Human Rotavirus Detection by Counterimmunoelectrophoresis Versus Enzyme Immunoassay and Electron Microscopy After Direct Ultracentrifugation

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The sensitivity of the counterimmunoelectrophoresis test with NCDV, Wa, and SA-11 rotavirus antisera was 60, 60, and 67%, respectively. The counterimmunoelectrophoresis specificity was greater than 99%, but the low sensitivity is a limiting feature of this test as a first-line immunodiagnostic test for rotavirus detection.

One of the earliest techniques for rotavirus detection was counterimmunoelectrophoresis (CIE) (8, 11, 12). This study was designed to evaluate CIE versus electron microscopy (EM) after direct ultracentrifugation of the virus (6) and versus direct enzyme immunoassay (EIA) (5, 10). We also sought to evaluate the CIE procedure with antisera to rotaviruses in each of the two human subgroups (7, 13).

Our study consisted of examination of human fecal specimens submitted to the Cadham Provincial Laboratory in Winnipeg, Manitoba, from patients with infantile gastroenteritis. A total of 174 specimens collected over a 7-week period in the winter of 1983 were examined by two reference methods and the CIE procedure. Reference method 1 was direct ultracentrifugation followed by examination by EM, as previously described (6). EM was performed as a routine laboratory procedure in a blinded fashion by four experienced technologists working on a rotational basis, with examination of two grid squares per specimen, at 30,000 magnification with a Philips 201 electron microscope. Reference method 2 was the direct EIA procedure with the Rotazyme test (Abbott Laboratories, Diagnostics Div.). All EIA results were read with a spectrophotometer in a blinded fashion. The blocking procedure for the Rotazyme test used a 1:25 dilution of anti-NCDV antiserum incubated with the bead for 16 to 18 h (after exposure to the specimen) versus a control bead of the test specimen incubated with phosphate-buffered saline before addition of the conjugate for both beads (10). A sample of approximately a 20% (wt/vol) fecal suspension in CIE buffer was saved at −70°C for the retrospective study of the CIE procedure by a single technologist. Antiserum used for the CIE procedure included the following: rabbit anti-NCDV and rabbit anti-SA-11 virus, kindly provided by L. Spence, Department of Microbiology, University of Toronto, who also provided the CIE protocol. The Wa strain of human rotavirus and guinea pig antiserum to the Wa strain were provided by the National Institutes of Health, Bethesda, Md. These antisera were titrated against their homologous antigens by the CIE procedure.

From 5 to 7 μl of a 20% fecal suspension in the CIE buffer and antisera (12) were placed in the cathode- and anode-facing wells. Electrophoresis was performed at 5 mA per slide for 90 min in 0.06 mol of Gelman barbital buffer per liter (pH 8.8)–0.05% sodium azide (NaN3). The slides were washed in saline overnight and stained the next day in 1% tannic acid for 90 min. The slides were read against a Shandon dark-ground viewer. The criteria for positivity included a precipitin line between the wells which did not arc around the wells (1). A reference positive control was run for each antiserum on each slide.

The results of antiserum titration for the CIE test revealed an endpoint titer of 1:256 (NCDV antiserum), 1:128 (SA-11 antiserum), and 1:640 (Wa antiserum). With the criterion of Spence et al. of a working concentration at 16 times the endpoint dilution (12), the working dilutions of antisera were 1:16, 1:8, and 1:40, respectively. A pool of NCDV and SA-11 antiserum was also used at a working concentration for each antiserum as described above.

The specificity of the rotavirus detection by the CIE procedure was 100% for the NCDV and Wa antiserum. Only one CIE-positive specimen with the SA-11 antiserum and the pooled SA-11 and NCDV antiserum was not confirmed by either EM or EIA. Therefore the overall specificity of the CIE procedure was 99%.

The sensitivity of rotavirus detection by CIE can be seen in Table 1 and was dependent upon the reference procedure used. The most sensitive reference procedure was EIA which detected 51 positive samples in the 174 specimens. However, as six of these could not be verified by the EM procedure, blocking assays were done on the specimens. Only three of the six positive specimens by EIA were considered to be true positives, as the optical density was reduced by >50% with the blocking assay. The optical density results of these six specimens incubated with phosphate-buffered saline (pH 7.4) versus NCDV antiserum were 0.399 versus 0.092, 0.238 versus 0.087, 2.0 versus 0.955, 0.189 versus 0.105, 0.239 versus 0.128, and 0.144 versus 0.105. Therefore, with 48 positive specimens, the sensitivity of the CIE detection of rotavirus with the NCDV and Wa antiserum was 60% and that of the CIE procedure with the SA-11 antiserum and the pool of NCDV and SA-11 antiserum was 67%.

Of the specimens, 45 were positive by EM after ultracentrifugation. Discrepancies between EM and the CIE procedure (CIE negative but EM positive) were reconfirmed as positive for rotavirus by EM in the suspension used for the CIE procedure in 12 of the 16 specimens. Therefore, the sensitivity of rotavirus detection by the CIE procedure was...
NOTES

TABLE 1. Sensitivity of CIE rotavirus detection

<table>
<thead>
<tr>
<th>Rotavirus antiserum</th>
<th>No. positive by CIE</th>
<th>% Sensitivity of CIE vs reference test:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EM from CIE suspension (n = 41)</td>
<td>EM total (n = 45)</td>
</tr>
<tr>
<td>NCDV</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>Human Wa</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>SA-11</td>
<td>32</td>
<td>78</td>
</tr>
<tr>
<td>Pooled NCDV and SA-11</td>
<td>32</td>
<td>78</td>
</tr>
</tbody>
</table>

64% for the NCDV and Wa antiserum alone and was 74% for the SA-11 antiserum used alone and for the pooled NCDV and SA-11 antiserum versus EM as the reference standard.

By EM, 41 specimens were initially positive for rotavirus versus 51 by EIA. Reexamination by EM of these 10 discrepant specimens detected an additional 4 rotavirus-positive specimens (3 of these 4 specimens were negative by CIE, suggesting that low titers of virus were present). The other six discrepant results (EM versus EIA) were reevaluated by the Rotazyme blocking assay, as described above.

In summary, in this study the CIE procedure with these antiseras detected between 60 and 67% of rotavirus-positive human fecal specimens in comparison with the Rotazyme direct EIA, in which results were verified by blocking assay or EM after direct ultracentrifugation. Early studies of CIE for the detection of human rotaviruses showed a 45% sensitivity (5 of 11 specimens positive by CIE) versus standard EM (11), a result which was markedly improved by staining of the immune complexes, which resulted in a 92% sensitivity (8 of 38 positive) and a 95.5% sensitivity (86 of 90 positive) (12). Grauballe et al. found CIE to be more sensitive than EM, with EM detecting only 77% (41 of 54) of specimens positive by CIE (2). However, they used a high-avidity second-generation antiserum prepared against immune complexes of human rotavirus and antibody prepared against bovine rotavirus, which resulted in antibody with four times the titer of the first-generation antiserum (3). The EM procedure used as a reference in their study included negative staining of virus fractions purified on a cesium chloride gradient, the sensitivity of which was not discussed. In 1981, with antiseras prepared against immunoprecipitates of human rotavirus and a new standard of EIA (which detected 42 of 44 rotavirus specimens), Grauballe et al. showed that the sensitivity of the CIE procedure was 80% (35 of 44 positive) (4). Obert et al. introduced a new reference standard, immunosorbtent electron microscopy, which detected 100% (39 of 39) of the rotavirus-positive specimens. CIE detected only 61% (24 of 39) of the rotavirus-positive specimens with rabbit anti-human rotavirus antiserum. Standard EM techniques detected 59% (23 of 39), and a direct EIA was positive for 95% (37 of 39) of the rotavirus-positive specimens (9).

Thus, variable sensitivities of the CIE procedure for rotavirus detection have been reported and partly accounted for by differences in reference methods. Also, previous CIE studies were done with anti-NCDV antiseras (11, 12) and anti-human rotavirus antiseras (3, 4, 8, 9) (of undetermined subgroup specificity). Some antiseras were used in these studies had undefined antibody titers.

The sensitivity of our CIE procedure for rotavirus detection did not vary markedly with the strain specificity of the antiseras, although we used antiseras prepared against subgroup 1 rotaviruses (NCDV [7]) and subgroup 2 rotaviruses (Wa [7]). This suggests that a pool of antiseras against subgroup 1 and subgroup 2 rotaviruses would not have increased the sensitivity of the CIE procedure and that antigenic cross-reactivity, presumably against a common antigen(s), is of major importance of rotavirus detection by the CIE test. However, we have not analyzed the subgroups or serotypes of rotaviruses from the time period during which these specimens were collected to determine whether more than one virus strain was present. Of interest was the fact that the use in the CIE test of SA-11 antiserum (of subgroup 1 [7] but belonging to the serotype 3 classification of human rotaviruses [13]) yielded three additional positive specimens. However, the sensitivity of the CIE procedure was substantially lower in comparison with early CIE studies for rotavirus detection in which unenhanced EM procedures were used as the reference standard. With more sensitive techniques of EIA, the direct ultracentrifugation preceding EM in this report, and previous reports in which the standards of EIA (4) and EIA and immunosorbent electron microscopy (9) have been used, a similar reduction in the sensitivity of the CIE procedure has been noted.

The marginally lower sensitivity of EM versus EIA results in this study may reflect the initial concentration of fecal suspension (5 versus 20%, respectively), the subjectivity of EM, or the structural degeneration of the virus particles (10). The sensitivity of the Rotazyme EIA was excellent, but three positive specimens could not be verified by a blocking assay (for a Rotazyme specificity of 98%). Most of these false-positive results occurred near the cutoff point. Recent suggestions that EIA procedures for rotavirus detection should be confirmed by blocking assay (T. H. Flewett and P. Halonen [Fourth International Conference on Comparative Virology, Banff, Alberta, October 17–23, 1983]) would markedly increase the time and expense of EIA systems, even if a blocking antiserum were provided with the commercial test systems.

The specificity of results, the speed and simplicity of the CIE procedure (1), and the economy of reagents make this a very attractive viral diagnostic test, as high-titer antiseras become commercially available. Many hospitals and regional laboratories currently possess CIE equipment which could be used for this diagnostic purpose. However, when compared with newer procedures, the lower sensitivity is a major limitation of the CIE assay.

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ADDENUM

After the above study was completed, we implemented rotavirus diagnosis in human fecal specimens by the CIE test in February 1983. Because of the high specificity of the CIE test shown in this report, we did not confirm results which revealed rotavirus by the CIE test. However, from specimens submitted from our nurseries for the newborn, we obtained several specimens positive by the CIE test for rotavirus. To evaluate possible problems with the rotavirus CIE test specificity, we collected fecal samples from asymptomatic newborn children in the nurseries for the newborn. By the CIE test, 7 of 23 fecal samples from asymptomatic newborn children were positive for rotavirus, but no rotavirus could be detected by the previously described EM technique after direct ultracentrifugation. Two specimens were positive by EM that were not detected by the CIE test. This poor specificity of the CIE test for fecal specimens from
the newborn would have been undetected in our initial CIE versus EM study, as no specimens were submitted from asymptomatic newborns in our specimen sample. Thus, the poor specificity of the Rotazyme test (P. J. Krause, J. S. Hyams, P. J. Middleton, V. C. Herson, and J. Flores, J. Pediatr. 103:259–262, 1983) may have a parallel in the poor specificity of the CIE test for rotavirus detection in a similar newborn patient population.

LITERATURE CITED