. D. Cles, K. Bruch, and W. E. Stamm (*J. Clin. Microbiol.* **26**:1735-1737, 1988) contains several inconsistencies in the methods, which invalidate the data and conclusions presented. These can be summarized as follows:

(i) The authors used 10 μ l to stain the slides, which is approximately threefold less than the volume recommended in Kallestad Diagnostics' package insert. This reduction in volume can adversely affect the performance characteristics of the staining reagent. In addition, the authors suggest diluting the staining reagent into a generic diluent. Each staining reagent is optimized to provide maximum specific fluorescence, minimum background, and maximum stability. Diluting the reagent into a generic matrix will undoubtedly alter the performance of the reagent.

(ii) Similarly, each manufacturer's mounting medium is formulated specifically for its staining reagent. The authors chose to use one manufacturer's mounting medium (Syva) for all six kits. The utilization of another manufacturer's or a generic mounting medium can have dramatic, deleterious effects on the observed fluorescence and lead to inaccurate interpretations.

(iii) The reagents used in this study are known to react with the lipopolysaccharide and/or the major outer mem-

brane protein of *Chlamydia trachomatis* (see each manufacturer's package insert). Treating the organisms with Formalin can cause cross-linking of both of these antigens and alter the epitope recognized by a specific antibody. Unfortunately, the authors elected to Formalin fix their specimens prior to evaluating the staining reagents rather than following each product's recommended method of fixation. This deviation could alter the presentation of lipopolysaccharide and/or major outer membrane protein and interfere with the binding of the antibody. In addition, the authors indicated that after Formalin fixation the organisms were treated according to each manufacturer's recommended protocol. This approach is inappropriate as most chemical fixations, such as Formalin, are irreversible.

Clearly, we were quite surprised at the methods used in this evaluation. We suggest that a more relevant and comprehensive evaluation would be to compare the six reagents on fresh preparations of all serovars of *C. trachomatis*. In addition, specimens, reagents, and procedures should be followed as directed by each manufacturer. Each of the tests reviewed by the authors has undergone careful scrutiny by U.S. licensing agencies and by credible investigators in a finalized format. Changing the test procedures or altering the components of the tests from the prescribed format consti-

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 μ l to stain the slides rather than 30 μ l 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 cephalixin-resistant flora, while no overgrowth was observed after storage at 4°C.

Practical experience shows that growth of cephalixin-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

1. Hoppe, J. E., and A. Weiss. 1987. Recovery of *Bordetella pertussis* from four kinds of swabs. *Eur. J. Clin. Microbiol.* 6:203-205.
2. Morrill, W. E., J. M. Barbaree, B. S. Fields, G. N. Sanden, and W. T. Martin. 1988. Effects of transport temperature and medium on recovery of *Bordetella pertussis* from nasopharyngeal swabs. *J. Clin. Microbiol.* 26:1814-1817.
3. Parker, C. D., and B. J. Payne. 1985. *Bordetella*, p. 394-399. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.

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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).