Comparison of Rotazyme and Direct Electron Microscopy for Detection of Rotavirus in Human Stools

EILEEN Y. CHEUNG,* STEPHEN I. HNATKO, HANS GUNNING, AND JAN WILSON

Microbiology Division, Department of Laboratory Medicine, Royal Alexandra Hospital, Edmonton, Alberta, Canada T5H 3V9

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A total of 115 stools were examined for Rotavirus, using direct electron microscopy (EM) and Rotazyme. The overall agreement was 88.7%. Of the negative results, there was 91.95% agreement. Rotazyme reactions of three-plus or more gave a 100% agreement with EM. The Rotazyme test is a useful diagnostic aid in laboratories not capable of performing EM.

Direct electron microscopy (EM) of human stools is a well-established test for the detection of rotavirus (1, 7, 8). The Rotazyme immunoassay is another recent test available for detecting the rotavirus antigen. To compare the results of the Rotazyme test with those of direct EM, 115 stool specimens from patients admitted from April to September 1981 were examined by both methods. (This paper was presented in part at the 49th Conjoint Meeting on Infectious Diseases, Ottawa, Ontario, Canada, November, 1981.)

The Rotazyme test, (Abbott Laboratories), was performed according to the manufacturer’s instructions using the visual method of reading. A reading of plus-minus (±) was treated as negative. Direct EM was done by examining two grids per specimen, five spaces per grid, using a Hitachi H600-4 electron microscope (Hitachi). Most of the specimens examined were also examined by the Virology Department, Provincial Laboratory of Public Health, Edmonton, Alberta, Canada. These were from 66 patients, varying in age from a few days to 70 years. Of all the patients included, 47 had clinically significant diarrhea, i.e., diarrhea occurring 1 to 2 days to about a week before admission to the hospital and lasting a few days to about a week. Other causes, such as antibiotics, food, and drugs, were excluded. All specimens were also examined for bacterial and parasitic pathogens. For the 115 specimens examined by the two methods, there was 100% agreement between the direct EM and ++++ and +++++ Rotazyme reactions (Table 1). The agreement was 77.78% between the two-plus Rotazyme results and direct EM. Negative EM had a 91.95% agreement with a negative Rotazyme reaction.

Table 2 shows the division of specimens into groups A to F. The overall agreement between direct EM and Rotazyme test results was 88.7%.

From 47 patients in group A, 80 stool specimens examined for Rotavirus by both direct EM and Rotazyme test were negative. Of the 47 patients, 21 had symptoms which did not fit into our definition of infectious diarrhea. Of the remaining 26 patients that fulfilled the diagnosis of infectious diarrhea, 5 had adenovirus by direct EM, 3 had enterovirus, 1 had Campylobacter jejuni, 1 had Staphylococcus aureus from stool cultures, and 1 had a one-plus Rotazyme reaction on one stool specimen examined the same day. Although the remaining 15 of the 26 patients had diarrhea, no pathogens were identified or isolated.

In group B, there were 22 stool specimens with positive EM and Rotazyme reaction (+ to ++++). These were from 17 patients, all of whom had clinically significant diarrhea. Their ages ranged from 3 weeks to 19 months, i.e., less than 2 years.

Groups C and D were considered together. There were seven specimens with positive EM and a negative or a plus-minus (±) (negative) Rotazyme reaction. These were from four patients, aged 2 to 14 months, all of whom had clinically significant diarrhea. However, three of these four patients had a one-plus, two-plus, and four-plus Rotazyme reaction on their stools examined 1 to 4 days previously (after 1 week, 3 days, and 3 weeks, respectively, of diarrhea). The probable explanation for these results is that during the acute phase of gastroenteritis there may be more rotavirus antigens to be detected by the Rotazyme test than are present later when the diarrhea subsides. The fourth patient had one ± Rotazyme reaction.

Groups E and F consisted of six specimens with negative EM and a one-plus or two-plus Rotazyme reaction. These were from six patients, three of whom had clinical infectious diarrhea. One of them was discussed in group A.
TABLE 1. Comparison of Rotazyme with EM results

<table>
<thead>
<tr>
<th>No. of specimens tested</th>
<th>Rotazyme result</th>
<th>No. in agreement with EM</th>
<th>% Agreement with EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>++++</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>++</td>
<td>7</td>
<td>77.78</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>± (−)</td>
<td>2+</td>
<td>25</td>
</tr>
<tr>
<td>79</td>
<td></td>
<td>74</td>
<td>93.67</td>
</tr>
</tbody>
</table>

* A reading of ± was considered negative.

The other two, aged 1 and 11 months, had two-plus Rotazyme reactions without other positive findings. They had diarrhea 3 and 7 days before the stools were collected.

Rotavirus is one of the most common causes of gastroenteritis, particularly in children under 2 years of age (2, 3, 5). In one study in Manitoba, it was the commonest viral pathogen, especially in infants (6). In Vancouver, British Columbia, it was also the commonest viral agent associated with gastroenteritis (9). In Rochester, Minn., it accounted for 35% of gastroenteritis in children (10). In our study, rotavirus was identified in 25.22% of our specimens by direct EM. The fact that this study was done in the summer period may explain the low incidence of positive findings. Direct EM offers a quick diagnostic tool for this virus. However, not every laboratory is equipped with an electron microscope. It would be helpful if there were other tests that could be used to detect this viral antigen within a short time, and that also correlated well with direct EM. This was the aim of this study.

We draw the following conclusions from our limited studies:

(i) The Rotazyme test correlates fairly well with direct EM for rotavirus detection. It appears that with a three-plus or four-plus Rotazyme reaction, there is excellent correlation and no false-positive Rotazyme reaction. Also, patients with a three-plus or four-plus Rotazyme reaction all had significant infectious diarrhea. A two-plus or lower Rotazyme reaction does not show sufficient correlation, and the specimen requires further testing, such as EM. We did not rule out false-positive Rotazyme reactions with either blocking enzyme-linked immunosorbent assay (ELISA) or confirmatory ELISA (4).

(ii) The negative Rotazyme test had a 91.95% correlation with direct EM. It appears from this study that it does not show cross-reactivity with adenovirus or Campylobacter sp. Our results are similar to those of Yolken and Leister (11), who found that the negative Rotazyme reactions correlate with direct EM in 95% of cases. Their positive Rotazyme reactions gave a 93% agreement with direct EM.

(iii) The Rotazyme test is easy to perform and read, and results can be obtained relatively rapidly. It is likely to become a useful diagnostic aid for laboratories that do not have an electron microscope or that have to send out stool specimens for viral examination.

LITERATURE CITED


