Comparison of Five Enzyme Immunoassays, Electron Microscopy, and Latex Agglutination for Detection of Rotavirus in Fecal Specimens

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Five different enzyme immunoassays, electron microscopy, and latex agglutination (Slidex; bioMérieux) were compared for the rapid detection of human rotavirus in fecal specimens. The enzyme immunoassay using rotavirus polyclonal antisera (Dakopatts) with simple in-house modifications was shown by the use of confirmatory tests to be the most sensitive and specific procedure.

Human rotavirus is the single most important etiologic agent of acute viral gastroenteritis, particularly in neonates and young children. Reliable diagnosis of human rotavirus infection is essential for patient management, particularly in the context of infection control within hospital wards. In recent years, human rotavirus antigen detection by various enzyme-linked immunoassay (ELA) procedures has become the most widely used approach, supplanting the more labor-intensive electron microscopic detection of virus particles. A number of comparisons between these methods and less widely available procedures, such as radioimmunoassay, solid-phase immune electron microscopy, immunofluorescence, immunoelectro-osmophoresis, dot blot hybridization, and polycrylamide gel electrophoresis, have been reported (1, 3, 5, 7–9). Particular problems encountered with ELA kits include false-positive reactions (2), particularly with neonates (6); lower sensitivity than that of a reference procedure such as solid-phase immune electron microscopy (4); and lack of generally available confirmatory assays. We report here the results of a comparison of seven different assays.

Specimens examined consisted of fecal samples from children with diarrhea or vomiting or both that were submitted for routine diagnosis during 1986 and 1987. Fecal suspensions (10% [wt/vol]) were made in phosphate-buffered saline (PBS) and clarified by centrifugation at 1,000 × g for 10 min in a bench centrifuge. Samples were stored at 4°C overnight or at −70°C for longer periods before testing. For electron microscopy (EM), samples were spun onto carbon-coated Formvar grids in an Autoanalyzer cup (Johns Plastics, Melbourne, Australia) at 20,000 × g for 5 min. These were negatively stained with 3% phosphotungstic acid (pH 6.5) and then examined for 5 min. Tests with Rotazyme II (a solid-phase ELA) were done according to the instructions of the manufacturer (Abbott Laboratories, North Chicago, III.), except that PBS was used instead of water for washing the beads because we have observed more consistent results with this modification in both this assay and other similar bead-based assays. The Dakopatts (Copenhagen, Denmark) ELA was slightly modified from that described by the manufacturer as follows. Microdilution wells were coated with the rabbit anti-rotavirus serum for 1.5 h at 37°C. Fecal samples were then incubated for 2 h at 37°C or overnight at room temperature; when a known rotavirus-positive speci-

men was titrated, the latter incubation conditions gave a slightly higher sensitivity. The diluent buffer used was 5% skim milk in PBS with 0.05% Tween 20 (PBS/T) instead of bovine serum albumin. The Slidex (bioMérieux, Lyon, France) agglutination assay and the other ELA procedures, viz. Wellozyme (Wellcome Diagnostics, Sydney, Australia), ICI (Melbourne, Australia), and Kadaicha (ICI), were used according to the instructions of the manufacturers except that PBS/T was used for washing whenever water was recommended by the manufacturer. For each ELA, we also assumed that each manufacturer’s recommended method for calculating cutoff values has been designed to yield optimum information within the limitations of the positive-negative discriminatory ability of the particular test procedure.

In selected cases, e.g., when two tests gave discordant results, the sample was examined by EM and, if negative by EM, by a confirmatory blocking test. The specimen (10% fecal suspension) was reacted with an equal volume of 1:10 dilution (in PBS) of anti-rotavirus serum (chick) (Wellcome Diagnostics) and then incubated in a water bath at 37°C for 1 h. Another portion of the specimen was also mixed with an equal volume of normal chicken serum and similarly treated. After incubation, the reaction mixtures were tested in the ELA. The specimen was considered positive for rotavirus if the absorbance reading of the specimen treated with antirotavirus serum (chick) was more than 50% lower than that of the normal-chicken-serum-treated specimen. Serotyping of the various positive samples was not performed, and for the purposes of this study we assumed that all the methods examined reacted primarily with the common inner-capsid group antigen of group A rotaviruses. Before the study described here, the blocking test described above demonstrated that all Rotazyme-reactive samples with an optical density reading greater than 20% above the calculated cutoff value could be confirmed as giving true-positive results. Accordingly, the criterion for a confirmed positive Rotazyme reaction was either (i) an optical density reading greater than 20% above the calculated cutoff value or (ii) a positive result in the blocking assay.

Three different panels of 1,052, 94, and 240 fecal suspensions were used to compare EM, the Kadaicha ELA, and the modified Dakopatts ELA with Rotazyme II (Table 1). These results demonstrated (i) that EM was significantly less sensitive than Rotazyme II, detecting only 39% of Rotazyme-
second fecal sample was found to have a titer of 80 in the Wellcozyme EIA and a titer of 320 in the Dakopatts EIA. Thus, the higher yield of confirmed positive results with the Dakopatts EIA when panels of fecal samples were examined was consistent with its higher relative titration sensitivity in comparison with those of the other two methods.

Thus, the assay supplied by Dakopatts, after simple modifications developed in our laboratory, was found to be superior to the other EIA procedures by the following criteria: (i) highest sensitivity, both in yield of confirmed positive results and in sensitivity on titration of a known positive sample; and (ii) high specificity, based on the fact that of 194 Dakopatts EIA-reactive samples examined, 154 were also positive in at least one other assay and the remaining 40 that were negative by other assays were confirmed by the blocking test with the Dakopatts assay.

The use of a comparative absorbance ratio between antibody-coated and normal-serum-coated wells as a routine criterion for sample reactivity in the Dakopatts EIA is likely to assist in maintaining specificity. In contrast, Rotazyme (which does not include a normal-serum-coated bead) gave eight positive results on Dakopatts EIA-negative samples that were considered to be false-positive reactions since they were not blocked by human rotavirus antiserum in the Rotazyme test and since they were negative by EM after concentration by ultracentrifugation. In more-recent experience with a different set of fecal samples from those shown in Tables 1 and 2, 254 samples of 1,968 tested (12.9%) gave positive results in the Dakopatts assay when the criteria described above were used (unpublished results). A further 11 samples (0.56%) gave strong reactions in both antibody-coated and normal-serum-coated wells. When retested after being diluted 1/10 and 1/100, 9 of the 11 samples gave unequivocal positive results based on the difference in optical density between test and control wells; for the remaining two samples showing no such difference, the reactions could not be neutralized in the blocking assay and were considered false-positive reactions.

This study demonstrated significant variation in sensitivity and specificity between various methods for rotavirus detection, including variations in performance between different commercial EIAs when they are used according to manufacturers' instructions. Secondly, the study demonstrated the use of blocking assays to confirm the specificity of positive EIA reactions. On the basis of the data obtained, the Dakopatts EIA method was demonstrably superior to other methods.

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LITERATURE CITED


