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Evaluation of the ImmunoCardSTAT! Rotavirus Assay for Detection of Group A Rotavirus in Fecal Specimens

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Rapid detection of group A rotavirus was performed by using ImmunoCardStat! Rotavirus (ICS-RV) (which uses immunogold-based, horizontal-flow membrane technology), two commercial enzyme immunoassays (Premier Rotaclone and TestPack Rotavirus), and electron microscopy. A total of 249 stool specimens collected from children with gastroenteritis between February and April 1997 were tested. After resolution of 19 of the 22 discordant results by reverse transcription-PCR for group A rotavirus, ICS-RV detected 125 positives while Rotaclone and TestPack detected 127 and 129 positives, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value were 94.0, 100, 100, and 93.4% for ICS-RV; 95.5, 100, 100, and 95.0% for Rotaclone; and 97.0, 96.5, 97.0, and 96.5% for TestPack. ICS-RV was sensitive and specific and was relatively simple to perform and interpret.

Group A rotavirus is a major cause of gastroenteritis in children throughout the world (2, 3, 14, 16). In addition, rotavirus is a common nosocomial infection on wards for young children (6, 17) and is a problem in the day care setting (1, 15). The accurate diagnosis of a rotavirus infection is important not only for the rapid identification of the patient with rotavirus gastroenteritis but also for the identification of infected individuals who are potential sources of infection to others.

Human rotaviruses are difficult to cultivate in commonly used cell culture systems (20); therefore, other methods of rotavirus identification have been developed. Originally, electron microscopy was used (18); however, in recent years immunoassays have become the standard method for the detection of group A rotavirus in stool specimens. Commercial immunoassay kits for detecting rotavirus are widely used by clinical laboratories (5, 10, 18, 19).

This study was undertaken to evaluate the performance of the ImmunoCard STAT! Rotavirus assay (Meridian Diagnostics, Cincinnati, Ohio), a novel system for the rapid detection of group A rotavirus using immunogold-based, horizontal-flow membrane technology. ImmunoCardSTAT! Rotavirus was compared with two widely used commercial enzyme immunoassays (EIAs), Premier Rotaclone (Meridian Diagnostics) and TestPack Rotavirus (Abbott Laboratories, Abbott Park, Ill.), with confirmation of results by electron microscopy.

MATERIALS AND METHODS

Patient population. Three clinical trial sites were included in this study. Stool specimens from children (ages 2 weeks to 15 years) with acute gastroenteritis were submitted to the Pediatric Gastroenteritis Research Laboratory at Rhode Island Hospital, Providence (n = 80), the Microbiology/Virology Laboratory of the Children's Hospital Medical Center, Cincinnati, Ohio (n = 80), and the Clinical Laboratory of the Children's Hospital, San Diego, California (n = 90), from February to April 1997 for rotavirus testing. A total of 250 fecal specimens were evaluated by all three assays, and 249 of those underwent electron microscopic evaluation. Swab specimens were excluded from the initial analysis. Stools were stored undiluted at 4°C until tested. For evaluation, stools were mixed to distribute virus throughout the specimens before being aliquoted and diluted for testing. After testing, the remaining stool was frozen at −20°C for retesting, if necessary.

Duplicate specimens, stool and a stool swab, were taken from 12 patients at Rhode Island Hospital to evaluate the performance of the ImmunoCardSTAT! Rotavirus assay concurrently with both types of specimens.

To determine whether ImmunoCardSTAT! Rotavirus would detect all rotavirus strains commonly circulating in the United States, representative patient strains were tested. Previously frozen stool samples with rotavirus G serotypes 1 through 4, ascertained by either EIA or reverse transcription (RT)-PCR serotyping assays, were selected for testing. These samples were retested for rotavirus integrity by using the Premier Rotaclone and were then tested by the ImmunoCardSTAT! Rotavirus assay.

ImmunoCardSTAT! Rotavirus. The ImmunoCardSTAT! Rotavirus assay uses immunogold-based technology in a horizontal-flow membrane to detect rotavirus. The stool specimen is diluted 1 to 15 in sample diluent supplied by the manufacturer. The suspension is vortexed and 150 μl is added to the bottom port of the device. The sample mixes with gold particles coated with antirotavirus monoclonal antibody and migrates along the nitrocellulose membrane through the capture antibody area and the control (goat anti-mouse antibody) area over a 10-min period at room temperature. After 10 min the test and control areas are observed for the presence of a red-purple line across the membrane surface. The control line serves as a procedural control to ensure that the sample has migrated the appropriate distance along the membrane. The test line contains antirotavirus polyclonal antibody (capture antibody). If rotavirus antigen is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-gold conjugate which can be seen as a red-purple line in the test area. The absence of a red-purple line in the test area indicates a negative result.

Stool swabs were prepared for testing by the ImmunoCardSTAT! Rotavirus assay by adding 700 μl of diluent to a test tube. The swab was placed into the diluent and allowed to soak for 5 min followed by vortexing for 10 s. The swab was then removed from the tube and 150 μl of the remaining solution was tested according to the above-described protocol.

Premier Rotaclone. The Rotaclone assay uses murine monoclonal antirotavirus antibody directed against VP6, the group-specific antigen for all group A human rotaviruses, as the solid phase (plastic microtiter wells are coated with the antibody), with the same monoclonal antibody conjugated to horseradish peroxidase as the detector antibody. The assay was run according to the manufacturer's directions. Results were read spectrophotometrically. Specimens with an absorbance at 450 nm (A_{450}) greater than 0.150 were considered positive as directed in the package insert.

TestPack Rotavirus. The TestPack Rotavirus assay is an EIA which uses guinea pig polyclonal antirotavirus antibody-coated latex particles as a solid-phase immunosorbent and both murine monoclonal and bovine polyclonal antirotavirus antibodies conjugated to alkaline phosphatase as detector antibodies. The assay was run according to the manufacturer's directions and results were read visually.

Electron microscopy. Specimens were evaluated by electron microscopy at the Providence and San Diego clinical sites. Frozen specimens from the Cincinnati

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TABLE 1. RT-PCR on specimens with discordant results from electron microscopy and rotavirus assays

Sample no.	Site[a]	PCR result	EM result[b]	Immuno-CardSTAT! result	Rotaclone result (A_{450})	TestPak result[c]
1-38	RI	+	Few	−	−	1+
1-68	RI	+	Few	−	−	1+
2-8	CN	+	Numerous	−	−	−
1-57	RI	+	Few	−	−	−
1-06	RI	+	Few	−	−	−
1-14	RI	+	Few	−	−	−
2-29	CN	+	−	+	3.383	4+
2-49	CN	+	−	+	3.392	4+
2-54	CN	+	−	+	3.353	4+
2-59	CN	+	−	+	3.347	4+
2-71	CN	+	−	+	3.312	4+
1-41	RI	+	−	−	0.287	1+
2-37	CN	+	−	−	0.152	1+
1-40	RI	−	Few	−	−	−
1-43	RI	−	Few	−	−	−
1-47	RI	−	Few	−	−	−
3-14	SD	−	−	−	−	4+
3-28	SD	−	−	−	−	4+
2-10	CN	−	−	−	−	1+

[a] RI, Rhode Island; CN, Cincinnati, Ohio; SD, San Diego, Calif.
[b] EM, electron microscopy. Positive results are expressed as amounts of particles.
[c] Positive specimens were scored as follows: 1+, weak; 4+, strong.

site were sent to San Diego for masked electron microscopic analysis. In all cases, electron microscopy technologists were unaware of laboratory results. Electron microscopy was carried out as described by Dennehy et al. (4). Grids were examined for virus at a magnification of ×52,000 for approximately 5 min. Photomicrographs were taken of all specimens where rotavirus was suspected and of any suspected viral particle in a specimen. Photomicrographs of specimens where the suspected viral particle was not unequivocally identified by the electron microscopy technologist at the initial reading were reviewed by a single experienced observer who determined the final reading of the specimen.

RT-PCR for group A rotavirus. Specimens whose results were discordant in the other assays were evaluated for group A rotavirus by RT-PCR. This procedure amplifies a 202-bp segment of the rotavirus VP4 gene (8a, 9).

Rotavirus double-stranded RNA was extracted from stool specimens with the RNeasy total RNA kit (Qiagen, Inc., Santa Clarita, Calif.). The manufacturer's protocol for isolation of total RNA from eukaryotic cells and tissues was followed. Samples were eluted in diethyl pyrocarbonate-treated double-distilled water and stored at −70°C.

Rotavirus RNA was amplified in a one-step process as described by Gouvea et al. (11). Samples were analyzed on a 1.6% agarose gel in TBE (90 mM Tris-borate, 2 mM EDTA [pH 8.0]). The gel was stained with 0.5% ethidium bromide. A sample was considered positive if a band was present at 202 bp.

To determine the sensitivity, specificity, and predictive values of both assays, each stool specimen was classified as either positive or negative. Specimens with a positive or negative result by electron microscopy and a positive result by RT-PCR were considered to be positive for rotavirus. Negatives were specimens with a negative or positive result by electron microscopy and a negative result by RT-PCR. Statistical comparisons of the assay results to electron microscopy results corrected by RT-PCR were done according to the method of Galen and Gambino (7, 8). Confidence intervals (95%) for performance statistics were calculated as recommended by Ilstrup (12).

RESULTS

Of the 249 stool samples from children with gastroenteritis tested by all three assays and electron microscopy, 120 were positive by all tests while 108 were negative. Nineteen of the 21 stool specimens which had discordant results by the three assays and electron microscopy were available for further testing for group A rotavirus by RT-PCR (Table 1). For nine specimens electron microscopy and RT-PCR results were in agreement. Seven RT-PCR-positive specimens were negative by electron microscopy; all were positive in at least two of the three assays. Three specimens did not contain group A rotavirus as determined by RT-PCR but were positive by electron microscopy. These specimens were negative by all other assays. Results for the 246 stool specimens of the three assays, compared with electron microscopy and after resolution of discordant results by RT-PCR, are shown in Table 2.

ImmunoCardSTAT! Rotavirus was run on matched stool swab and whole fecal samples obtained from 12 patients. ImmunoCardSTAT! Rotavirus results for swab specimens were in complete agreement with the assay results for the eight positive and four negative fecal specimens. The intensities of the positive reaction were identical for swab and whole fecal pairs in two cases; the positive reactions were less intense for the swab specimen in the remaining six cases.

ImmunoCardSTAT! Rotavirus detected all group A rotavirus strains commonly circulating in the United States. Fifteen previously frozen stool samples with G serotypes 1 through 4 were tested, and all samples were positive for rotavirus with both Rotaclone and ImmunoCardSTAT! Rotavirus assays.

DISCUSSION

The results of this study indicate that ImmunoCardSTAT! Rotavirus is as sensitive and specific as two widely used commercial EIAs for detection of group A rotavirus. Rotaclone has been found to be sensitive and specific compared with electron microscopy and with other commercially available immunoassays in several prior studies (4, 5).

The combination of electron microscopy plus RT-PCR to evaluate specimens with discordant results was chosen as the method to determine positives and negatives in order to improve sensitivity and to avoid identifying a non-group A rotavirus seen on electron microscopy as a positive. At the Rhode Island site group C rotaviruses have routinely been found during rotavirus outbreaks (13). Three specimens which were negative by all assays and RT-PCR and positive by electron microscopy were seen in Rhode Island, suggesting that a non-group A rotavirus was present. In addition, RT-PCR was used

TABLE 2. Comparison of results of ImmunoCard STAT! Rotavirus, Rotaclone, and TestPack Rotavirus after resolution of discordant results by RT-PCR

Test	No. of:				Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Diagnostic accuracy (%)
	True positives[a]	True negatives[b]	False positives	False negatives					
ImmunoCard STAT! Rotavirus	125	113	0	8	94.0	100	100	93.4	96.7
Rotaclone	127	113	0	6	95.5	100	100	95.0	97.6
TestPack Rotavirus	129	109	4	4	97.0	96.5	97.0	96.5	96.7

[a] True positives are specimens either positive or negative by electron microscopy and positive by PCR.
[b] True negatives are specimens either negative or positive by electron microscopy and negative by PCR.

to evaluate specimens with discordant results since electron microscopy was found to be less sensitive than Rotaclone in a previous study at the Rhode Island site (4).

Of the 19 specimens with discordant results in the three assays or electron microscopy, 13 were positive by RT-PCR and 8 of these were ImmunoCardSTAT! Rotavirus negative (Table 1). Of these eight specimens, four were TestPack Rotavirus positive. The low intensity of the TestPack Rotavirus assay results in the positive specimens indicates that a small amount of antigen was present in these specimens. In three specimens negative by ImmunoCardSTAT! Rotavirus but positive by electron microscopy, only a few viral particles were present. These results suggest that ImmunoCardSTAT! Rotavirus is capable of detecting rotavirus except in a few specimens where only a small amount of rotavirus is present.

Three specimens contained adenovirus as determined by electron microscopy; in two of the three rotavirus was found as well. ImmunoCardSTAT! does not react differently with stool specimens positive for viruses other than rotavirus. The manufacturer conducted extensive preclinical testing on ImmunoCardSTAT! with rotavirus-negative and -positive stool specimens which had been "spiked" with the following viruses: adenovirus types 2, 40, and 41; coronavirus; coxsackievirus types A9, B1, and B6; echovirus types 22 and 32; enterovirus type 69; and poliovirus type 1 (16a). No interactions were seen.

Other factors related to test performance, such as speed, expense, and simplicity, also need to be evaluated. The times required to test 10 specimens were 22 min for ImmunoCardSTAT! Rotavirus, 1 h 55 min for Rotaclone, and 53 min for TestPack Rotavirus. The estimated hands-on times for 10 specimens were 12 min for ImmunoCardSTAT! Rotavirus, 15 min for Rotaclone, and 18.5 min for TestPack Rotavirus.

In addition to producing accurate results, ImmunoCardSTAT! Rotavirus was easy to use. Little technical expertise was needed to perform the assay since it requires only the preparation and application of the diluted specimen to the device, and no additional washes or additions of reagents are needed. Rotaclone requires manual washes and reagent additions, and multiple reagents must be added to the Testpack Rotavirus device during testing.

ImmunoCardSTAT! Rotavirus was convenient to use since the test kit and reagents can be kept at room temperature, while TestPack Rotavirus and Rotaclone both require refrigeration. Kit components for TestPack Rotavirus and Rotaclone need to be brought to room temperature prior to use, adding time from receipt of the specimen to obtaining the final result of the assay.

Rotaclone was able to test larger volume of specimens than either ImmunoCardSTAT! Rotavirus or TestPack Rotavirus. While one Rotaclone kit can test 46 specimens plus 2 controls, the TestPack Rotavirus and ImmunoCardSTAT! Rotavirus assays are limited by the need to manually add the specimen and/or reagents to each individual test device, which limits the number of specimens per run. The Rotaclone assay has breakaway strips of eight wells which allow fewer than 46 tests to be run at one time. The ImmunoCardSTAT! Rotavirus and TestPack Rotavirus assays are run in individual devices and thus can be run on any number of specimens.

The choice of rotavirus assay will depend to a great extent on the requirements of the individual laboratory. Each laboratory must consider its needs on the basis of patient population, pricing, and technical help to determine what is best for its specific environment.

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