JOURNAL OF CLINICAL MICROBIOLOGY, June 1990, p. 1132–1134
0095-1137/90/061132-03$02.00/0

Vol. 28, No. 6

Occurrence of Nonspecific Reactions among Stool Specimens Tested by the Abbott TestPack Rotavirus Enzyme Immunoassay

STEVEN M. LIPSON,[1,2]* GARY P. LEONARDI,[1] RICHARD J. SALO,[3] TED E. SCHUTZBANK,[4] AND MARK H. KAPLAN[2]

Virology Laboratory, Departments of Pathology and Laboratories[1] and Pediatrics,[3] Nassau County Medical Center, East Meadow, New York 11554; Department of Medicine, North Shore University Hospital-Cornell University Medical College, Manhasset, New York 11030[2]; and Technicon Instruments Corporation, Tarrytown, New York 10591[4]*

Received 30 November 1989/Accepted 12 February 1990

Sixty-five stool specimens obtained from children suffering from gastroenteritis were tested for the presence of antigen to rotavirus by the Abbott TestPack Rotavirus (TestPack) enzyme immunoassay kit. The Kallestad Pathfinder enzyme immunoassay, polyacrylamide gel electrophoresis, immune electron microscopy, and virus isolation were utilized as reference assays. Fifty-four specimens were in accord by TestPack and Kallestad Pathfinder. Among 11 discordant specimens positive with TestPack but negative by Kallestad Pathfinder, rotavirus was not identified by polyacrylamide gel electrophoresis, immune electron microscopy, or isolation in primary African green monkey kidney cell cultures. TestPack displayed a performance specificity of 83%. The inordinately high number of stool specimens reported as false-positive by TestPack precludes the incorporation of this antigen detection kit into our routine regimen of diagnostic virologic testing.

Rotavirus is a major etiologic agent of diarrhea and gastroenteritis in infants, young children, and the elderly (5). Although rotavirus infections are usually self-limiting, high mortality rates occur in Third World countries and, to a lesser extent, in the United States (9). An accurate and rapid laboratory diagnostic test for this infectious agent is needed to assist the physician or health care worker in the administration of the appropriate therapeutic modalities.

The Abbott TestPack Rotavirus (TestPack) enzyme immunoassay (EIA) is a relatively new, rapid, and easy-to-perform test for the detection of rotavirus antigen in stool. The sensitivity of the assay has been found to be satisfactory for the diagnosis of rotavirus gastroenteritis. However, the specificity of TestPack, at selected geographic locations, has been shown to vary from 90 to 100% (2, 3, 15). The purpose of this study was to investigate the performance of the TestPack in the Long Island, N.Y., area, to identify any recrementitious variability in the specificity of the test.

MATERIALS AND METHODS

Stool specimens. Stool specimens were collected from symptomatic patients (i.e., 3 to 5 days after onset of gastroenteritis or diarrhea) who were admitted to or treated in the emergency rooms of the Nassau County Medical Center or at the North Shore University Hospital-Cornell University Medical College during the winter season of 1988 to 1989. Some specimens from the previous season were utilized. All specimens were placed in sterile containers, refrigerated (4°C), and delivered to the respective virology laboratory within 24 h of collection. Several specimens not tested within 3 days of arrival in the laboratory were divided into aliquots, frozen at −20°C, and subjected to parallel testing, using the TestPack and Kallestad Pathfinder (PTH) EIAs. Previously identified discordant stool specimens were thawed and retested by both EIAs, as well as by polyacrylamide gel electrophoresis (PAGE), immune electron microscopy, and isolation.

EIAs. The PTH EIA for the detection of rotavirus antigen in stool is performed routinely at the Nassau County Medical Center and the North Shore University Hospital-Cornell University Medical Center virology laboratories. The TestPack (lots 20522M100 and 20521M100) and PTH (lots 88015803, 88013585, and 88009483) assays were performed strictly by the manufacturer's specifications.

(i) TestPack. Briefly, a fecal filtrate was treated with TestPack guinea pig antirotavirus antibody-coated particles and antirotavirus alkaline phosphatase conjugate (mouse monoclonal and bovine polyclonal antibodies). After a 5-min incubation, the complex was added to a reaction disk. The reaction disk surface was washed with guanidine hydrochloride, and chromagen was then added. The appearance of a "+" (positive) symbol on the reaction disk was indicative of a positive reaction, and a "−" (negative) symbol (compared with the control) indicated nonreactivity.

(ii) PTH. The PTH EIA consisted of rabbit antirotavirus immunoglobulin G-coated tubes into which diluted stool and horseradish peroxidase-conjugated murine monoclonal antibody were added. The tubes were gently mixed and incubated for 1 h at room temperature. After thorough washings, the tubes were visually read 15 min after the addition of the chromogen (tetramethylbenzidene). Specimens displaying a blue color were considered positive. Specimens displaying no blue color were interpreted as negative. Previous studies in this laboratory showed no differences between our ability to visually interpret the results of the assay and spectrophotometric readings (14).

PAGE. Discordant stool specimens, which had been repeated at least twice by each EIA, underwent further testing at least two times to identify the presence of viral RNA by PAGE (4, 11). Briefly, stool specimens were suspended in 0.05 M Tris-NaCl (20% suspension, pH 8.0), sonicated in an ice bath at 40 W for 30 s (Sonifier cell disrupter; Heat Systems-Ultrasonics, Inc., Plainview, N.Y.), and centrifuged at 3,000 × *g* for 30 min at 4°C. The resultant supernatant was then centrifuged at 99,000 × *g* for 1 h at 4°C to pellet the virus. The RNA was extracted from the pellet by the phenol-chloroform method (RNAzol; Cinn/Biotecx Laboratories International, Friendswood, Tex.), using the manufac-

* Corresponding author.

turer's recommendations. The RNA was suspended in sample buffer (0.0625 M Tris base [pH 6.8], 20% glycerol, 0.5% sodium dodecyl sulfate, and 0.1% bromphenol blue) for loading onto a discontinuous system consisting of a 3% stacking and a 10% running gel (12).

Cells infected with laboratory-adapted strains of simian rotavirus SA-11 (SA-11; kindly supplied by Mary K. Estes, Baylor College of Medicine, Houston, Tex.) and reovirus type 1, Lang strain (ATCC VR-230), served as controls. Flasks containing primary African green or rhesus monkey kidney cells were inoculated with SA-11 or reovirus (13, 14) and incubated until there was a 1+ or 2+ cytopathic effect. The infected cells were treated with RNAzol (0.2 ml of RNAzol per 10^6 cells) and then with 0.2 ml of chloroform, with subsequent precipitation of the viral RNA in isopropanol. The RNA was washed in 75% ethanol, suspended in sample buffer, and loaded onto the gel. RNA segments were visualized by silver staining (catalog no. AG-25; Sigma Chemical Co., St. Louis, Mo.). Data analysis was performed as described previously (14).

Immune electron microscopy. A 20% stool suspension was prepared in phosphate-buffered saline. The suspension was mixed thoroughly on a Vortex mixer and then subjected to additional mixing with a cell disrupter (Mini Bead Beater; Biospec Products, Bratleville, Okla.) for 1 min (14). The suspension was then centrifuged at 10,000 × *g* for 10 min. Equal volumes of the supernatant and rotavirus antiserum (rabbit immunoglobulin to human rotavirus [Dako Corp., Santa Barbara, Calif.]; diluted 1:100 in phosphate-buffered saline) were gently vortexed and incubated for 30 min at 37°C. The suspension was centrifuged at 10,000 × *g* for 1 h at room temperature, and then the supernatant was aspirated and the pellet was suspended in 25 μl of phosphate-buffered saline. An equal volume of 4% phosphotungstic acid was added to the virus-antibody complex. One drop of the complex was placed onto a carbon-Formvar-coated, 400-mesh grid for a period of 3 min. Bibulous paper was used to remove the excess inoculum. The controls consisted of a pooled stool mixture of five fecal specimens, which were PTH EIA and isolation positive, and SA-11, which had been prepared in primary African green monkey kidney cell cultures. A known negative control stool specimen was processed in parallel with the positive controls and the discordant clinical specimens in question.

Virus isolation. Attempts to isolate rotavirus from stool were performed by procedures described earlier (14). Briefly, trypsin-treated stool extracts were inoculated into culture tubes containing primary African green monkey kidney cell monolayers. The tubes were placed into a roller drum apparatus and incubated for 10 days. The controls consisted of SA-11 and a pooled positive stool specimen. After 10 days of incubation, cells were scraped from the tubes and then washed and stained by immunofluorescence to detect the presence of cell-associated rotavirus antigen. A cytopathic effect was visualized after 3 days in the SA-11-inoculated culture tubes only.

RESULTS AND DISCUSSION

The evaluation of any assay system is contingent upon the accuracy of the reference standard. Since no single assay is perfect, a combination of confirmatory methodologies must be used (8). The use of PTH and PAGE as confirmatory assays was based on the superior performance of the PTH EIA in two prior evaluation studies (6, 14) and our extraction/concentration procedure of the infectious agent from stool to enhance detection of the genomic double-stranded RNA. The rotavirus isolation assay approximates 5×10^2 infectious particles per ml (17), perhaps approaching that sensitivity reported with polymerase chain reaction (J. A. Wilde, J. J. Eiden, R. P. Viscidi, and R. H. Yolken, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 456, 1989). Our additional confirmatory assay of immune electron microscopy complemented the use of the PTH EIA, PAGE analyses, and isolation.

FIG. 1. Testing of 11 discordant stool specimens by PAGE. Lane a, Rotavirus SA-11; lanes b to j, l, and m, stools that contained no detectable RNA; lanes k and n, negative specimens; lane o, rotavirus SA-11; lane p, reovirus type 1 Lang strain.

Of 65 specimens tested by TestPack and PTH, 54 were in accord. Eleven discordant specimens, positive by TestPack but negative by PTH, were negative by PAGE (Fig. 1), immune electron microscopy, and isolation. In our hands, TestPack failed to approach the near-perfect test specifications reported by others (3, 15). However, our results showing an 83% specificity are more in agreement with those of workers reporting a specificity of 90% (2). Two of our 11 discordant specimens were obtained from 1987–1988 frozen stock. In the clinical setting, however, a rapid specimen turnaround time is the rule rather than the exception. Consequently, our testing of primarily fresh specimens addressed those needs of the physician and the laboratory worker.

Disparities reported between our work and the earlier studies (3, 15) are difficult to explain. However, critical examination of the signal produced on the TestPack reaction disk (i.e., the + and − symbols) cannot help but ignite a nidus of interpretive discord. Some workers, for example, may interpret (in violation of the manufacturer's specifications) a faint + signal on the reaction disk as nonreactive. Such interpretive difficulties by the novice or perhaps the experienced laboratory worker with minimal exposure to the assay in question would not be unexpected (M. Chernesky, personal communication). Importantly, incorporation of rotavirus TestPack into the laboratory environment is contingent upon the level of experience of the individual performing the test in question.

Approximately one-third of our false-positive specimens took 1 min or longer to pass through the TestPack reaction disk focuser. Although the manufacturer recommends resampling and retesting of such "suspect" specimens, the suggested protocol negates the rapidity of the test and requires

TABLE 1. Age distribution of patients suffering from gastroenteritis or diarrhea

Patient age (mo)	No. of patients tested	No. of false-positives
≤1	28	5
2–3	10	1
4–6	0	0
7–9	1	0
10–12	4	2
24–36	7	1
48–60	2	1
>60	13	1

additional effort on the part of the physician, the nursing staff, and the laboratory support personnel. Differences in filtrate behavior (i.e., the migration rate) in the disk focuser were not found to reflect variations in stool pigment intensities.

Reduced rotavirus EIA specificity has been ascribed some years ago to confounding nondiscrete substances in stool such as fibers, protein metabolites, enzymes, and antiglobulins (1, 10, 16, 18). One may not rule out an effect of these and perhaps other ill-defined stool components which might be responsible, in part, for the appearance of a faint + signal on the reaction disk. Another possible reason for the high false-positivity rate identified in this study was thought to have been our relatively large symptomatic neonatal and infant (≤3 months of age) patient populations (Table 1). However, comparison of data obtained from these populations with those obtained from our >10-month-old patients revealed no differences in the specificity of TestPack. More data are needed from patients of various age groups, however, before a more definitive statement can be made. Previous studies evaluating the TestPack among symptomatic neonatal or infant patients had been ill-defined or only minimally addressed (2, 3, 15).

The TestPack has the obvious advantage of rapidity and simplicity of performance. Clearly, such an assay has considerable appeal to health officials in rural areas throughout the Third World. In the fully equipped virology/microbiology laboratory, however, the questionable specificity of TestPack limits its incorporation into the routine testing regimen when other relatively simple assay systems are available (6, 14).

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Meadowbrook Medical Education and Research Foundation, Inc.

We appreciate the excellent technical assistance of H. Middleton, K. Zelinsky-Papez, and Z. Ciamician.

LITERATURE CITED

1. **Brandt, C. D., H. W. Kim, W. J. Rodriguez, L. Thomas, R. H. Yolken, J. O. Arrobio, A. Z. Kapikian, R. H. Parrott, and R. M. Chanock.** 1981. Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. J. Clin. Microbiol. **13**:976–981.
2. **Brooks, R. G., L. Brown, and R. B. Franklin.** 1989. Comparison of a new rapid test (TestPack Rotavirus) with standard enzyme immunoassay and electron microscopy for the detection of rotavirus in symptomatic hospitalized children. J. Clin. Microbiol. **27**:775–777.
3. **Chernesky, M., S. Castriciano, J. Mahony, M. Spiewak, and L. Schaefer.** 1988. Ability of TESTPACK ROTAVIRUS enzyme immunoassay to diagnose rotavirus gastroenteritis. J. Clin. Microbiol. **26**:2459–2461.
4. **Croxson, M. C., and A. R. Bellamy.** 1981. Extraction of rotavirus from human feces by treatment with lithium dodecyl sulfate. Appl. Environ. Microbiol. **41**:255–260.
5. **Davidson, G. P.** 1986. Viral diarrhea. Clin. Gastroenterol. **15**: 39–53.
6. **Dennehy, P. H., D. R. Gauntlett, and W. E. Tente.** 1988. Comparison of nine commercial immunoassays for the detection of rotavirus in fecal specimens. J. Clin. Microbiol. **26**:1630–1634.
7. **Flores, J., O. Nakagomi, T. Nakagomi, R. Glass, M. Gorziglia, J. Askaa, Y. Hoshion, I. Perez-Schael, and A. Z. Kapikian.** 1986. The role of rotaviruses in pediatric diarrhea. Pediatr. Infect. Dis. **5**(Suppl.):53–62.
8. **Forrer, C. B., J. M. Rodden, H. F. Clark, and H. M. Friedman.** 1989. Discrepant rotavirus results in two laboratories using the same enzyme immunoassay. Am. J. Clin. Pathol. **91**:85–87.
9. **Ho, M.-S., R. I. Glass, P. F. Pinsky, and L. J. Anderson.** 1988. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. J. Infect. Dis. **158**:1112–1116.
10. **June, M. N., S. S. Morse, and D. M. Stark.** 1988. Identification of nonspecific reactions in laboratory rodent specimens tested by Rotazyme rotavirus ELISA. Lab. Anim. Sci. **38**:273–278.
11. **Kasempimolporn, S., S. Louisirirotchanakul, P. Sinarachatanant, and C. Wasi.** 1988. Polyacrylamide gel electrophoresis and silver staining for detection of rotavirus in stools from diarrheic patients in Thailand. J. Clin. Microbiol. **26**:158–160.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Lipson, S. M., and G. Stotzky.** 1983. Adsorption of reovirus to clay minerals: effects of cation-exchange capacity, cation saturation, and surface area. Appl. Environ. Microbiol. **46**:673–682.
14. **Lipson, S. M., and K. Zelinsky-Papez.** 1989. Comparison of four latex agglutination (LA) and three enzyme-linked immunosorbent assays (ELISA) for the detection of rotavirus in fecal specimens. Am. J. Clin. Pathol. **92**:637–643.
15. **Marchlewicz, B., M. Spiewak, and J. Lampinen.** 1988. Evaluation of Abbott TESTPACK ROTAVIRUS with clinical specimens. J. Clin. Microbiol. **26**:2456–2458.
16. **Sambourg, M., A. Goudeau, C. Courant, G. Oinon, and F. Denis.** 1985. Direct appraisal of latex agglutination testing, a convenient alternative to enzyme immunoassay for the detection of rotavirus in childhood gastroenteritis, by comparison of two enzyme immunoassays and two latex tests. J. Clin. Microbiol. **21**:622–625.
17. **Ward, R. L., D. R. Knowlton, and M. J. Pierce.** 1984. Efficiency of human rotavirus propagation in cell culture. J. Clin. Microbiol. **19**:748–753.
18. **Yolken, R. H., and P. J. Stopa.** 1979. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus. J. Clin. Microbiol. **10**:703–707.