Comparative Evaluation of a Commercial Enzyme-Linked Immunoassay and Solid-Phase Immune Electron Microscopy for Rotavirus Detection in Stool Specimens

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Using solid-phase immune electron microscopy (SPIEM) as a reference test, we examined 151 stool specimens from infants and young children with acute gastroenteritis for rotavirus detection by a one-step commercial enzyme-linked immunosorbent assay (ELISA) with labeled monoclonal antibody. Of the 83 samples determined to be positive for rotavirus by SPIEM, 82 were detected as positive by the monoclonal antibody ELISA (sensitivity, 98.7%), while 67 of the 68 specimens determined to be negative by SPIEM were correctly detected as negative by the ELISA (specificity, 98.5%). The diagnostic accuracy of the ELISA kit was 98.6%. Thus, the one-step monoclonal antibody ELISA, which can be completed in less than 90 min, appears to be highly suitable for the rapid and reliable detection of rotavirus in stools.

The diagnosis of human rotavirus (HRV) infections is usually accomplished by rotavirus particle (1) or antigen (9) detection in stools. Antigen detection is largely preferred over direct detection of virus particles by electron microscopy (EM), which is still a cumbersome and time-consuming technique. Among different immunological techniques proposed for antigen detection, enzyme-linked immunosorbent assays (ELISAs) have been widely used and with results considered to be highly satisfactory (9, 10). In the last few years, commercial kits have become available, but there have been problems with specificity (false-positive results) or sensitivity (false-negative results) (6, 7). Recently, we had the opportunity to evaluate a commercial ELISA kit (SPIEM) for the detection of HRV in stools. To investigate the sensitivity and specificity of the assay, we evaluated it by using solid-phase immune EM (SPIEM) as the reference technique for HRV detection. As previously reported, SPIEM increases the sensitivity of EM 10^2 to 10^3 times (3, 8). Thus, the SPIEM technique is considered to be the most sensitive and specific assay now available for rotavirus detection, since false-positive results are avoided and false-negative results are greatly reduced, if not eliminated.

A total of 151 stool specimens, taken from infants and young children 1 to 3 days after the onset of acute gastroenteritis, were tested for the presence of rotavirus. All samples were collected from 1982 to 1985 from patients admitted to hospitals in northern Italy.

The specimens were examined for rotavirus by the monoclonal antibody ELISA kit and, as a reference test, SPIEM. Detection was also performed by direct EM and by an indirect double-antibody sandwich ELISA. Samples under study were never frozen and thawed more than twice before being tested. The Pathfinder kit for HRV detection (Kallestad Laboratories, Inc., Austin, Tex.), a one-step monoclonal antibody ELISA which can be completed in 1.25 h, was performed in accordance with the instructions of the manufacturer. Briefly, 100 μl of peroxidase-conjugated mouse monoclonal antibody to EDIM rotavirus (2) and 300 μl of 10% stool suspensions were added to polystyrene tubes precoated with rabbit antrotavirus immunoglobulin. The tubes were then mixed and incubated for 60 min at room temperature. After five washings with distilled water, chromogenic substrate was added, and the results were read visually and spectrophotometrically after 15 min. SPIEM for HRV detection was performed with a mixture of unabsorbed rabbit antisera to the four known HRV serotypes as a trapping antibody bound to staphylococcal protein A previously adsorbed on carbon-Formvar-coated grids (3). Direct EM examination and scoring of specimens was performed essentially as described by Brandt et al. (1). Stool preparations were observed with a Philips EM-201 electron microscope, and the average number of virus particles per grid square was determined after examination of 10 to 20 grid squares at a magnification of 45,000×. The indirect double-antibody sandwich ELISA for HRV detection was done with either polyclonal antisera as previously described (3) or mouse monoclonal antibodies prepared in our laboratory (unpublished observations). All HRV-positive samples were tested for subgrouping by the ELISA (5). Stool specimens which could not be subgrouped by the ELISA were examined by SPIEM for HRV subgrouping with either absorbed subgroup-specific rabbit antisera (5) or subgroup-specific monoclonal antibodies produced in our laboratory (unpublished observations). In addition, 29 HRV-positive samples were serotyped by SPIEM with absorbed polyclonal antisera (4).

In preliminary experiments, log10 dilutions of two highly positive rotavirus specimens from a stool sample and a cell culture-adapted HRV strain were tested by direct EM, SPIEM, the indirect double-antibody sandwich ELISA with polyclonal antisera and monoclonal antibodies, and the Pathfinder ELISA kit. The results (Table 1) showed that direct EM and the indirect ELISA reached a level of sensitivity of 10^3 with both samples, while the Pathfinder ELISA reached a level of sensitivity of 10^2. The sensitivity of SPIEM was 10^2 to 10^3 times higher, reaching 10^-6.
prozone phenomenon was observed in the Pathfinder test with both samples. On the basis of results from multiple tests, 68 of the 151 stool specimens tested were determined to be true-negatives by SPIEM, direct EM, and the indirect ELISAs, whereas 83 were found to be true-positives. Of these 83, 77 were determined to be positive by direct EM and the indirect ELISAs and 6 were determined to be positive by SPIEM. Of the 83 positive specimens, only 25 (30.1%) were found to be rich in rotavirus particles by EM (greater than or equal to five particles per grid square), whereas all of the remaining 58 specimens had less than five rotavirus particles per grid square. Of these 58 samples, 22 (36.2%) had less than one rotavirus particle per grid square and 6 (7.2%) were found to have rotavirus particles by SPIEM only. Of these six samples found to be positive by SPIEM only, three had less than one virus particle and three had about one virus particle per grid square (Fig. 1). When assayed with the Pathfinder kit, as many as 82 of the 83 positive samples were positive; the one resulting negative was a false-negative. Thus, the sensitivity of the Pathfinder ELISA was 98.7%. The number of rotavirus particles detected by direct EM or SPIEM did not correlate with the degree of staining in the Pathfinder ELISA (Fig. 1). Of 36 samples found to contain few virus particles by EM (less than or equal to one virus particle per grid square), 22 (61.1%) were strongly reactive (3 to 4+) in the Pathfinder ELISA. On the other hand, 19 of 25 samples (76.0%) found to contain many virus particles by EM (3 to 4+) reacted weakly to moderately (1 to 2+) in the Pathfinder ELISA. In addition, of the six stool samples found to be positive by SPIEM only, two were scored with 1+ staining in the Pathfinder ELISA, one had 2+ staining, and two had 3+ staining; only one could not be detected as positive.

Among the 68 negative samples examined with the kit, 67 were found to be negative for rotavirus antigen; the 1 resulting positive was a false-positive. This specimen was determined to be negative by direct EM, SPIEM, and the indirect ELISAs. Thus, the specificity of the Pathfinder kit was 98.5%. The concordance between the Pathfinder kit and SPIEM was 98.6%. Of the 83 positive samples tested, 75 were subgrouped (5): 64 (78.6%) were found to belong to subgroup II, and 11 (13.7%) were found to belong to subgroup I. Furthermore, 29 of the positive samples were serotyped by SPIEM: 17 (58.6%) belonged to serotype 1, 9 (31.0%) belonged to serotype 2, and 3 (10.4%) belonged to serotype 4. In addition, four serotype 3 and seven serotype 4 cell culture-adapted HRV strains which were not available as stool specimens were tested by the Pathfinder ELISA and correctly identified as rotavirus. Thus, the Pathfinder ELISA detected rotavirus strains regardless of subgroup and serotype. This is not surprising, because the monoclonal antibody used in the kit is specific for the common rotavirus group antigen coded for by the sixth viral genome segment (2).

On the basis of the results reported above, the Pathfinder ELISA kit for the direct detection of rotavirus in stool shows a high degree of specificity and sensitivity (>98.0%). The level of concordance with the results obtained by EM and SPIEM is superior to 98.0%. The Pathfinder ELISA can reliably replace, for the diagnosis of rotavirus gastroenteritis, EM techniques, which are available only in highly specialized virus laboratories. Thus, a simple and rapid method such as the Pathfinder ELISA appears to be highly suitable for the routine diagnosis of HRV infections. In a recent report comparing the sensitivities and specificities of two polyclonal antibody-based commercial enzyme immunoassays, the Pathfinder ELISA, and a latex agglutination assay, it was shown that, while the specificities of the four assays were grossly comparable, ranging from 88 to 98%, the sensitivity of the monoclonal antibody-based assay was greatly superior to those of the other assays (6). In this

![Fig. 1. Correlation between direct EM or SPIEM score (according to Brandt et al. [1]) and degree of staining by the Pathfinder ELISA for 151 stool specimens, of which 6 (○) were found to be positive by SPIEM but not by direct EM.](http://jcm.asm.org/)
report, we showed that the Pathfinder ELISA is as specific as but more sensitive than direct EM.

The lack of correlation in the positive stool specimens between the number of virus particles detected by EM and the degree of staining in the Pathfinder ELISA may have been for different reasons. First, in some samples determined to contain few virus particles by EM, many unassembled or ruptured virions not detectable by EM could be highly reactive in the Pathfinder ELISA. On the other hand, some samples weakly reactive in the Pathfinder ELISA could have a high EM score owing to the presence of many intact double-shelled virus particles. Thus, in these samples the common rotavirus group antigen would not be readily available for binding of the monoclonal antibody used in the Pathfinder ELISA. Finally, in the Pathfinder ELISA some specimens very rich in rotavirus particles could produce prozone effects similar to those shown in Table 1.

High levels of sensitivity in ELISAs for rotavirus detection are highly desirable, not only for diagnosing acute HRV infections but also for examination of stool specimens taken as rectal swabs from immunized children in vaccination field trials. A poorly sensitive ELISA would drastically reduce the number of positive samples and thus probably alter conclusions regarding the effectiveness of rotavirus vaccines. The Pathfinder ELISA, which is rapid, sensitive, and specific, would be particularly useful in the detection of HRV in stool specimens among low-level virus shedders, allowing timely isolation of infected patients and thus prevention of disease transmission.

Although a single lot of the Pathfinder ELISA was evaluated in our study, our conclusions can probably be generalized, since the use of a monoclonal antibody minimizes variations in reagent production among different lots of commercial assays.

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