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Direct Appraisal of Latex Agglutination Testing, a Convenient Alternative to Enzyme Immunoassay for the Detection of Rotavirus in Childhood Gastroenteritis, by Comparison of Two Enzyme Immunoassays and Two Latex Tests

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During February and March 1984, 207 fecal samples from infants and children with gastroenteritis were tested for rotavirus with four techniques: two enzyme immunoassays (Rotazyme; Abbott Laboratories, North Chicago, Ill., and Enzygnost-Rotavirus; Calbiochem-Behring, La Jolla, Calif.) and two latex agglutination tests (Rotalex; Orion Research, Inc., Cambridge, Mass., and Slidex Rota-Kit; Biomérieux). All stool samples were also tested for yeasts and bacterial pathogens. Electron microscopy was used to investigate discrepant results. We found 47% positive samples with Enzygnost-Rotavirus, 38% with Rotazyme, 37% with Slidex Rota-Kit, and 34% with Rotalex. No specimen was found positive by Rotazyme only or Slidex Rota-Kit only. On the contrary, 12 samples which were positive with Enzygnost-Rotavirus only and 3 which were positive with Rotalex only were not confirmed as positive by electron microscopy. Both enzyme immunoassays gave 6% equivocal results; Slidex Rota-Kit gave significantly fewer equivocal results than did Rotalex: 2.9% versus 9.7% ($P < 0.01$). The sensitivity and specificity of latex tests compared favorably with that of enzyme immunoassays. Latex agglutination tests can be performed by unskilled personnel and are rapid and relatively cheap. They appear to be very suitable for routine laboratory work and may prove useful for large-scale screening in developing countries.

In 1973, Bishop et al. (3) reported the presence of a virus which they described as an orbivirus in duodenal biopsy specimens from children with gastroenteritis. Since then, human rotavirus (HRV) has been recognized as the major etiological agent of diarrhea in infants and young children, with peak infection occurring in winter months in temperate climates (4, 13). The highest rate of infection with HRV is observed in infants between 6 and 24 months of age (14, 18). In France, Aymard (2) reported HRV infection to account for 23.1% of childhood gastroenteritis.

Although HRV has been successfully grown in cell cultures, routine isolation is not performed (6, 12, 19). Electron microscopy (EM) and immune EM were for some time the only methods available to provide a positive diagnosis of rotavirus infection (9). Numerous assays were later developed for detecting HRV in stool samples (13); radioimmunoassay and enzyme immunoassay (EIA) are generally recommended for routine diagnosis. However, these techniques can only be carried out in microbiological laboratories with highly trained personnel. They are expensive and ill-suited for small numbers of samples. Latex agglutination tests have been recently introduced that can be performed by nonspecialized hospital laboratories. Latex agglutination is simple and can provide to the clinician a rapid etiological diagnosis. However, some questions have been raised as to the sensitivity and specificity of these tests.

In the present work, we compared the results obtained in 207 stool samples from infants and children with gastroenteritis, using two EIAs and two latex agglutination tests.

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MATERIALS AND METHODS

Clinical specimens. Two hundred and seven stool samples were collected from children (newborn through 15 years of age) with diarrhea at the Centre Gatien de Clocheville, Tours, France, during February and March 1984. Each stool sample was processed and frozen at −20°C until assayed. The specimens were subjected to only one thawing. All stool samples were tested with four techniques to detect HRV. Two commercially available EIAs (Rotazyme; Abbott Laboratories, North Chicago, Ill., and Enzygnost-Rotavirus; Calbiochem-Behring, La Jolla, Calif.) and two latex agglutination tests, one commercially available (Rotalex; Orion Research Inc., Cambridge, Mass.) and one under development (Slidex Rota-Kit; Biomérieux), were each performed as recommended by the manufacturer. All specimens were also tested for yeasts and bacterial pathogens, including *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species and toxigenic *Escherichia coli* and *Staphylococcus aureus*. EM was used only to investigate discrepant results.

EIAs. An outline of the two test procedures is presented in Table 1. Results were considered equivocal when stool supernatant fluids had an absorbance value within the "gray zone." Samples giving equivocal results were considered negative for the analysis of specificity and sensitivity.

Latex agglutination tests. An outline of the two test procedures is presented in Table 2. Results were considered equivocal when stool supernatant fluids agglutinated both reactive and control latex. Samples giving equivocal results were considered negative for the analysis of specificity and sensitivity.

EM. This was carried out as described by Drucker et al. (8). Specimens were diluted to 20% in 0.01 M phosphate-buffered saline (pH 7.3) with 1% Tween 80. They were

TABLE 1. EIA procedures[a]

Condition	Procedure with:	
	Rotazyme	Enzygnost-Rotavirus
Preparation of specimens	Dilute stools to ca. 10% with the sample diluent provided in the kit Mix with vortex apparatus Centrifuge at 2,000 × *g* for 10 min Use supernatants for the test	Dilute stools to ca. 20% with the dilution buffer provided in the kit Mix with vortex apparatus Centrifuge at 2,000 × *g* for 10 min Use supernatants for the test
Solid phase	Beads precoated with a guinea pig antiserum to simian rotavirus SA-11	Microplate wells precoated with rabbit antiserum to simian rotavirus
Positive control	Inactivated SA-11 (10^8 viral particles/ml)	Inactivated NCDV
Negative control	Sample diluent Rotazyme	Negative feces control must be selected by the user
Test procedure	Incubate the bead for 3 ± 0.5 h at 45°C with 200 μl of stool supernatant Wash the bead four times with 1 ml of water Incubate for 1 h at 45°C with 200 μl of rabbit anti-rotavirus serum conjugated to horseradish peroxidase Wash again six times Transfer beads to fresh tubes Incubate for 15 min at room temperature in 200 μl of orthophenylene diamine-2 HCl substrate Stop the reaction by adding 1 ml of 1 N HCl Read at 492 nm	Add in a well 150 μl of stool supernatant fluid and incubate for 2 h at 37°C Wash the well three times with 200 μl of the provided washing solution Incubate for 1 h at 37°C with 50 μl of calf antiserum to NCDV conjugated to alkaline phosphatase Wash again three times Incubate for 45 min at room temperature with 100 μl of paranitrophenyl-phosphate substrate Stop the reaction by adding 50 μl of 2 N NaOH Read at 405 nm
Determination of cutoff value	NC OD < 0.070 PC OD > 0.150 Cutoff = NC + 0.075 Samples with absorbance values within ± 25% of the cutoff value are considered suspect and noted GZ	NC OD < 0.2 PC OD > cutoff Cutoff = NC + 0.1 Pitfall. Since there was no recommendation from the manufacturer for the choice of the negative control, we established a cutoff value for each run of the test by the following procedure: For a microplate (96 samples), we calculated the mean (m) of OD under 0.2 Positive specimen: OD ≥ (m + 25%) + 0.1 Negative specimen: OD ≤ (m − 25%) + 0.1 Samples with absorbance values within this range were considered suspect and noted GZ

[a] Abbreviations: NC, negative control; OD, optical density; PC, positive control; GZ, gray zone; and NCDV, Nebraska calf diarrhea rotavirus.

mixed with a vortex apparatus and clarified by low-speed centrifugation for 10 min at 2,000 × *g* and 30 min at 12,500 × *g*. Supernatant fluids were dropped on carbon-coated 400-mesh copper grids and adsorbed for 30 s. Grids were negatively stained with 1% uranyl acetate. Specimens were examined with a JEOL 100 B electron microscope at a ×50,000 magnification. A specimen was considered positive or negative after the examination of five acceptable squares and at least 15 min of observation time.

RESULTS

Overall results. Detailed results are shown in Table 3. Concordant data with the four tests were obtained in 151 stool samples (73%): 66 were positive and 85 were negative. Forty-four samples (21%) yielded equivocal results with at least one of the tests; 2 samples yielded equivocal results with both latex tests, and one yielded equivocal results with both EIAs.

Of 207 stools, 101 were found positive with at least one of the methods. The relative sensitivity of Rotazyme was 77% (78 of 101) versus 97% (98 of 101) for Enzygnost-Rotavirus, 70% (71 of 101) for Rotalex, and 76% (77 of 101) for Slidex Rota-Kit (Table 4).

Analysis of discrepancies. Only one stool sample was positive with both EIAs and negative with both latex tests; it was confirmed positive by EM. No sample was positive with both latex tests but negative with both EIAs. No specimen was found to be positive by Rotazyme only or by Slidex Rota-Kit only. Eighteen samples were positive with Enzygnost-Rotavirus only; in one of these, the presence of HRV was confirmed by EM examination. Three samples were positive with Rotalex only.

Coinfections. Bacterial pathogens were found in 14 stool samples: 10 *Salmonella* spp., 3 *Yersinia enterocolitica*, and 1 *Campylobacter* sp. One stool sample contained yeasts. Three of these samples were also positive for HRV by at least one technique.

DISCUSSION

EM has long been the standard method for detecting rotavirus in stool samples. However, many factors affect the accuracy of this technique, such as viral concentration, adsorbing properties of the grids, observation time, and

operator skill. EM is long and fastidious, requires expensive equipment, and is ill-suited for large series of samples. However, owing to the characteristic morphology of HRV, EM is highly specific.

More handy and less expensive tests such as EIA have been introduced in hospital laboratories during the past few years. Both EIAs used in the present study have been found to be very specific (7, 15, 16) and at least as sensitive as EM. The standard procedure for Enzygnost-Rotavirus has no zone of uncertainty (gray zone) and thus does not yield equivocal results. Owing to the relatively high variation of results of intra- and interassays (variation coefficient of up to 25%), the recommendations of the manufacturer appeared to us to be overly optimistic. As a safeguard for better accuracy, we introduced a gray zone into which 6% of the samples fell. The same percentage of equivocal results was observed with Rotazyme.

Rotazyme and Enzygnost-Rotavirus require at least 4 h to be completed. They are designed for large numbers of samples, and costwise it is difficult with an average of 100 to 200 stool samples a month (our practice) to run more than one test per week. Since rotavirus infection is usually of short duration (8), laboratory results will very often reach the clinician too late to be useful. Latex agglutination tests have several advantages over EIAs. They are less expensive, can be performed within 30 min, and do not require major laboratory equipment. Several studies have pointed out that latex tests can be used reliably for rotavirus infection diagnosis (1, 5, 10, 15, 17). We assayed two latex agglutination tests kits that were very similar in their principle.

Concordance and discordance between latex tests and EIAs were not significantly different from what was observed between EIAs. Equivocal results, when the stool samples agglutinated the control latex, have been noted with variable frequencies by several authors (1, 10, 11, 15). They usually amounted to ca. 5% of samples. In the present study, Slidex Rota-Kit yielded significantly fewer nonspecific reactions than did Rotalex: 2.9% versus 9.7% ($P < 0.01$). According to the Rotalex manufacturer, nonspecific results

TABLE 2. Latex agglutination tests procedures

Condition	Procedure with:	
	Rotalex	Slidex Rota-Kit
Preparation of specimens	Dilute stools to ca. 10% with buffer provided in the kit Let stand 30 min at room temperature and mix again Centrifuge at 1,200 × *g* for 20 min Use supernatant for the test	Dilute stools to ca. 10 to 20% with buffer provided in the kit Let stand 5 to 10 min at room temperature and mix again Centrifuge at 800 × *g* for 10 min Use supernatant for the test
Reactive latex	Latex particles precoated with rabbit anti-NCDV[a] antibodies	Latex particles precoated with rabbit antiserum raised again a strain of rotavirus of bovine origin adapted to cell culture by the Institut Merieux
Positive control	Suspension of purified rotavirus	Suspension of purified rotavirus
Control latex	Latex particles coated with nonimmune rabbit immunoglobulin	Latex particles coated with nonimmune rabbit immunoglobulin
Test procedure	Mix carefully on a glass slide 50 μl of supernatant with one drop of reactive latex or control latex Read the agglutination within 2 min	As Rotalex, but read within 2 to 3 min
Interpretation	The test is positive if a distinct agglutination is observed, provided the control latex remained milky The result is equivocal if the control latex is agglutinated	As Rotalex

[a] NCDV, Nebraska calf diarrhea rotavirus.

TABLE 3. Application of two EIAs (Rotazyme and Enzygnost-Rotavirus) and two latex agglutination tests (Rotalex and Slidex Rota-Kit) to the detection of HRV in 207 stool samples from children with gastroenteritis

No. of specimens	Reaction with:				
	Rotazyme	Enzygnost-Rotavirus	Rotalex	Slidex Rota-Kit	EM[a]
66	+	+	+	+	
1	+	+	+	E[b]	
9	+	+	E	+	
1	+	+	E	E	
1	+	+	−	−	1 POS
1	GZ[c]	+	+	+	
1	GZ	+	E	+	
1	GZ	+	E	−	1 POS
1	GZ	+	−	E	1 NEG
4	GZ	+	−	−	4 NEG
1	GZ	GZ	−	−	
4	GZ	−	−	−	
9	−	+	−	−	9 NEG
3	−	+	E	−	3 NEG
85	−	−	−	−	
1	−	−	−	E	
4	−	−	C	−	
1	−	−	E	E	
2	−	−	+	−	2 NEG
9	−	GZ	−	−	
1	−	GZ	−	E	
1	−	GZ	+	−	1 NEG

[a] EM, Electron microscopy: positive (POS) and negative (NEG).
[b] E, Equivocal result (agglutination of the control latex).
[c] GZ, Gray zone (see the text for definition).

TABLE 4. Applicaton of two EIAs (Rotazyme and Enzygnost-Rotavirus) and two latex agglutination tests (Rotalex and Slidex Rota-Kit) to the detection of HRV in 207 stool samples from children with gastroenteritis

Test	No. (%) of positive stools	No. (%) of equivocal results
Rotazyme	78 (37.7)	13 (6.3)
Enzygnost-Rotavirus	98 (47.3)	12 (5.8)
Rotalex	71 (34.3)	20 (9.7)
Slidex Rota-Kit	77 (37.2)	6 (2.9)

TABLE 5. Sensitivity and specificity of Rotazyme, Rotalex, and Slidex Rota-Kit as compared with Enzygnost-Rotavirus[a]

Test	% Sensitivity	% Specificity
Rotazyme	79.6 (70–87)	100 (97–100)
Rotalex	69.4 (59–78)	97.2 (94–100)
Slidex Rota-Kit	78.6 (69–86)	100 (97–100)

[a] 95% confidence intervals are shown in parentheses.

can occur with protein A of *S. aureus*, haptoglobin, fibronectin, and fibers present in stool supernatant fluids (personal communication). Sixty-seven samples (32%) were positive with both latex and confirmed positive by at least one EIA. One hundred and thirteen stool samples (55%) were found to be negative with both latex tests; 85 of them (75%) were true negatives confirmed by both EIAs; 1 (0.5%) was found to be positive with EIAs and EM; 27 others (13%) gave discordant results with the two EIAs. Three samples (1.5%) which were positive with Rotalex but negative with Slidex Rota-Kit were confirmed negative by EIAs and EM and can be considered as false-positives with the Rotalex kit. Such false-positive reactions with Rotalex have already been reported (1, 10, 11).

Enzygnost-Rotavirus yielded 18 (9%) positive reactions that were not confirmed by other tests. Six of the stool samples were in the gray zone of Rotazyme but gave negative or equivocal results with both latex tests; the other 12 were negative by Rotazyme and latex tests. It was unclear whether such results reflected a greater sensitivity of Enzygnost-Rotavirus or nonspecific reactions. One of the samples was found to be positive by EM; 17 others were negative despite lengthy and careful observation. Similar results have been recently reported by Morinet et al. (15). Greater relative sensitivity of Enzygnost-Rotavirus as confirmed by a specific blocking assay was thought to be related to reactions with an antigenic subunit of HRV inner capside that was present in large amounts in stool samples from infected children. Taking Enzygnost-Rotavirus as a standard for the presence of rotavirus in stool samples, we calculated the sensitivity and specificity of the three other tests (Table 5). It appeared that, although they were less sensitive than Enzygnost-Rotavirus, Rotazyme and Slidex Rota-Kit had comparable performances. Rotalex sensitivity fell below 70%, partly due to the number of equivocal results observed with this test.

In conclusion, both latex kits compared favorably with EIAs. A slight advantage could be assigned to Slidex Rota-Kit which gave fewer equivocal and false-positive reactions. Both kits were found to be very suitable for the rapid diagnosis of rotavirus gastroenteritis in children. The rapidity of the latex agglutination technique is its main attribute. Now that the sensitivity and specificity of these tests approach that of more sophisticated EIAs, they represent a very convenient alternative for routine laboratory use. They may also prove to be very useful for large-scale infield screening of acute diarrhea in developing countries.

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