Comparative Efficiency of Commercial Immunoassays for the Diagnosis of Rotavirus Gastroenteritis during the Course of Infection

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Received 24 May 1985/Accepted 22 July 1985

We evaluated the performance characteristics of three commercially available immunoassays for the detection of rotavirus antigens in stool samples obtained from infants during the course of rotavirus gastroenteritis. Two of the assays, Bio-EnzaBead (Litton Bionetics, Charleston, S.C.) and Rotazyme (Abbott Laboratories, North Chicago, Ill.), are enzyme immunoassays, while the third, Rotalex (Medical Technology Corporation, Somerset, N.J.), is a latex agglutination assay. We tested a total of 122 samples obtained from 26 children with gastroenteritis; 56 samples, obtained from 21 children, were found to contain rotavirus antigen by a reference microplate enzyme immunoassay. Rotavirus antigen was found by the Bio-EnzaBead, Rotazyme, and Rotalex assays in 53, 42, and 29 samples, respectively. The true positivity of samples which were positive by the reference microplate assay but negative by the other assays was confirmed by a specific neutralization assay or by the visualization of bands of double-stranded RNA by polyacrylamide gel electrophoresis or both. No false-positive assay results were noted with any of the commercial assays. The sensitivity of the assays was determined to a great extent by the time after the onset of illness at which the specimen was collected. Thus, the sensitivity of commercial assays with specimens collected early in the course of illness did not differ significantly from that of the microplate assay. However, significantly lower degrees of sensitivity were noted later in the course of illness. Results of our studies indicate that all three commercial assays can accurately detect rotavirus in stools from children with rotavirus gastroenteritis. However, the choice of assay systems for use in the clinical laboratory will depend on the conditions in which stool specimens are collected and tested in the laboratory.

Rotavirus is a major cause of symptomatic gastroenteritis in children throughout the world (1, 3, 14). Rotavirus infection is particularly problematic in hospitalized children and other children living in closed environments (2, 14). The accurate diagnosis of rotavirus infections thus is important not only for the rapid diagnosis of infection in children with gastroenteritis but also for the identification of children who could potentially spread infection to other individuals.

Since human rotaviruses are generally fastidious agents and are difficult to cultivate in commonly used tissue culture systems (22), the diagnosis of rotavirus infection relies greatly on noncultivation methods of identification. Originally, rotaviral diagnosis was accomplished by means of electron microscopic techniques (4, 15). In the last few years, immunoassay techniques have been widely used for the detection of rotavirus infection in human stool samples (4, 7, 10, 11, 13). Recently a number of commercial immunoassays have also become available for the detection of rotaviruses in stool samples (9, 17–19, 25). These assays make the diagnosis of rotavirus possible in virtually any clinical laboratory. We evaluated the sensitivity and specificity of three commercially available assays for the detection of rotavirus in samples obtained from children with rotavirus gastroenteritis at different points in the course of their rotavirus infection.

MATERIALS AND METHODS

Clinical samples. One hundred twenty-two stool specimens were collected from 26 children, aged 1 day to 24 months, with symptomatic gastroenteritis during the winter of 1983–1984.

The first specimen was obtained within 3 days of the onset of diarrhea, and subsequent samples were obtained daily during the course of hospitalization when possible. The patient population consisted of patients admitted because of acute gastroenteritis and of children hospitalized for other causes who developed diarrhea during the course of hospitalization.

The samples were prepared as 10% suspensions in phosphate-buffered saline (PBS; pH 7.4) and stored at −70°C until they were tested. Specimens from 50 healthy newborns were also obtained during the same period to serve as control samples. Samples from newborns were selected as controls since previous reports have suggested a high rate of false-positive reactions in such specimens (5, 16). The SA-11 strain of rotavirus (originally provided by H. Mahlerbe, Gull Laboratories, Salt Lake City, Utah) was prepared by cultivation in MA-104 cells as previously described (22). The viral preparation used in the assays described below had a titer of approximately 10⁶ 50% tissue culture infective dose per 0.1 ml in this cell line. Serum from germ-free calves infected with human rotavirus was obtained originally from Richard Wyatt, National Institutes of Health, Bethesda, Md.

Assay systems. (i) Reference assay. All samples were originally tested in a reference solid-phase enzyme immunoassay by previously described methods (24). This latter assay was selected as the reference method since previous studies have documented that it is equivalent to other standard reference methods such as electron microscopy (4, 18). Briefly, alternate rows of polyvinyl microtiter trays were coated and incubated overnight at 4°C with hyperimmune goat antirotavirus antibody and preimmune goat antibody. The reaction was started by the addition of the fecal sample to duplicate wells coated with antirotavirus and control sera. After incubation for 1 h at 37°C, the sample was removed by

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washed five times in PBS containing 0.5% Tween 20 with an automatic microplate washer (Microwash II; Skatron Instruments, Lier, Norway). The reaction was completed by subsequent incubation with guinea pig antirotavirus antibody, alkaline phosphatase-labeled goat antiguinea pig immunoglobulin, and p-nitrophenyl phosphate substrate. These incubations were performed for 1 h at 37°C and were separated by a washing procedure performed as described above. The absorbance in each well was measured at a wavelength of 405 nm in a microplate colorimeter (MR 580; Dynatech Laboratories, Inc., Alexandria, Va.). For each sample a specific activity was determined by subtracting the mean optical density generated in the wells coated with control antibody from that generated in the wells coated with specific antirotavirus antibody. A specimen was considered to be positive if it yielded an activity equal to or greater than two standard deviations above the mean activity of negative control samples. A subset of samples which yielded discrepant results between different assay systems was further evaluated by means of a confirmatory enzyme inhibition immunosassay, as described previously (24). The samples were incubated with highly specific antibody generated from the infection of gnotobiotic calves with human rotavirus as well as nonimmune calf serum. After an overnight incubation at 4°C the amount of unneutralized antigenic activity was determined by the transfer of the antigen-antibody mixture to microtiter wells coated with antirotavirus and control sera, and the subsequent measurement of rotavirus antigen was performed as described above. A specimen was considered to contain rotavirus activity if there was a 50% decrease in the amount of antigen detected in samples reacted with specific antirotavirus antibody as compared with the control antibody.

(ii) Commercial assays. The characteristics of the assays used in this study are described in Table 1. The Rotazyme assay was obtained from Abbott Laboratories, North Chicago, Ill. The Rotalex latex agglutination assay was obtained from Medical Technology Corporation, Somerset, N.J. These assays were performed following the manufacturers instructions as described previously (18, 20). The Litton Bio-EnzaBead assay was obtained from Litton Bionetics, Charleston, S.C. This assay uses metal beads as a solid phase and a magnetic transfer device as described previously (20). For the enzyme immunosassays the results were measured spectrophotometrically, and the cutoff points were determined as indicated in the information provided with the assays. For the latex agglutination assay the degree of agglutination was visually determined following the manufacturers’ recommendations.

Detection of rotavirus nucleic acid. Rotavirus double-stranded RNA was recovered from fecal specimens by CF-11 cellulose chromatography (21). RNA preparations were electrophoresed on 7.5% polyacrylamide gels (1.5-mm thickness) at 30 mA constant current for 15 h. Electrophoresis buffer was 0.14 M Tris base and 0.002 M disodium EDTA with glacial acetic acid (pH 7.8). Gels were stained with silver nitrate as described previously (12).

RESULTS

The three commercial assay systems were compared with the reference microplate enzyme immunoassay. Initial comparisons were made by means of serial dilutions of the SA-11 strain of rotavirus prepared as described above. The virus preparation was evaluated diluted either in PBS or in a pool of human stool samples obtained from healthy individuals. The preparation, containing 10⁵ 50% tissue culture infective dose per 0.1 ml of viable virus, could be detected at a dilution of 1:300 by the Bio-EnzaBead and microplate assays, 1:30 by the Rotazyme assay, and 1:10 by the Rotalex system. The detection levels were similar when the SA-11 strain of virus was tested in PBS or in stool samples (Fig. 1).

A total of 122 samples serially obtained from 26 hospitalized children were then analyzed by the available assay systems. The sensitivity of the assay systems for the detection of rotavirus in these samples is depicted in Table 2. A total of 56 of the 122 samples, obtained from 21 of the 26 study patients, had rotavirus detectable by the microplate confirmatory enzyme-linked immunosorbent assay (ELISA). All but 1 of the 56 samples were from stools considered to be diarrheal by the clinician caring for the patient. A total of 53 of these 56 samples (95%) were also positive by the Bio-EnzaBead assay system. On the other hand, only 42 (75%) of the samples were positive by the Rotazyme assay, and only 29 (52%) of the samples were positive by the Rotalex agglutination assay. The agglutination assay was significantly less sensitive than the microplate assay (P < 0.01) by the chi-square test (6). The ability of the agglutination assay to detect rotavirus antigen correlated significantly with the level of antigen in the stool sample, as determined by the specific activity measured by the microplate ELISA. Thus the ELISA activity of positive samples by the Rotalex assay (mean ± standard deviation) was 0.415 ± 0.123 U, and the mean activity of the negative samples by this assay was 0.292 ± 0.123 U (P < 0.05) by Student’s t test (6). The lower sensitivity of the Rotazyme assay approached but did not achieve statistical significance (0.1 > P > 0.05). None of the 66 samples negative for rotavirus antigen by the confirmatory ELISA was positive by any of the other assay systems. The specificity of the assay was further evaluated by testing 50 stool samples from healthy newborns. None of the samples were positive by the reference assay or any of the three commercial assay systems.

To determine whether the samples positive by the refer-
ence microplate ELISA and negative by the other assays represented true or false-positive reactions, additional assays were performed on 15 samples which were positive by the microplate assay but negative by one or more of the other assay systems (Table 3). All 15 samples were investigated by the ELISA neutralization assay with sera obtained from gnotobiotic calves experimentally infected with human rotavirus as described above. In 13 cases, the presence of rotavirus antigen was documented by the ability of the rotavirus antibody to specifically neutralize antigenic activity. In addition, in 12 cases segments of double-stranded RNA typical of rotaviruses could be demonstrated by silver staining polyacrylamide gel electrophoresis preparations (Fig. 2). By the combined use of these two assays it could be confirmed that rotavirus antigen was present in all samples positive by the reference assay but negative by the other assay systems.

To further investigate the performance characteristics of the assay systems, we determined the sensitivity of the assays for the detection of virus in stool samples collected at different times after the onset of gastroenteritis. This analysis revealed that the ability of the assay systems to detect positive samples depended to a great extent on the time of collection of the samples. All of the assays displayed high degrees of sensitivity when samples obtained early in the course of disease were tested (Table 4). The sensitivity of the Bio-EnzaBead, Rotazyme, and Rotalex assays in specimens collected less than 3 days from the onset of illness was 100, 95, and 80%, respectively, as compared with the reference microplate ELISA. These differences were not statistically significant. However, the sensitivities of these systems showed a decrease when later specimens were collected. The sensitivity of the Rotazyme assay was only 82% for

![Graph](https://via.placeholder.com/150)

**FIG. 1.** ELISA dose-response curve and latex agglutination reactivity of tissue culture Rotavirus antigen (SA-11). Dotted and continuous lines represent titration of viral preparation added to PBS and to a stool sample pool obtained from healthy individuals, respectively. Mean and standard deviation of triplicate measurements are indicated. Assay values for uninfected tissue culture fluids for each assay system are depicted in the lower left corner. Latex reactivity was determined diluted in the stool sample pool. Symbols: ●, confirmatory microplate ELISA; □, Bio-EnzaBead; ○, Rotazyme.

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**TABLE 2.** Comparison of immunoassays for detection of rotavirus

<table>
<thead>
<tr>
<th>Assay</th>
<th>No. of positive and negative samples by the following immunoassays:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio-EnzaBead</td>
</tr>
<tr>
<td>Confirmatory ELISA</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Bio-EnzaBead</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>41</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Rotazyme</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

* * x² = 0.66, P not significant.
* * x² = 2.880.05 < P < 0.1
* * x² = 12.20, P < 0.01.
* * x² = 1.40, P not significant.
* * x² = 8.96, P < 0.01.
* x² = 2.86, 0.05 < P < 0.1.

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**TABLE 3.** Confirmatory tests performed on 15 fecal specimens yielding conflicting results by the commercial assays and the reference assay

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Tests positive</th>
<th>Tests negative</th>
<th>Result of blocking assay</th>
<th>Presence of RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, RZ</td>
<td>L, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>L, RZ, LA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>L, RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>RZ, LA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>M, L, RZ</td>
<td>LA</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>M, L</td>
<td>RZ, LA</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Abbreviations: M, Microplate ELISA; L, Bio-EnzaBead; RZ, Rotazyme; LA, Rotalex.
* ELISA blocking assay performed as described in the text.
* Identification of segmented double-stranded RNA after polyacrylamide gel electrophoresis and silver staining were performed as described in the text.
* ND, Not done.
The Rotazyme assay requires the use of only a single plastic bead which can be manipulated without a specific transfer device. While the current formulation of this assay required a performance time somewhat shorter than that of the Bio-EnzaBead assay, it appeared to be less sensitive than that assay system and the reference enzyme immunoassay. In addition, the Rotazyme assay does not provide a control bead coated with nonimmune serum to distinguish specific from nonspecific reactions. While nonspecific reactions were not noted in our study, the lack of a simple control reaction might still be judged a disadvantage when large numbers of heterogeneous samples are to be tested. The latex agglutination assay system (Rotalex) was by far the most rapid assay, requiring less than 10 min, and was the simplest to perform. However, it displayed the lowest degree of sensitivity when compared with the reference enzyme immunoassay. It should be noted that the agglutination assay system which we tested differs somewhat from previous formats of the original assay in that filtration of the fecal sample through a device provided with the assay kit was used instead of the centrifugation procedure used in previous studies (9, 17, 19). The filtration was easy to perform and obviated the need for access to a high-speed centrifuge. However, the effect of the filtration procedure on the sensitivity of the assay could not be definitively determined, but it should be examined in future studies. The specimens that were tested were stored frozen at −70°C prior to testing. However, additional evaluation of a panel of fresh specimens indicated that the relative degree of sensitivity of the commercial assays was similar to that found for the frozen samples (P. G. Miotti and R. H. Yolken, unpublished data).

In our evaluation we tested samples obtained at different time points in the course of rotavirus infection. This allowed us to determine the sensitivity of the different assay systems at different times after the onset of gastroenteritis and the collection of the initial sample. Thus, while the Rotazyme and Rotalex assays displayed a lower overall sensitivity than the other assay systems, they did display comparable levels of sensitivity when used to test the initial samples. Both assays thus might be efficiently used to test samples collected early in the course of disease. However, both assays displayed decreased levels of efficiency later in the course of illness. Negative assays should be interpreted with caution under these circumstances.

The decreased efficiency of these assays is due to lower amounts of antigen present in the later samples because of either the decreased production of infectious virus or the complexing of antigen with endogenous intestinal antibody.

**DISCUSSION**

This report describes a comparative evaluation of three commercially available immunoassay systems for the detection of rotavirus antigen in stool samples.

Although all the assays displayed good degrees of specificity, as confirmed by the absence of false-positive reactions, the three assays were found to differ in their relative sensitivities. This is not surprising since the three assays use different reaction formats.

The characteristics of the commercial assays are depicted in Table 4. The Bio-EnzaBead assay system proved to be the most comparable in sensitivity to the microplate ELISA immunoassay in use in our laboratory. The Bio-EnzaBead assay utilizes iron-containing plastic beads which can be manipulated by means of a magnetic transfer device. Use of this bead system allows more flexibility in assay performance while retaining the advantages of the ability to perform large numbers of reactions and to perform quantitative control reactions. However, the performance of this assay system does require multiple incubations and washing steps, as well as access to the magnetic transfer device. On the other hand, the Rotazyme assay requires the use of only a single plastic bead which can be manipulated without a specific transfer device. While the current formulation of this assay required a performance time somewhat shorter than that of the Bio-EnzaBead assay, it appeared to be less sensitive than that assay system and the reference enzyme immunoassay. In addition, the Rotazyme assay does not provide a control bead coated with nonimmune serum to distinguish specific from nonspecific reactions. While nonspecific reactions were not noted in our study, the lack of a simple control reaction might still be judged a disadvantage when large numbers of heterogeneous samples are to be tested. The latex agglutination assay system (Rotalex) was by far the most rapid assay, requiring less than 10 min, and was the simplest to perform. However, it displayed the lowest degree of sensitivity when compared with the reference enzyme immunoassay. It should be noted that the agglutination assay system which we tested differs somewhat from previous formats of the original assay in that filtration of the fecal sample through a device provided with the assay kit was used instead of the centrifugation procedure used in previous studies (9, 17, 19). The filtration was easy to perform and obviated the need for access to a high-speed centrifuge. However, the effect of the filtration procedure on the sensitivity of the assay could not be definitively determined, but it should be examined in future studies. The specimens that were tested were stored frozen at −70°C prior to testing. However, additional evaluation of a panel of fresh specimens indicated that the relative degree of sensitivity of the commercial assays was similar to that found for the frozen samples (P. G. Miotti and R. H. Yolken, unpublished data).

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**TABLE 4. Comparison of immunoassays for the detection of rotavirus antigen during the course of illness**

<table>
<thead>
<tr>
<th>Day of collection</th>
<th>No. of samples positive (%)</th>
<th>Confirmatory ELISA</th>
<th>Bio-EnzaBead</th>
<th>Rotazyme</th>
<th>Rotalex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial specimen*</td>
<td>20</td>
<td>20 (100)</td>
<td>19 (95)</td>
<td>16 (80)</td>
<td></td>
</tr>
<tr>
<td>2 to 4</td>
<td>17</td>
<td>17 (100)</td>
<td>14 (82)</td>
<td>7 (41)</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>19</td>
<td>16 (84)</td>
<td>9 (47)</td>
<td>6 (31)</td>
<td></td>
</tr>
</tbody>
</table>

* In the three commercial assays, the percentage refers to the number of samples positive as compared with the confirmatory ELISA.

* The initial specimen was always collected within the first 3 days of recognized gastroenteritis. The following specimens were collected 2 to 4 days and >4 days after the initial specimen was collected.
It should be noted that it is likely that even the most sensitive enzyme immunoassays might fail to detect low amounts of available antigen which might be present late in the course of illness, and that more sensitive immunologic (26), cultivation (23), or nucleic acid detection (8) systems might be useful in detecting low amounts of virus in infected individuals.

It should also be noted that the various amounts of antigen found at different times during the course of infection might explain discrepancies which have been reported in the literature concerning the relative sensitivities of the available assay systems. For example, some investigators have found the Rotazyme and Rotaalex assays to be as sensitive as reference methods (17, 18), while others have documented lower degrees of sensitivity (9, 19).

Differences in the time of specimen collection as well as the clinical state of the patient populations should be considered in future evaluations of assay systems for the detection of rotavirus and other viruses in clinical samples.

Our findings suggest that the choice of rotavirus assay system will depend to a great extent on the conditions of the individual laboratory. For example, the Bio-EnzaBead assay might be advantageous in situations in which large numbers of specimens collected at several points in the course of rotavirus gastroenteritis are to be tested, as might occur in a reference laboratory. On the other hand, the Rotazyme and Rotaalex assays might be efficiently utilized in situations in which only smaller numbers of specimens obtained early in the course of disease are to be tested, as might occur in a smaller clinical laboratory or in a physician’s office. The Rotaalex assay might be particularly useful in the latter diagnostic environment since it can be performed rapidly without the need for any specialized equipment.

The successful application of these assay systems should thus allow for the improved management of patients with suspected rotavirus gastroenteritis as well as for the prevention of nosocomial rotavirus infection and a decrease in morbidity associated with rotavirus infection in hospitalized patients. However, additional efforts should be directed at providing practical assays of improved sensitivity and decreased reaction time so that the largest number of cases of rotavirus infection can be diagnosed and prevented in these populations.

LITERATURE CITED


