Ability of TESTPACK ROTAVIRUS Enzyme Immunoassay To Diagnose Rotavirus Gastroenteritis

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TESTPACK ROTAVIRUS, a simple 10-min enzyme immunoassay, was compared with electron microscopy and Pathfinder enzyme immunoassay on feces from 172 patients of various ages with gastroenteritis. The percent sensitivities and specificities before blocking with antiserum were as follows: TESTPACK, 100% sensitivity and 99% specificity; Pathfinder, 95% sensitivity and 98% specificity. After blocking, the sensitivity and specificity, respectively, were 100% and 100% for TESTPACK and 95% and 99% for Pathfinder. TESTPACK ROTAVIRUS was more sensitive, but not significantly, than Pathfinder (P > 0.1) and the direct electron microscopy technique (P > 0.1).

Rotavirus can be a major cause of gastroenteritis in the very young (4, 17) and in the elderly (8, 14). Because the virus does not grow well in cell culture, a diagnosis is usually made by direct visualization of virus particles by electron microscopy (EM) (3, 5, 6) or by the use of immunological techniques using polyclonal or monoclonal reagents to indicate the presence of viral antigens (2, 9, 11, 18). In recent years, efforts have been made to produce simple and rapid enzyme immunoassays (EIA) (12, 13, 15). This paper reports the accuracy of a new simple, short-incubation, commercially available solid-phase EIA (TESTPACK ROTAVIRUS; Abbott Laboratories, North Chicago, Ill.) to detect rotaviruses in clinical specimens from patients which were found to be positive or negative by direct negative-staining EM. A second commercially available EIA (Pathfinder Rotavirus; Kallestad, Austin, Tex.) which we were accustomed to using in our laboratory was performed and compared. Where discordant results arose in the comparison, a standard blocking test was used as a deciding factor.

Fecal specimens from 172 patients of various ages who were admitted to the hospital in Hamilton, Ontario, Canada, with gastroenteritis from 15 October 1987 to 15 February 1988 were examined in the study. Approximately half of the specimens were frozen and thawed before examination, and this procedure was random for age and virus.

EM was performed as described previously (6). A 10% suspension of feces was made in distilled water. A drop was placed onto the surface of a 400-mesh copper grid previously coated with Formvar. Each specimen was stained with 2% potassium phosphotungstate (pH 7.2) and examined in a Philips 300 EM for a minimum of 10 min. The TESTPACK ROTAVIRUS and Pathfinder Rotavirus assays were performed by the instructions of the manufacturers. In the TESTPACK assay, 0.1 ml of feces was mixed with 0.01 M phosphate-buffered saline in a dilution cup. By using a filter tube to provide a clarified specimen, the microparticles coated with antisera to human rotavirus (produced in guinea pigs) and antirotavirus (mouse monoclonal antibody) conjugated to bovine alkaline phosphate were added. After sitting for 5 min at room temperature, the contents were poured into the center of the TESTPACK reaction disk through a focusing apparatus which was then removed. The reaction disk was then washed with 1.0 M guanidine hydrochloride followed by the addition of the chromogen, a 2-min incubation, and then a final rinse of guanidine hydrochloride. The test was read immediately as a cross or a dash on the pad. The total test took 7 to 10 min, depending on the number of specimens tested. In the Pathfinder EIA, 100 µl of peroxidase-conjugated mouse monoclonal antibody to EDIM rotavirus (9) and 300 µl of 10% stool suspension were added to a plastic tube previously coated with rabbit antirotavirus (SA-11) immunoglobulin. The tube was mixed and then incubated at room temperature for 60 min. After five washings (4 ml each) with deionized water, chromogenic substrate was added, and 15 min of reaction time was allowed before stopping with acid; then, the results were read at 450 nm. Positive and negative controls were included, and cutoff values and gray zones (±10% of the cutoff value) were calculated. Gray-zone specimens were repeated. A positive specimen was greater than or equal to the upper gray-zone cutoff value. The whole test took 1.5 h. A blocking EIA was performed to confirm the presence of rotavirus antigens in discordant specimens. Duplicate samples of fecal extract supernatants were incubated with 1:10 dilutions of nonimmune rabbit serum and of hyperimmune antirotavirus rabbit serum (Behring, Marburg, Federal Republic of Germany). Incubation was at 37°C for 1 h. A sample of each was then put into the respective EIAs. A specimen was confirmed as positive if the result with the hyperimmune serum was reduced by at least 50% of that achieved with the nonimmune serum.

The presence of adenoviruses or enteroviruses was documented by direct EM or tissue culture. Of the specimens, 20 contained adenoviruses (presumably types 40 and 41) which did not grow in conventional cell cultures and another 10 contained enteroviruses which grew well in monkey kidney cell cultures and were also visualized by EM.

The distribution, according to patient age, of viral infections diagnosed by EM or tissue culture is summarized in Table 1. Of specimens submitted from patients less than 2 years of age, 58% (55 of 95) were positive for rotavirus by EM, and this represented 75% (55 of 73) of all of the rotavirus infections in the study. Adenovirus and enterovirus infections were also concentrated in the younger pa-
patients. Specimens without viruses were represented in fairly equal distribution in children and adults.

Table 2 summarizes the characteristics of 6 specimens which exhibited discordant rotavirus results. Patient age did not appear as a factor. The first 4 specimens were missing by the Pathfinder assay, and 3 of the 4 were just weakly positive by TESTPACK. The fifth specimen was missed by EM and recorded minimal positive reactions in the two EIAs. All of these specimens were blocked in the EIAs by the rabbit polyclonal anti-rotavirus serum. The last specimen (6277) was negative by EM and TESTPACK and repeatedly positive in the Pathfinder assay, and it did not exhibit specific blocking.

Discrepancies between results achieved by EIA and those for specimens visualized by EM for rotavirus detection have been reported previously (6, 13). Results for specimens positive by EM and negative by EIA may be due to the use of less than optimal capture antibodies reflected by low avidity, by limited strain specificity, or by the presence of coproantibodies in the stool interfering with the EIA. Results for specimens positive by EIA and negative by EM may be a reflection of EM procedure (3, 11) or of the presence of unrecognizable low-molecular-weight subunits of rotavirus (1). A blocking test such as that used in this study will determine the specificity of the EIA reaction. Continuously positive specimens after blocking would suggest nonspecific binding (false-positive test results) influenced by ingredients in that stool or would suggest the presence of reactive material which may be detected by the use of nucleic acid gels. Insufficient material from specimen 6277 prevented the pursuit of this hypothesis.

Table 3 presents the calculated sensitivities and specificities of the two EIAs after setting EM as the standard (standard A) and then combining EM with the results of the blocking test to set the standard (standard B). In the first calculation, TESTPACK was 100% sensitive and 99% specific. With the corrected standard, the sensitivity remained the same and the specificity reached 100%.

To our knowledge, this is the first report of the performance of TESTPACK ROTAVIRUS compared with that of direct EM. Future studies will allow additional comparisons. The Pathfinder results were also impressive, at a sensitivity of 94.6% and a specificity of 99.0% by standard B. Two other evaluations have appeared comparing the Pathfinder EIA with EM. Kniessley et al. (13) showed Pathfinder to have a sensitivity and specificity of 95% when compared with the pseudoreplica EM technique on stools from children with diarrhea. Gerna et al. (11) compared the Pathfinder assay with immune and direct EM and found that the sensitivity and specificity of Pathfinder were 98%.

Several commercial companies are in the process of introducing simplified technology for the diagnosis of infectious diseases. TESTPACK was introduced as a kit for the diagnosis of group A streptococcus infections. On laboratory strains or clinical specimens (10, 16), the test demonstrated a sensitivity of 90% and a specificity of 97 to 100% compared with culture. The procedure in that kit began with a 5-min nitrous acid carbohydrate extraction step which was then neutralized. The extracted fluid was then layered onto the reaction disk before adding an anti-streptococcus A enzyme conjugate. The first filter was then removed before adding the chromogen and reading the test. TESTPACK for rotavirus detection is more simplified. The capture and detector reagents in the assay are added together with the specimen in a tube, and this mixture is layered onto the pad before adding the chromogen. The same plus-minus result format is present.

Laboratories which have access to EM facilities will continue to examine stool specimens by EM because it is a universal method for determining viral etiology (3, 7). However, for laboratories without EM facilities or during outbreaks of rotavirus when the numbers of specimens to be examined by EM are unmanageable, these accurate rapid assays can be used for the diagnosis of rotavirus infection, allowing appropriate isolation of patients on hospital wards and reduction of nosocomial spread of the disease.

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