Evaluation of New Commercial Enzyme Immunoassay for Rotavirus Detection

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We evaluated a new commercial enzyme immunoassay (EIA) for rotavirus (Rotavirus EIA; International Diagnostic Laboratories, Chesterfield, Mo.), a total of 161 consecutive stool samples (including 18 from infants less than 30 days old) submitted to the diagnostic laboratory at Children's Hospital, Washington University Medical Center, St. Louis, Mo., for rotavirus detection were tested by Rotavirus EIA and by Rotazyme II (Abbott Laboratories, North Chicago, Ill.) according to the instructions of the manufacturer. In addition, 16 samples from infants less than 30 days old without diarrhea were tested by both assays. Samples showing discrepant results after repeat testing were examined by electron microscopy. Nine samples yielding discrepant results were also tested by using a reference EIA directly on the specimen and on culture supernatants from two passages in MA 104 cells. Rotavirus EIA and Rotazyme II yielded concordant results for 85% of the samples. All of the 26 discrepant samples tested negative by Rotavirus EIA and positive (15 samples) or equivocal (11 samples) by Rotazyme II. These samples included 11 from symptomatic infants more than 30 days old, 2 from symptomatic infants less than 30 days old (neonates), and 2 from neonates without diarrhea. Rotavirus was not detected in any of the 24 that were examined by electron microscopy or in any of the 9 that were tested by the reference EIA. The sensitivity, specificity, positive predictive value, and negative predictive value were 100% for Rotazyme EIA and 100, 90, 70, and 100%, respectively, for Rotazyme II. Rotavirus EIA was comparable to Rotazyme II in ease of performance. We conclude that Rotavirus EIA is equally sensitive and more specific than Rotazyme II for detecting rotavirus. Rotavirus EIA is a practical and accurate rotavirus assay for use in clinical laboratories.

Human rotavirus is an important cause of diarrhea in young children (8). The diagnosis of rotavirus infection has been greatly facilitated by the development and widespread application of enzyme immunoassay (EIA) techniques (2). In the present study, we evaluated the performance of a new commercial EIA (Rotavirus EIA; International Diagnostic Laboratories, Chesterfield, Mo.) for rotavirus by comparing it with another widely used commercial EIA (Rotazyme II; Abbott Laboratories, North Chicago, Ill.). Samples yielding discrepant results were evaluated by electron microscopy. In addition, a subgroup of these samples was also tested by a reference EIA (Dakopatts, Glostrup, Denmark), both directly and after two passages in MA 104 cells (7).

MATERIALS AND METHODS

Specimens. Specimens included in the study consisted of 161 consecutive stool samples (including 18 from infants less than 30 days old) submitted to the diagnostic laboratory at Children's Hospital, Washington University Medical Center, St. Louis, Mo., for rotavirus detection during the winters of 1984 to 1985 and 1985 to 1986. In addition, 16 stool samples were obtained from infants less than 30 days old without diarrhea who were being cared for in the hospital's neonatal intensive care unit during the winter of 1985 to 1986. All specimens were refrigerated overnight or frozen for several days at −20°C before testing.

Assays. Rotavirus EIA uses a microtiter plate in which alternate wells are precoated with rabbit rotavirus antibody and preimmune rabbit serum. The assay was performed precisely according to directions of the manufacturer. Liquid stools were tested undiluted, and semiformalized stools were diluted to approximately 1:5 with distilled water to reach a consistency suitable for pipette usage and then vortexed. Twenty microliters of the diluted sample was then added to each of two adjacent wells: one was coated with antirotavirus antibody, and the other was coated with preimmune serum. The plate was incubated at room temperature for 60 min. The contents of the wells were aspirated, and the wells were washed three times with wash solution. Fifty microliters of biotin-conjugated anti-rotavirus antibody was added to each well, followed immediately by 50 µl of avidin-horseradish peroxidase conjugate. The plate was incubated at room temperature for 30 min. After this incubation, the wells were washed four times, and 100 µl of ortho-phenylenediamine substrate was added. After 15 min of incubation at room temperature, the reaction was stopped by adding 100 µl of stop solution (1 M H2SO4). All tests were read visually and spectrophotometrically at 492 nm. Tests were considered positive by visual examination when the color in the test well was distinctly more intense than the color in the negative control well and spectrophotometrically if the difference between the A492 of the test well and that of the negative control well was >0.1 and if the A492 of the test well was at least six times that of the negative control well. All samples were also tested by using the Rotazyme II EIA (Abbott Laboratories, North Chicago, Ill.). The

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Rotavirus II assays were carried out precisely according to the instructions of the manufacturer. Each sample was diluted to an approximate 10% suspension with the specimen diluent supplied by the manufacturer. An antibody-coated bead was added to 200 μl of the sample in a reaction tray and incubated in a 37°C water bath for 60 min. After three washes with distilled water, 200 μl of horseradish peroxidase enzyme conjugate was added. The tray was again incubated in a 37°C water bath for 60 min. The bead was then washed three times with distilled water and transferred to a plastic test tube to which 300 μl of ortho-phenylene-diamine substrate was added. After a 30-min incubation at room temperature, 1 ml of sulfuric acid was added to each tube to stop the reaction. The tube was then vortexed, and the A492 was read on a Quantum spectrophotometer. The cutoff value was determined by adding 0.075 to the A492 of the sample diluent control. A sample was considered positive if its A492 was more than 10% greater than the cutoff. A sample was considered negative if its A492 value was more than 10% below the cutoff. Samples with an A492 within 10% of the cutoff were considered equivocal.

Discrepant samples. All samples yielding discrepant results were retested by both assays. In all cases, the result of the second test was used for calculations. Most samples with discrepant discrepancies were prepared for electron microscopy. In addition, nine discrepant samples and one sample that was positive by both assays were evaluated for the presence of rotavirus by using a reference EIA (Dakopatts) and cultivation in MA 104 cells.

Electron microscopy. Electron microscopy was performed by Grady W. Phillips, Jr., Department of Genetics, Washington University School of Medicine. Samples tested were diluted with water and centrifuged at a low speed to deposit particulate material. If the supernatant remained turbid, additional water was added, and the procedure was repeated until the supernatant was no longer turbid. Five microliters of the diluted sample was placed on fresh nitrocellulose-coated grids. After approximately 10 s, the excess was removed with filter paper, and 5 μl of 2% phosphotungstic acid (pH 7.2) was placed on the grid for 10 s and then removed with filter paper. The preparation was then viewed with a Philips 201C transmission electron microscope for approximately 10 min per sample. All specimens that were initially negative for rotavirus were also examined by the pseudo-replica technique (4). Twenty-five microliters of the diluted stool sample was placed on a disk of 2% agar and allowed to dry. One drop of 1% nitrocellulose in amyl acetate was placed on the agar disk. The nitrocellulose membrane that formed was floated off in water and captured on a 300-mesh copper grid. One drop of 0.5% uranyl acetate was added and removed by filter paper after 10 s. The grid was then examined as described above.

Reference EIA. Nine discrepant samples and one negative sample were sent frozen to the laboratory of one of us (R.I.G.) for further testing, using an independent EIA (Dakopatts) and cultivation. Specimens were thawed, diluted 1:10 in veal infusion broth, and activated with trypsin (10 μg/ml) for 1 h at 37°C. Inocula were prepared by diluting the activated specimens 1:10 in Eagle minimal essential media containing glutamine (2 mM), chlorotetracycline (25 μg/ml), penicillin (250 U/ml), amphotericin (2.5 μg/ml), and trypsin (0.5 μg/ml). These inocula (200 μl) were added to roller tubes of MA 104 cells that had been washed twice with the same medium used to dilute the samples. The tubes were incubated on roller drums at 37°C for 1 h, washed with the diluent, refed, and returned to the roller drums for 7 to 10 days. At that time, material from each tube was passaged into an additional tube of MA 104 cells and incubated under similar conditions for an additional 10 days. After this passage, the supernatant was removed and tested by EIA.

Test parameters. To determine the sensitivity, specificity, and predictive values of both assays, each sample was classified as either true-positive or true-negative. Samples were considered true-positive if they were positive by both assays or if they were positive by one assay and by the confirmatory procedures (electron microscopy and, on selected samples, reference EIA). Samples were considered true-negative if they were negative by both assays or if they were negative by one assay and by the confirmatory procedures. The two samples that yielded discrepant results on the Rotavirus EIA and the Rotazyme II assays but which were not tested by the confirmatory procedures were excluded from this analysis. For each assay, the sensitivity was determined by dividing the number of true-positive samples by the number of true-positive plus false-negative samples. The specificity was determined by dividing the number of true-negative samples by the number of true-negative plus false-positive samples. The positive predictive value was determined by dividing the number of true-positive samples by the number of true-positive plus false-positive samples. The negative predictive value was determined by dividing the number of true-negative samples by the number of true-negative plus false-negative samples. In the calculations of the Rotazyme II test parameters, samples yielding equivocal results were included for the determination of sensitivity but were excluded from the determination of specificity.

RESULTS

This study included 143 samples from patients more than 30 days old. Of those samples, 30 (21%) were positive and 99 (69%) were negative by both assays (Table 1). No samples were positive by Rotavirus EIA and negative by Rotazyme II. However, 14 samples (10%) were negative by Rotavirus EIA and either positive (11 samples) or equivocal (3 samples) by Rotazyme II. Of these samples, 12 were examined by electron microscopy; rotavirus was not detected in any of the 12. Samples from 34 neonates (infants less than 30 days old), including 18 who were asymptomatic and 16 who did not have diarrhea, were also evaluated by both assays (Table 1). All 34 were negative by Rotavirus EIA, while 12 were either positive (4) or equivocal (8) by Rotazyme II. Discrepant results occurred in samples from neonates both with and without diarrhea. Rotavirus was not detected by electron microscopy in any of the 12 discrepant samples.

Ten samples, including one positive by both assays and nine that had yielded discrepant results in the two EIA, were studied by using the reference EIA directly on the specimen and on culture supernatants from two passages of the stool samples in MA 104 cells. Rotavirus was detected only in the sample that was positive by both EIAs. For the determinations of test parameters, the 30 samples that were positive both by Rotavirus EIA and by Rotazyme II were considered true-positive. The 121 samples that were negative by both assays were considered true-negative, as were the 24 samples that were negative by Rotavirus EIA, positive by Rotazyme II, and negative by confirmatory procedures (Table 2). For Rotavirus EIA, the sensitivity, specificity, and positive and negative predictive values were all 100% (30/30, 145/145, 145/30, and 145/145). For Rotazyme II, the sensitivity was 100% (30/30), the specificity was
TABLE 1. Tests of stool samples from older infants and neonates for rotavirus, using Rotavirus EIA and Rotazyme II

<table>
<thead>
<tr>
<th>Infant age (days)</th>
<th>Assay result</th>
<th>No. of samples</th>
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<tbody>
<tr>
<td>&gt;30</td>
<td>+</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>11*</td>
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<td></td>
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<td>19</td>
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<tr>
<td>≤30 (symptomatic)</td>
<td>+</td>
<td>0</td>
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<tr>
<td></td>
<td>-</td>
<td>2*</td>
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<tr>
<td></td>
<td>+</td>
<td>10</td>
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<tr>
<td></td>
<td>±</td>
<td>2*</td>
</tr>
<tr>
<td>&lt;30 (asymptomatic)</td>
<td>+</td>
<td>0</td>
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<td></td>
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<td>2*</td>
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<tr>
<td></td>
<td>+</td>
<td>8</td>
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<td>12</td>
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</tbody>
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* ±, Equivocal.
* Rotavirus not detected in nine samples examined by electron microscopy.
* Rotavirus not detected by electron microscopy.

90.3% (121/134), the positive predictive value was 70% (30/43), and the negative predictive value was 100% (121/121). When the analysis was restricted to the 34 samples from neonates, only the determination of specificity was meaningful, since all of the samples were true-negative. For those samples, the specificities of Rotavirus EIA and Rotazyme II were 100% (34/34) and 85% (22/26), respectively (equivocal samples excluded).

The time required to perform EIAs on 10 specimens was 2.75 h for Rotazyme II and 2.25 h for Rotavirus EIA. The estimated "hands on" time was 15 min for Rotazyme II and 30 min for Rotavirus EIA.

DISCUSSION

In this study, we evaluated a new commercial EIA, Rotavirus EIA, that is designed to detect human rotavirus in stool samples. We found Rotavirus EIA to be equally sensitive and more specific than Rotazyme II, a widely used commercial EIA (1). For all discrepancies that occurred in testing samples by both assays, Rotavirus EIA was negative and Rotazyme II was either positive or equivocal.

Our belief that Rotavirus EIA is more specific than Rotazyme II rather than less sensitive is based on the results of independent tests to detect rotavirus in stool samples yielding discrepant results in the two assays. Electron microscopy did not reveal rotavirus in any of the 24 samples that were examined (of 26 samples that yielded discrepant results). Because some EIAs are more sensitive than direct electron microscopy for detecting rotavirus (6), we also used a reference EIA to test nine of the samples yielding discrepant results. The reference EIA was used directly on the stool samples as well as on culture supernatants from two passages in MA 104 cells, a cell line known to support the growth of human rotaviruses (7). Rotavirus was not detected in any of the nine stool samples tested in this manner.

Concern has been raised in regard to the specificity of Rotazyme for testing stools from neonates (3, 5; I. L. Christie, B. M. Totterell, and J. E. Banatvala, Letter, Lancet ii:1028–1029, 1983). Accordingly, we tested 34 samples from neonates in this evaluation, including 16 from neonates without diarrhea. All 34 were negative by Rotavirus EIA, while 12 of the 34 were positive or equivocal by Rotazyme II. All 12 were negative for rotavirus by electron microscopy. Thus, in this evaluation, we found no evidence that Rotavirus EIA produced nonspecific results when used to test stool samples from neonates, even when it was used on stool samples yielding nonspecific results in the Rotazyme II assay. In contrast, it appears that, like the original Rotazyme assay, Rotazyme II also can produce false-positive tests when used to test stools from neonates.

In addition to producing accurate results, Rotavirus EIA was convenient to use, with results available in less than 2.5 h. The results were easy to read, with clear-cut differences between positive and negative samples. Equivocal results were not encountered. In summary, Rotavirus EIA was a practical and accurate assay for rotavirus that is suitable for use in clinical laboratories.

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LITERATURE CITED


