Letters to the Editor
Diagnosis of Rotavirus Infection

Kasempimolporn et al. (3) recently reported the use of polyacrylamide gel electrophoresis and silver staining for the routine diagnosis of rotavirus infection in Thailand. They reported that the “simplicity, economy, and efficacy” of polyacrylamide gel electrophoresis diagnosis of rotavirus makes this technique a viable alternative to the use of expensive enzyme-linked immunosorbent assays for the rapid diagnosis of rotavirus infection. They also noted that enzyme-linked immunosorbent assays need expensive equipment and highly skilled technicians (3).

While it is true that most diagnostic techniques used routinely for the detection of rotavirus are fairly sophisticated and require advanced technology and qualified personnel, another major drawback to these techniques is that they require several hours to perform. This performance time becomes a problem in patient care and management because the results of the assay are not available until after the patient has left the clinic or hospital (unless admitted). Rapid and simple tests that can be used in nonspecialized hospital laboratories, clinics, and even physicians’ offices are needed (2).

Although reductions may be made in the financial cost, polyacrylamide gel electrophoresis is a labor-intensive technique which still requires experienced personnel and expensive reagents (phenol and silver nitrate, for instance): it has to be delayed until a “batch” is ready for testing and then still requires some hours before a result is ready. As such, this technique is not suited to small hospital laboratories or outlying clinics in rural communities, such as are found in South Africa and Thailand. As a major etiological agent of gastroenteritis, rotavirus needs rapid and accurate diagnosis for proper patient management.

A number of commercially available latex agglutination tests for the detection of rotavirus became available recently (4, 5). These tests depend on the agglutination of antibody-coated latex particles by rotavirus antigens present in fecal extracts. Among the advantages claimed for the latex tests are that they are simple to perform, can be read by eye, do not require expensive equipment, and are suitable for use on the ward and in the consulting room and that the reagents are stable at 4°C (2, 4).

An early diagnosis of rotavirus infection for the physician could affect patient management in that (i) the unnecessary administration of antibiotics would be avoided, and (ii) unnecessary hospitalization with the concomitant risk of nosocomial spread of rotavirus could be prevented. Laboratories attached to small hospitals and outlying clinics catering to the needs of rural communities would also benefit from a rapid and easy-to-perform test for the detection of rotavirus. In South Africa, gastrointestinal diseases are still of primary importance in black children and are second only to respiratory infections in white children (8). Several studies have shown that infants and young children with rotavirus infection and up to 5 to 10% dehydration can be treated with oral sucrose or glucose electrolyte solutions, if they are not in shock (1, 6). This means that an early diagnosis of rotavirus gastroenteritis, which poses a greater risk of dehydration than with bacterial infections, may be treated with oral rehydration mixtures at an early stage.

Latex agglutination is one of the tests that offers promise in this context, since it is an inexpensive, simple, and reliable assay. Although the sensitivity of the test for rotavirus was inferior to that of the enzyme-linked immunosorbent assay, it is sufficiently specific and sensitive to be used reliably in clinics, and it is certainly more sensitive than polyacrylamide gel electrophoresis (7, 9). The latex assay was easily the least complex assay that we tested, requiring the least amount of apparatuses and giving a very rapid result (9). Latex agglutination assays are suitable for the screening of rotavirus gastroenteritis and should be easily implemented in the smaller hospital laboratory and clinic.

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A. D. Steele
Division of Virology
Department of Microbiology
Medical University of Southern Africa
P.O. MEDUNSA 0240
Pretoria, Republic of South Africa

Author’s Reply
I appreciated reading Dr. Steele’s comments. We also prefer to use the latex agglutination (LA) test for screening of rotavirus in stool samples because of its rapidity and simplicity. The LA test is available commercially in Thailand, and it is used in most private hospitals and clinics when
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.

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Pantipa Sinachatchanant Department of Microbiology Faculty of Science Mahidol University Rama VI Road Bangkok 10400, Thailand

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 µl 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 membrane 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...