Rapid Diagnosis of Rotavirus Gastroenteritis by a Commercial Latex Agglutination Test

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The Rotalex test, a commercial latex agglutination test for rotavirus, was compared with direct electron microscopy (EM) and the Rotazyme test to a commercial enzyme immunoassay, for detection of rotavirus in stools of children and neonates. For initial stool specimens from 265 children (<3 years old) with diarrhea, the Rotalex test had a sensitivity of 81.7% and specificity of 99.5% compared with EM results. Positive and negative predictive values were 98 and 94.9%, respectively. The Rotalex test was slightly more sensitive and specific than the Rotazyme test. When daily stool specimens from patients with rotavirus gastroenteritis were examined, the sensitivity of the Rotalex test varied depending on the time of stool collection relative to the onset of symptoms. Sensitivity was 100 (20/20), 96 (23/24), and 54% (7/13) during 1 to 4, 5 to 7, and 8 to 18 days, respectively, after the onset of symptoms. The specificity of the Rotazyme test varied similarly with days from onset. We also examined 214 EM-negative stool specimens from asymptomatic newborns. False positivity by the Rotalex test was only 3.3% (7/214) compared with 4.2% (9/215) for the Rotazyme test. The Rotalex test was as sensitive and specific as EM for detection of rotavirus during the acute stage of illness and much faster and cheaper than EM or the Rotazyme test. The test appears to be suitable for routine use in small hospitals, emergency wards, or even the physician’s office for rapid diagnosis of rotavirus gastroenteritis.

Since the discovery of rotavirus by direct electron microscopy (EM) more than 10 years ago (3, 8), the technique has been used routinely for diagnosis of rotavirus gastroenteritis. However, EM is not available to most hospitals, and to examine a large number of stool specimens by EM is time consuming. For these reasons, a number of immunoassays have been developed to detect rotavirus antigen in stool specimens (2, 5, 10, 12, 15, 18, 21, 22, 24). Rotazyme (Abbott Laboratories, Diagnostics Div., North Chicago, Ill.), a commercially available enzyme immunoassay, has been evaluated extensively and found to be comparable to EM in sensitivity and specificity (6, 16). However, the drawback to Rotazyme is that the test requires several hours to perform and, therefore, would not be cost effective for small hospitals where one or two specimens would be examined at one time. On the other hand, the latex agglutination test is much simpler and faster and has the potential of providing a diagnosis within minutes of collecting stool specimens (10, 12, 21).

Recently, a commercial latex agglutination kit has become available and is marketed as Rotalex by Orion Diagnostica of Helsinki, Finland. In this study, we evaluated the Rotalex test by comparing it with EM and Rotazyme.

MATERIALS AND METHODS

Stool specimens. Three groups of stool specimens (not rectal swabs) were examined in the study: (i) initial (first available) stool specimens from children (<3 years of age) with diarrhea submitted to the microbiology laboratory of the Alberta Children’s Hospital (Calgary, Alberta, Canada) for routine culture or virus examination or both; (ii) follow-up specimens from children whose initial stool specimens were positive for rotavirus by EM, Rotalex, and Rotazyme tests; and (iii) stool specimens from newborns (<4 months of age) without diarrhea. Initial stool specimens were received during two rotavirus seasons. Stool specimens received during the 1982–1983 season were diluted 1:5 with phosphate-buffered saline (0.01 M, pH 7.2) and stored at −20°C for 1 to 4 months. From mid-December of 1983 to the end of April, 1984, initial stool specimens were studied prospectively. When initial specimens from hospitalized patients were positive for rotavirus by EM, follow-up specimens were obtained daily or as frequently as possible until the patients were discharged. Patients with mixed infections of rotavirus and adenovirus or a bacterial pathogen were excluded from the follow-up study because the date of the onset of symptoms due to rotavirus infection could not be determined in these patients. Stools from newborns without diarrhea were collected in weekly batches during the 1983–1984 season at the intensive care unit of the Foothills Hospital, Calgary, Alberta, Canada.

Processing of stool specimens. Stools diluted 1:5 with phosphate-buffered saline were centrifuged at 12,000 × g for 2 min (microcentrifuge model 235A; Fisher Scientific Co., Pittsburgh, Pa.), and the supernatant fluids were tested immediately for rotavirus or stored at 4°C for later tests.

Rotalex test. Rotalex reagents (Orion) include test latex coated with rotavirus antiserum, control latex coated with preimmune serum, positive control, and buffer solution. Supernatant fluids from 20% stool suspensions were further diluted 1:2 with the Rotalex buffer to make a final dilution of 10%. A drop (about 50 μl) of the supernatant fluids was mixed well with a drop of test latex on a slide, and reaction (development of precipitates) was read in 2 min. A mixture of test specimen and control latex was set up at the same time. The test was considered positive for rotavirus if distinct agglutination was observed with test latex but not with control latex. If agglutination was observed in the mixture containing control latex, the test was considered uninterpretable.
The Rotazyme test was slightly less sensitive and specific than the Rotalex test, although the difference was not significant (Table 1). When 1+ results were considered positive, it was more sensitive (51/60) but less specific (186/205) than the Rotalex test.

There were 21 specimens for which EM results (17 positive and 4 negative) did not agree with one or both of the other two tests. Of 17 EM-positive specimens, 9 were negative by both the Rotalex and Rotazyme tests, 6 were negative by Rotazyme only, and 2 were negative by Rotalex only. Of four EM-negative specimens, three were positive by Rotazyme only, and one was positive by Rotalex only. No specimen was EM negative when with the two other tests were positive.

Blocking tests were performed in 11 specimens; 5 specimens (control) were positive by all three tests, 1 was positive by EM and Rotazyme, 1 was positive by EM and Rotalex, three were positive by Rotazyme only, and 1 was positive by Rotalex only. All EM-positive specimens were positive by the confirmatory test, whereas the test was negative in the EM-negative specimen. There were another six EM-positive specimens for which only one of the other two tests was positive, but blocking tests were not done owing to insufficient quantity.

Follow-up specimens. We followed up 20 patients whose initial stool specimens were positive for rotavirus by all three tests but negative for bacterial pathogens or adenovirus to evaluate the performance of the Rotalex test during the acute and convalescent periods of illness. A total of 81 stool specimens were collected at 1 to 18 days from the onset of diarrhea, and the results were grouped into four periods (Fig. 1). Each period was 3 days long, except the first and last periods. There was only one specimen collected on day 1 after onset, which was included in the first period, and all specimens collected on day 11 or thereafter were included in one period because of an insufficient number of specimens.

Rotavirus was detected by EM in 100, 86, 63, and 18% of

### TABLE 1. Comparison of the Rotalex and Rotazyme tests with EM in detection of rotavirus in initial stools from 265 children with diarrhea

<table>
<thead>
<tr>
<th>EM</th>
<th>Rotalex</th>
<th>Rotazyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Positive*</td>
</tr>
<tr>
<td>Positive</td>
<td>49</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

* Greater than or equal to 2+ by visual reading.

**Rotazyme test.** Supernatant fluids from 20% stool suspensions were further diluted 1:2 with Rotazyme sample diluent to make a final dilution of 10%. Rotazyme Diagnostic Kit I was purchased from Abbott, and the test was performed according to manufacturer instructions. Reactions were read visually and, unless otherwise indicated, tests were considered positive only when color intensity was 2+ or greater according to the color chart provided by the manufacturer.

**Blocking test.** Stool specimens that were positive by the Rotalex or Rotazyme test or both but negative by EM were further examined by a blocking test. For the Rotalex test, equal amounts of stool filtrates and a 1:100 dilution of rabbit antiserum to Nebraska calf diarrhea virus (Orion) were mixed and incubated at room temperature for 1 h before the slide agglutination test. Stool filtrates were also incubated with preimmune serum as control. Specimens were considered positive for rotavirus if latex agglutination was blocked by preincubation with the antiserum.

For the Rotazyme test, the procedures described by Rubinstein and Miller (18) were used. Antibody-coated beads were incubated with stool filtrates for 3 h and washed four times with distilled water. The beads were then incubated with 200 μl of a 1:100 dilution of rabbit antiserum to Nebraska calf diarrhea virus at room temperature for 16 h. As a control, the beads were also incubated with preimmune serum. The beads were again washed twice with distilled water and allowed to react with anti-rotavirus-peroxidase conjugate according to the manufacturer-recommended procedure. Reactions were read photometrically at 492 nm. Specimens were considered positive for rotavirus if the optical density produced by the specimens incubated with preimmune serum was above a minimum acceptable density and was reduced to 50% or less by hyperimmune serum.

**Direct EM.** A small drop of stool supernatant fluids (10%) was placed on a Formvar-coated grid (200 mesh) and stained with 2% phosphotungstic acid. The specimens were examined in an electron microscope (Philips EM400). Tests were considered negative if no rotavirus particles were seen in five acceptable (containing fecal material) grid squares.

**RESULTS**

**Initial stool specimens.** Initial stool specimens were obtained from 268 children (49 children in the 1982–1983 season and 219 children in the 1983–1984 season) and examined for rotavirus by EM and the Rotalex and Rotazyme tests. Each test was performed blindly. The Rotalex test was positive for 49 of 60 patients positive for rotavirus by EM and negative for 204 of 205 patients with negative EM results (Table 1). The results of the Rotalex test on three stool specimens were unacceptable because both the test and control latexes were positive (one was positive and two were negative by EM). Excluding these three specimens, the Rotalex test had a sensitivity of 81.7%, a specificity of 99.5%, a positive predictive value of 98%, and a negative predictive value of 94.9% compared with EM results.

![FIG. 1. Sensitivities of Rotalex and Rotazyme tests for detection of rotavirus in stools from children with rotavirus gastroenteritis at various times after onset.](http://jcm.asm.org/)
stools collected during 1 to 4, 5 to 7, 8 to 10, and 11 to 18 days after onset, respectively. Of 81 specimens, 57 were positive for rotavirus by EM compared with 50 by Rotalex and 44 by the Rotazyme test. However, examination of the data for each period revealed that the sensitivities of both tests varied depending on the time of stool collection relative to the onset of illness. The sensitivity (number of test-positive samples/number of EM-positive samples × 100) of the Rotalex test was 100 (20/20), 96 (23/24), 60 (6/10), and 33% (1/3) during 1 to 4, 5 to 7, 8 to 10, and 11 to 18 days after onset, respectively. The difference in the sensitivities of the test between 1 to 7 and 8 to 18 days from onset was highly significant (P < 0.001 by chi-square test). No false-positive (EM-negative and Rotalex-positive) results were observed.

The Rotazyme test had a similar pattern of sensitivities, which varied from 91% (40/44) during 1 to 7 days after onset of symptoms to 31% (4/13) during 8 to 18 days after onset of symptoms (P < 0.001). Two specimens were negative by EM but positive by the Rotazyme test only; both specimens were negative by the blocking test.

**Stool specimens from newborns.** Stool specimens from newborns without diarrhea were examined because a significant false-positive rate by the Rotazyme test was reported previously (7, 14). A total of 219 specimens from 105 newborns were available (Table 2). All stool specimens were negative by EM, but nine were positive (=2+) by the Rotazyme test and seven were positive by the Rotalex test. None of the 16 specimens were positive by the blocking test. Excluding the five specimens that yielded equivocal results because both the test and control latexes were positive, the Rotalex test had a false-positive rate of 3.3% (7/215) compared with 4.2% (9/215) for the Rotazyme test.

**Turnaround time and cost.** EM and the Rotazyme and Rotalex tests were compared for turnaround time and cost (Table 3). We estimated turnaround time by performing each test with five stool specimens at a time. The Rotalex test was much faster than EM or the Rotazyme test. The Rotazyme test requires a considerable incubation period, which was excluded from technologist time. Although the cost for EM was difficult to calculate, it was estimated to be about $40.00/h if EM time was paid on an hourly basis. Total cost for the Rotalex test was less than one-half that of the Rotazyme test and less than one-third that of EM.

**DISCUSSION**

EM or enzyme immunoassay including the Rotazyme test is commonly used for diagnosis of rotavirus gastroenteritis, with results available in a day or two because these tests are usually performed in batches. A delay in the diagnosis of rotavirus gastroenteritis may pose the potential problem of unnecessary hospitalization, thereby introducing a risk of nosocomial transmission of the virus to children admitted with other illnesses. A number of studies have demonstrated that nosocomial transmission of rotavirus can be a serious problem (9, 19, 23). In 1984, 74 children with rotavirus gastroenteritis were admitted to the Alberta Children’s Hospital, and 13 hospital-acquired rotavirus infections were documented in spite of stringent infection control measures. Numerous studies have shown that infants and young children with rotavirus gastroenteritis and 5 to 10% dehydration, if not in shock, can be treated with oral glucose or sucrose electrolyte solution (4, 17, 20). Since oral therapy can be given at home, many hospital admissions might be avoided altogether, provided that etiological diagnosis is established. Rapid diagnosis may have resulted in fewer admissions and prompt introduction of infection control measures. An accurate, inexpensive diagnostic test that can be completed within minutes or while patients are waiting at emergency wards is desirable.

The data from this study demonstrate that the Rotalex test, a commercial latex agglutination kit, is highly accurate, inexpensive, and extremely rapid. False-positive results were seen in only 1 of 265 initial (first available) stools from children with diarrhea (Table 1) and in none of 81 follow-up stools from children with rotavirus gastroenteritis (Fig. 1). Although the sensitivity of the Rotalex test was 82% (49/60) for initial stool specimens, it was much higher when tested on stool specimens obtained during an early stage of rotavirus infection. Sensitivity was 100 and 96% during 1 to 4 and 5 to 7 days, respectively, after the onset of symptoms and then fell to lower levels thereafter. These findings are consistent with a previous report that the concentration of virus particles shed in stools is decreased significantly after the first week of illness in the majority of patients (13).

The sensitivities of latex agglutination tests for rotavirus reported in the literature varied from 91 to 100% (1, 10, 11, 16, 21). The data from our study suggest that the varying sensitivities reported could be due to different timing of stool collection relative to the onset of illness. Sensitivity of the Rotalex test on the initial stool specimens in this study was 81.7%, which is lower than on the follow-up specimens on days 1 to 7. Some of the initial specimens could have been collected in the second week or during a later period of the illness, but information was not available. We recommend that the Rotalex testing be performed for children with diarrhea seen within a week of the onset of symptoms. The Rotalex test may not be reliable in the second week of illness.

The Rotazyme tests performed on initial stools showed a sensitivity of 75.0%, which was considerably lower than

### Table 2. Comparison of the Rotalex and Rotazyme tests with EM in 219 stools from 105 newborns without diarrhea

<table>
<thead>
<tr>
<th>Rotalex</th>
<th>Rotazyme</th>
<th>EM</th>
<th>No. of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>187</td>
</tr>
<tr>
<td>-</td>
<td>1+</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>-</td>
<td>&gt;2+</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>1+</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>&gt;1+</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

*a Results were equivocal because both the test and control latexes were positive.

### Table 3. Turnaround time and cost

<table>
<thead>
<tr>
<th>Test</th>
<th>Equipment</th>
<th>Turnaround time (min)</th>
<th>Technology time (min)</th>
<th>EM Costs</th>
<th>Materials Costs</th>
<th>Labor Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM</td>
<td>EM, centrifuge</td>
<td>127</td>
<td>110</td>
<td>$50.00</td>
<td>$ 2.00</td>
<td>$27.50</td>
</tr>
<tr>
<td>Rotalex</td>
<td>Water bath</td>
<td>360</td>
<td>130</td>
<td>$21.00</td>
<td>$32.50</td>
<td></td>
</tr>
<tr>
<td>Rotalex</td>
<td>Centrifuge</td>
<td>23</td>
<td>20</td>
<td>$20.00</td>
<td>$ 5.00</td>
<td></td>
</tr>
</tbody>
</table>

*a Turnaround time and cost for five stool specimens.

b Excluding waiting periods between procedures.

* Based on the $40/h rate charged by the EM unit of the Health Sciences Centre, University of Calgary, Alberta, Canada.

Based on a rate of $3/h.

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those reported in other studies (1, 6, 16). The difference in the criteria for positivity used in these studies may account for the disagreement. For example, Cheung et al. (6) reported the sensitivity of the Rotazyme test to be 91.7% when a color intensity of 1+ or greater was considered positive. However, sensitivity was reduced to 75.0% when positivity was defined the same way as in the present study, i.e., 2+ or greater. Other reasons for the difference in sensitivities observed in various studies may be that stools were collected at different times relative to the onset of diarrhea. It was shown in the present study that the sensitivity of the Rotazyme test varied, as did that of the Rotalex test, depending on the timing of stool collection during illness.

Enzyme immunoassays for rotavirus have been reported to be more sensitive than EM (2, 5, 18, 24). However, a problem we encountered in our study was that a large number of stool specimens produced a visual reading of 1+, of which the majority were EM negative. We found the suggestion of the manufacturer that tests with a reading of 1+ be repeated on specimens collected 24 h after the first sampling to be impractical. If a reading of 1+ had been considered positive for rotavirus, the sensitivity of the Rotazyme test could have increased significantly, but false positivity would also have increased. Another problem was that the Rotazyme test kit does not include control beads coated with preimmune sera. The number of specimens with equivocal results might be reduced if these were available.

Stool specimens from asymptomatic newborns were examined by the Rotalex test because reports have indicated unreliability (15 to 20% false positivity) of the Rotazyme test performed on stool specimens from newborns (7, 14). Our study showed a false positivity of only 4.2% (9/215) by Rotazyme and 3.3% (7/215) by the Rotalex test (Table 2). The significant difference between the false positivities reported could not be explained. Regardless, we found the Rotalex test reliable in screening newborn stools for rotavirus.

The test of the Rotalex test was about one-half to one-third of that of EM or Rotazyme. The test can be completed within 5 min for a single specimen compared with 1 h to several hours for EM or the Rotazyme test. Since the completion of this study, a new version of the Rotalex test kit has been marketed in the United States only that uses filtration rather than centrifugation for sample preparation. Vials with predispensed dilution buffer are included in the new kit, and stool suspension is clarified as it is pushed through a filtration device attached to the cap of the vial and dropped onto a test slide. The new kit may cost slightly more than the one used in this study, but it eliminates the need for a centrifuge and pipettes and may reduce turnaround time.

In conclusion, the Rotalex test was shown to be highly accurate, inexpensive, and extremely rapid in detecting rotavirus in stools. However, when the test is performed on stool specimens from the children who are in the second week of diarrheal illnesses, negative results should be confirmed by EM. The Rotalex test appears suitable for rapid diagnosis of rotavirus gastroenteritis in small hospitals, emergency wards, or even in the physician’s office.

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LITERATURE CITED