Comparison of a New Rapid Test (TestPack Rotavirus) with Standard Enzyme Immunoassay and Electron Microscopy for the Detection of Rotavirus in Symptomatic Hospitalized Children

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We compared a new, rapid, qualitative test for rotavirus (TestPack Rotavirus; Abbott Laboratories, North Chicago, Ill.) with another enzyme immunoassay (Pathfinder Rotavirus; Kallestad Laboratories, Inc., Austin, Tex.) and electron microscopy to determine its clinical utility in a population of symptomatic hospitalized children. In the first part of the study, 100 frozen stool samples were tested. The results after resolution with a blocking reagent showed a sensitivity of only 50% and a specificity of 88% for TestPack Rotavirus. In the second part of the study, we tested TestPack Rotavirus on 100 fresh, unfrozen samples. The results (sensitivity/specificity) were as follows: TestPack Rotavirus, 95/90%; Pathfinder Rotavirus, 84/98%; direct electron microscopy, 63/100%. Although it was not as sensitive or specific as immune electron microscopy, TestPack Rotavirus was more sensitive than direct electron microscopy or Kallestad Pathfinder Rotavirus. TestPack Rotavirus represents a rapid, qualitative method for the detection of rotavirus in stools of symptomatic children.

Rotaviruses are among the most important pathogens known to infect infants and children (8). Because of the common occurrence of this organism as a cause of diarrhea in hospitalized children, its prompt identification is of major importance to clinicians. The diagnosis of human rotavirus infection is dependent upon finding viral particles, antigens, or RNA in stool samples obtained early in the course of clinical symptoms. A number of techniques have been developed to this end, including enzyme immunosorbent assays (EIAs) using polyclonal (2, 12, 15) or monoclonal (4, 9) antibodies, latex agglutination (7, 16, 17), dot hybridization (5), polyacrylamide gel electrophoresis (11), and direct (1, 2) and immune (6, 14) electron microscopy (EM). We compared a new, qualitative test for rotavirus (TestPack Rotavirus; Abbott Laboratories, North Chicago, Ill.) (ATR) with EIA (Pathfinder Rotavirus; Kallestad Laboratories, Inc., Austin, Tex.) (KPF) and EM (immune and direct) to determine the sensitivity, specificity, and clinical utility of this new test.

All patients in the study were hospitalized at Orlando Regional Medical Center between January 1987 and March 1988 and were symptomatic for gastroenteritis. All specimens were collected by container (rectal swabs were excluded). In the first part of the study, the performance of ATR was compared with that of EIA (KPF) and immune EM (IEM) on 100 frozen stool samples received from children (≥4 weeks of age) during the 6 months preceding the study. Many of these samples had been frozen at −70°C and thawed for rotavirus assay as part of a previous study. In the second part of the study, ATR was compared with KPF and direct EM (DEM) on 100 fresh stool samples from children (96 children, 4 neonates). In this part of the study, some specimens were frozen (−20°C) for less than 48 h before assay.

For both parts of the study, samples were tested by ATR according to the manufacturer’s recommended guidelines.

Approximately 100 μl of stool was placed into a specimen dilution cup containing 1 ml of sample diluent (0.01 M phosphate-buffered saline [PBS]). After thorough mixing, the fecal suspension was clarified by use of the filter tube. Three drops each of guinea pig antirotavirus antibody-coated particles and antirotavirus alkaline phosphatase conjugate (mouse monoclonal and bovine polyclonal) were added to the clarified specimen, and the mixture was incubated for 5 min at room temperature. The contents were then poured through a focuser onto the reaction surface and allowed to completely flow through. After removal of the focuser, the reaction surface was washed with 1 ml of guanidine hydrochloride (1.0 M), and 3 drops of chromogen were then added. After a 2-min color development and a final wash of guanidine hydrochloride, the specimen was read visually. The appearance of a purple cross (+) indicated the presence of rotavirus antigen. The appearance of a purple horizontal bar (−) indicated a properly run, but negative, sample. Specimens were graded from 1+ to 3+ on the basis of the intensity of the positive reaction. KPF was performed according to the manufacturer’s recommendations, using the spectrophotometric method for interpretation of results. Specimens with an absorbance within 10% of the cutoff value were rerun to determine positivity or negativity.

IEM was performed as follows for the first part of this study. Formvar-coated 300-mesh copper grids were incubated for 30 min at room temperature with goat antirotavirus antibody (Kallestad Laboratories) diluted 1:3 in PBS. After three washes in PBS, antibody-coated grids were placed on fecal suspension (30% in PBS) and incubated overnight at room temperature in a humid atmosphere. After being washed three times in distilled water, the grids were stained for 10 s with 2% phosphotungstic acid in distilled water (pH 4.5) (13) and blotted dry. Grids were read with a Zeiss EM-109 electron microscope at a magnification of ×30,000 for at least 10 min (or until rotavirus particles were seen). For the second part of the study, DEM was performed by placing 40 μl of a 30% fecal suspension in PBS on a

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Formvar-coated copper grid for 10 s. Grids were blotted dry and stained with 2% phosphotungstic acid (pH 4.5) for 10 s and then read as described above for the IEM method.

To determine sensitivity, specificity, and predictive value, each sample was classified as either true-positive or true-negative. Two separate methods were used to assess results. In method A, EM alone was considered the standard for true positivity and true negativity. In method B, the calculations were based on the standard of EM plus results of a blocking test for discordant specimens with a dilution of the American Type Culture Collection antirotavirus serum (catalog no. VR-971) (15). Samples were retested with ATR or KPF after preincubation of the specimen with this serum. A 50% or greater reduction in signal intensity was considered a positive blocking assay and confirmed the sample to be a true-positive.

Table 1 shows the results of testing 100 frozen stool samples. All three tests were concordant in 70 samples (9 positive, 61 negative). With the use of IEM as the standard (method A), the sensitivity and specificity of ATR were 45 and 86%, respectively, and those of KPF were 85 and 86%, respectively. Twenty-seven of the discrepant samples were retested (three samples positive by IEM and negative by both EIAs were not retested). Of the 24 samples which initially gave different results when tested by ATR and KPF, repeat testing alone resolved the discrepancy in 18 samples. In 6 of the 24 samples, resolution was made by blocking assay. The remaining 18 samples, which were positive by KPF and one sample which was positive by ATR blocked with antibody and were considered true-positive samples. Two additional KPF-positive samples did not block and were considered to be falsely positive. Of three samples which were positive by both EIAs but negative by IEM, two did not block in either IEM and were also considered falsely positive. The use of blocking antibody for resolution actually changed test results very little. Most notable were an increase in the sensitivity of ATR to 50% and a decrease in the sensitivity of IEM to 91%.

Because of the low concordance rate between test methods and the relatively low sensitivity of each EIA on frozen samples, we tested an additional 100 fresh stool samples for the presence of rotavirus. The results of ATR, KPF, and DEM are shown in Table 2. All three tests were concordant in 78 samples (23 positive, 55 negative). With the use of DEM as the standard (method A), the sensitivity and specificity of ATR were 100 and 76%, respectively. KPF had a sensitivity of 96% and a specificity of 87% by this method. Eighteen of the twenty-two discordant samples were retested. Four samples which were strongly positive in both EIAs but which were negative by DEM were not available for retesting. (Based on the strong positive EIA results and the ability to block three other samples with similar results [ATR positive, KPF positive, DEM negative], we considered these four samples to be falsely negative in DEM.) Repeat testing resolved the discordance in 3 of 18 samples. Of the remaining 15 samples, blocking assay revealed ATR and KPF to be truly positive in 9 and 5 samples, respectively. The results after retesting and use of blocking assay revealed a sensitivity and specificity of ATR of 95 and 90%, respectively. The specificity of KPF increased to 98%, but its sensitivity decreased to 84%. The sensitivity of DEM decreased to only 63%.

Rotavirus infection remains an important cause of diarrhea in hospitalized children (8). Each of the methods to diagnose this disease has demonstrated certain advantages and disadvantages. Since 1973, when Bishop and her colleagues first identified rotaviral particles in the intestines of infants with diarrhea (1), EM has been an important technique for the diagnosis of rotavirus infection. Its rapidity of performance and ability to detect non-group-A rotaviruses and other enteric viruses have been overshadowed, however, by its lack of availability and relative insensitivity. This latter problem has been improved by use of IEM, which has increased the sensitivity of this technique 100- to 1,000-fold (6, 14). Because of these limitations, most laboratories use either EIA or latex agglutination procedures for the diagnosis of rotavirus infection. EIA procedures appear more sensitive than DEM (2, 15) but not necessarily more sensitive than IEM (15). Although particularly suited for testing large numbers of clinical specimens, EIAs often require several hours to complete, and their complexity limits their use to larger laboratories. In 1986, Yolken et al. described a self-contained enzymatic membrane immunoassay system which was rapid and simple to use and which possessed sensitivities similar to those of another EIA (18). We evaluated a similar solid-phase EIA (ATR) for the detection of rotavirus and compared these results with those obtained with another EIA and with EM.

In the first part of our study, frozen stools were tested by ATR, KPF, and IEM. The overall concordance between the three tests was low (70%), and a relatively low sensitivity (50 to 82%) was seen with each of the EIAs. Because many of these stool samples had been previously freeze-thawed and because the EIAs had only a modest sensitivity, we initiated a second part of the study in which we assayed fresh stool samples. In order to process these stool samples simultaneously by EM and by EIA, we performed DEM rather than IEM for this latter part. With this approach, concordance between tests improved and we believe a more accurate comparison of the tests emerged. After resolution with a
blocking assay. ATR had a sensitivity of 95% and a specificity of 90%. Of the six falsely positive samples, five were only weakly (1+) positive. Although these reactions could represent detection of rotavirus antigens below the threshold of EM or the other EIA, the inability to block the reaction with a competitive blocking assay would suggest these to be nonspecific reactions due to immunoglobulin M antibodies (19) or other proteins.

These results are similar to those recently described by Marchlewicz et al., who found an ATR sensitivity of 89% and a specificity of 88% when testing stools from symptomatic children (10). Chernesy et al. also recently described their experience with the use of ATR on stool samples from 172 patients (3). The sensitivity and specificity were both 100% when compared to a standard (DEM and blocking assay) similar to that used in the second part of our study. Since freeze-thawing of many of their samples did not appear to affect the results of testing, we are led to believe that the markedly better results achieved by ATR in the second part of our study are due mainly to its comparison with the less-sensitive (compared with IEM) DEM.

The ease of performance and time to completion (10 min), however, clearly favored the use of ATR over KPF and IEM. Approximately 10 stool samples were able to be run simultaneously by ATR. This resulted in an average of 1.5 to 2 min of technician “hands-on” time per stool sample. In addition, because this is a qualitative test, no special equipment or expertise was needed to carry out the assay. The ATR reagents are stable for at least 90 days when refrigerated at 2 to 8°C. The cost of ATR ($11.00) compared favorably with those of KPF ($11.00) and IEM ($8.50) at our hospital. We feel that these characteristics make the test particularly suited not only for major laboratories but also for physicians’ offices, clinics, and field work in rural or tropical settings. Further evaluation of this test and, in particular, comparison with other rapid diagnostic tests for rotavirus, such as the latex agglutination test, appear warranted.

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