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Comparison of Nine Commercial Immunoassays for the Detection of Rotavirus in Fecal Specimens

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One hundred fecal specimens obtained from patients with acute gastroenteritis were tested for rotavirus with nine commercial immunoassays to evaluate the sensitivity, specificity, predictive value, and diagnostic accuracy of these assays. Kits evaluated included two monoclonal antibody-based enzyme immunoassays (EIAs) (Rotaclone and Pathfinder Rotavirus), three polyclonal antibody-based EIAs (Rotavirus Immunoassay, Rotazyme II, and Wellcozyme Rotavirus), and four latex agglutination assays (Rotastat, Virogen Rotatest, Meritec-Rotavirus, and The Wellcome Latex Test). Thirty-eight of the 100 specimens were found to contain rotavirus by a reference microplate EIA. The accuracy of the reference assay was determined by RNA electrophoresis and a blocking assay on discordant specimens. The two monoclonal antibody EIAs had superior sensitivities (100%) and identified two positive specimens which were negative by the reference method but positive by the blocking assay. Among the polyclonal EIAs, all had sensitivities of greater than 90%, but specificities were variable; Rotazyme II, with a specificity of 50%, showed considerable discrepancy from other polyclonal EIAs. The latex tests had sensitivities ranging from 70 to 90% and specificities of 80 to 100%. Latex agglutination tests were more rapid than EIAs and did not require expensive equipment. The final choice of assay system will depend on the cost, speed, and accuracy requirements of the clinical laboratory.

Rotavirus is a major cause of gastroenteritis in children throughout the world (4, 15). In addition, rotavirus is a common nosocomial infection on wards for young children (7, 31, 33) and is a problem in the day-care setting (16). Recently outbreaks of rotavirus infection have also been identified in elderly patients (13, 20). The accurate diagnosis of rotavirus infections is important not only for the rapid diagnosis of infection in patients with 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 (37, 39); therefore other methods of rotavirus identification have been developed. Originally, electron microscopy was used (9), but recently many different techniques, including radioimmunoassay (22), enzyme immunoassay (EIA) (40), immune adherence hemagglutination (21), reversed passive hemagglutination (35), staphylococcal coagglutination (38), latex agglutination (36), agglutination of antibody-coated erythrocytes (2), counterimmunoelectrophoresis (23), complement fixation (24), immunofluorescence in cell culture (1), fluorescent virus precipitation (29), and polyacrylamide gels to detect rotaviral nucleic acid (14), have been employed for the detection of rotaviruses in stool samples.

Immunoassays have become the standard method for the detection of rotavirus in stool specimens. Commercial immunoassay kits for detecting rotavirus are now available and include monoclonal antibody-based EIAs, polyclonal antibody-based EIAs, and latex agglutination assays (30). This study was undertaken to determine the sensitivity, specificity, and predictive values of nine commercial assays with a group of stool specimens representative of stools likely to be submitted to a diagnostic facility for rotavirus testing and to compare results obtained with each type of assay as well as results obtained with assays of similar methodology.

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MATERIALS AND METHODS

Clinical specimens. A total of 100 stool specimens from adults and children (ages 2 weeks to 93 years) with acute gastroenteritis submitted to the Diagnostic Virology Laboratory at Rhode Island Hospital were used in the evaluation. Stools were stored undiluted at −20°C until tested.

Reference assay. All specimens were tested by the reference method, a direct double-antibody sandwich microplate EIA described by Grauballe et al. (12). This assay was selected as the reference method because it is more sensitive than electron microscopy (12) and is comparable to the indirect enzyme immunoassay used as the World Health Organization reference standard (12). The reference assay was performed as follows. Alternate wells of a polyvinyl 96-well plate were coated with a 1:50 dilution of hyperimmune rabbit anti-human rotavirus antibody (DAKO Corp., Santa Barbara, Calif.) and normal rabbit immunoglobulin. The plate was incubated for 1 h at room temperature. The wells were emptied and washed five times with phosphate-buffered saline containing 0.5% Tween 20 with a hand-held wash apparatus (Miniwasher-12; Skatron, Inc., Sterling, Va.) The fecal specimens, prepared as 10% suspensions in phosphate-buffered saline–0.5% Tween 20 containing 0.5% bovine serum albumin, were added to duplicate wells coated with anti-rotavirus and control sera. After incubation for 1 h at room temperature the specimen was aspirated, and the plates were washed as described above. A 0.04% solution of normal rabbit serum was added to each well to block nonspecific reactions, and the plates were incubated for 1 h at room temperature. A 1:250 dilution of horseradish peroxidase-labeled rabbit anti-human rotavirus antibody (DAKO) was added to each well and incubated for 30 min at room

temperature. After washing as described above, an *o*-phenylenediamine substrate was added, and plates were incubated for 15 min in the dark. The color development was stopped with 1 M H_2SO_4, and absorbance in each well was measured at a wavelength of 490 nm on a microplate reader (Micro-ELIA Reader; Fisher Scientific Co., Medford, Mass.). A specimen was considered positive if the absorbance of the test well minus the absorbance of the control well was greater than 0.1 and the absorbance of the test well divided by the absorbance of the control well was greater than 6.

Commercial immunoassays. Nine commercial assays were evaluated. The evaluator of each commercial assay was blinded to the results of the reference assay. All evaluators had prior experience in running both plate and bead EIAs as well as latex agglutination tests.

Commercial EIAs. Five of the kits evaluated were EIAs. Two kits (Rotaclone; Cambridge Bioscience Corp.; Pathfinder Rotavirus, Kallestad Laboratories) use monoclonal antibodies directed against VP6, the group-specific antigen for all group A human rotaviruses. Rotaclone has both monoclonal capture and enzyme-conjugated antibodies, whereas Pathfinder Rotavirus uses a polyclonal capture antibody with a monoclonal antibody conjugate. The three remaining EIAs (Rotazyme II, Abbott Laboratories; Rotavirus EIA, International Diagnostic Laboratories [herein referred to as the IDL assay]; Wellcozyme Rotavirus, Wellcome Diagnostics) use polyclonal antisera produced in a variety of animal species for both capture and conjugated antibodies. EIAs were run according to the manufacturers' instructions and were read by spectrophotometer. Cutoff values were calculated as directed in the package inserts. Specimens giving results falling in the grey zone were retested.

Commercial latex agglutination assays. Four of the kits evaluated were latex agglutination tests (Rotastat, International Diagnostic Laboratories; Meritec-Rotavirus, Meridian Diagnostics; Virogen Rotatest, Wampole Laboratories; Wellcome Latex Test, Wellcome Diagnostics). All latex agglutination tests evaluated use polyclonal antisera on the test latex. The manufacturer's instructions were followed for each kit. The degree of agglutination was visually determined as indicated in the package insert. Specimens giving nonspecific results were retested.

Detection of rotavirus nucleic acid. Specimens with disagreement between the reference assay and at least one commercial immunoassay underwent further evaluation by polyacrylamide gel electrophoresis (PAGE) of extracted stool by a modification of the method of Laemmli (19) with silver staining of rotaviral RNA as follows. Stool specimens were suspended in EDTA and then sequentially extracted with 1,1,2-trichloro-1,2,2-trifluoroethane and phenol-chloroform to obtain rotaviral RNA. The RNA was ethanol precipitated and dissolved in Laemmli buffer. Each sample was applied to a discontinuous 7.5% polyacrylamide–7 M urea gel with a 3% stacking gel and run at 95 V for 17 h. Gels were stained by using a silver stain kit (Bio-Rad Laboratories, Richmond, Calif.).

Blocking assay. Specimens positive by one or both of the monoclonal antibody-based EIAs but negative by the reference EIA and PAGE were further evaluated by a blocking assay. The specimens were incubated with 1:5 dilutions of bovine anti-rotavirus antiserum (NIAID V-710-501-553; American Type Culture Collection, Rockville, Md.) or normal bovine serum negative for rotavirus antibody for 1 h at 37°C before the performance of the EIA as directed by the manufacturer. A stool sample having a reduction in absorbance of at least 50% in the anti-rotavirus serum-treated specimen as compared with the normal serum-treated specimen was considered positive for rotavirus antigen.

Data analysis. Results of the commercial assays were compared with results obtained with the reference assay and the blocking assay. True-positives are defined as specimens positive by both the reference or blocking assay and the commercial assay, whereas false-positives are positive by the commercial assay but not by the reference or blocking assays. False-negatives are specimens with positive results on the reference or blocking assay but negative results on the commercial assay. Sensitivity or the true-positive rate was determined by dividing the number of specimens positive in both the commercial and reference or blocking assay by the total number positive in the reference or blocking assay. Specificity or the true-negative rate was determined by dividing the number of specimens negative in both the commercial and reference or blocking assay by the total number negative in the reference or blocking assays. The positive predictive value, the percentage of truly infected individuals among those with positive tests, was determined by dividing the number of specimens positive in both the commercial and reference or blocking assay by the total number positive in the commercial assay. The negative predictive value, the percentage of truly uninfected individuals among those with negative tests, was determined by dividing the number of specimens negative in both the commercial and reference or blocking assay by the total number negative in the commercial assay. Diagnostic accuracy was determined by dividing the number of specimens with positive results in both tests plus the number of specimens with negative results in both tests by the total number of specimens tested. All of the above measures were multiplied by 100 and are expressed as percentages.

RESULTS

The reference EIA found 38 of the 100 stool samples from patients with gastroenteritis to be positive for rotavirus, whereas 62 were negative. PAGE was done on 41 specimens in which the results of the reference assay and at least one commercial immunoassay were discordant. The results of PAGE were in complete agreement with the reference EIA; the 6 specimens positive by the reference assay all contained rotaviral RNA, whereas none of the 35 negative specimens had detectable RNA. Three additional specimens which were positive by a monoclonal antibody-based EIA but negative by the reference EIA and PAGE were further tested by a blocking assay. In two of the three specimens the blocking assay found rotavirus antigen which was not detected by the reference assay or PAGE. One of the two specimens positive by blocking assay had low absorbance readings in both monoclonal antibody-based EIAs, whereas the other specimen had been read as negative in the reference EIA because of greater absorbance in the control well than that in the test well.

The results of the two monoclonal antibody-based EIAs are compared with the reference and blocking assays in Table 1. Both Rotaclone and Pathfinder detected all 40 true positives. Two specimens falsely positive by both Rotaclone and Pathfinder when compared to the reference EIA were blocked by rotavirus-specific antisera and are considered positive for rotavirus antigen. One specimen positive by Pathfinder but negative by Rotaclone did not block and was confirmed as a false-positive. No specimen required retesting by Rotaclone, whereas four specimens fell into the grey zone in the Kallestad assay and were resolved on retesting.

TABLE 1. Results of assays for detection of rotavirus compared with the results of the reference and blocking assays

Test[a]	True-positives (no.)	False-positives (no.)	False-negatives (no.)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Grey zone reactions (no.)	Diagnostic accuracy (%)
MAb-based EIAs									
Rotaclone	40	0	0	100	100	100	100	0	100
Pathfinder	40	1	0	100	98	98	100	4	99
PAb-based EIAs									
IDL assay	38	0	2	95	100	100	97	12	98
Wellcozyme	37	0	3	93	100	100	95	9	97
Rotazyme II	37	30	3	93	50	55	91	7	67
LA tests									
Rotastat	36	0	4	90	100	100	94	1	96
Meritec	32	5	8	80	92	87	87	0	87
Virogen	37	12	3	93	80	76	94	0	85
Wellcome	28	0	10	70	100	100	85	4	90

[a] MAb, Monoclonal antibody; PAb, polyclonal antibody; LA, latex agglutination.

The results of the three polyclonal antibody-based EIAs are shown in Table 1. The IDL assay identified 38 positives and agreed completely with the reference assay but failed to identify the two specimens positive in the blocking assay. Wellcozyme and Rotazyme II identified 37 true positives and thus had identical sensitivity (93%). However Rotazyme II had 30 false-positives, which reduced the specificity of the test to 50%, the positive predictive value to 55%, and the diagnostic accuracy to 67%. Sixteen (53%) of the 30 specimens falsely positive by Rotazyme II had high background activity in control wells of the reference EIA or the IDL assay. Grey zone results were obtained with all of the polyclonal assays requiring retesting of specimens to clarify assay results.

Results of the four latex agglutination tests are presented on Table 1. The number of true-positives varied from a high of 37 in the Virogen assay to a low of 28 in the Wellcome latex test. Virogen gave the largest number of false-positives (12), whereas Wellcome had the largest number of false-negatives (8). Nonspecific reactions were seen uncommonly. Overall, Rotastat had the highest diagnostic accuracy (96%) of the latex agglutination assays.

DISCUSSION

The choice of a reference standard for the evaluation of rotavirus assays is important in determining the clinical utility of assays and in comparisons of evaluations of assays done in a variety of laboratory settings. The reference method must be able to accurately predict the presence of rotavirus in clinical specimens. Reference standards used in prior evaluations of rotavirus assays have included electron microscopy (5, 17, 26, 28, 32, 34), a reference EIA (6, 8, 11, 25, 41), or PAGE of rotaviral RNA (27). The reference microplate EIA used in this evaluation was selected on the basis of its superiority over electron microscopy (12) and its comparability with PAGE; however it has been combined with a blocking assay because that assay identified as positive two specimens positive by monoclonal antibody-based EIAs and negative by reference EIA-PAGE. These specimens are interpreted as true positives with very small amounts of viral antigen not detectable by the reference techniques. Monoclonal antibody-based EIAs have been found to be capable of detecting rotavirus in specimens containing 10-fold fewer virions than those positive by electron microscopy or an indirect enzyme-linked immunosorbent assay similar to the reference EIA (10).

The results of this study indicate that the EIA kits are, in general, more sensitive and more specific and have greater diagnostic accuracy than latex agglutination tests. There is an exception however: one polyclonal antibody-based EIA, Rotazyme II, had lower specificity and diagnostic accuracy than the latex agglutination assays evaluated. Lack of sensitivity has been a problem in many evaluations of latex agglutination assays (3, 30) and has been particularly apparent when testing stool from patients late in the course of illness when smaller amounts of antigen may be present (25, 28).

The monoclonal antibody-based EIAs evaluated in this study appear to be superior to the polyclonal antibody-based tests, including the reference EIA, for detection of rotavirus. The superiority of the monoclonal antibody-based assays is likely due to the differences in antigen recognition by monoclonal and polyclonal antisera. Polyclonal antisera contain antibodies directed against multiple antigenic determinants and with a wide range of affinities, whereas monoclonal antibodies are directed against a single epitope and have a single affinity. As a result, EIAs employing monoclonal antibodies are usually more specific and have fewer grey zone results than EIAs with polyclonal antibodies, which may have cross-reactivity with other components of stool. Improvements in the technology of monoclonal antibody production in recent years have produced higher-affinity monoclonal antibodies which have sensitivity equal to or even greater than that of polyclonal antisera from a highly immunized animal. High levels of sensitivity are desirable in rotavirus testing of specimens from patients late in the course of the illness or in adults where levels of viral shedding may be low. Accurate identification of rotavirus in stool specimens facilitates the prompt isolation of infected patients and prevents disease transmission. If further studies confirm the greater sensitivity and specificity of monoclonal antibody-based assays for rotavirus detection, then these assays may become the reference methods of choice.

In this study Rotazyme II had an unexpected high false-positive rate when compared with other polyclonal antibody-based EIAs. This problem has been previously reported with Rotazyme I in neonates (18) and may be due to rheumatoid factor-like substances in stool (42). The specificity problem was seen in all age groups tested, including adults, not exclusively in neonatal specimens, as has been reported by Krause et al. (18). The inclusion of a preimmune serum control, as is done in the reference assay and the IDL assay,

TABLE 2. Comparison of commercial kits for detection of rotavirus

Test	Type[a]	Manufacturer	Prepn time (min)		Test performance time	No. of tests per kit	Cost per test ($)[b]	Equipment needed
			Specimen	Reagent				
Reference method	PAb EIA	DAKO	15	20	4.5 h	240	1.01	Centrifuge, wash apparatus, spectrophotometer
Rotaclone	MAb EIA	Cambridge Bioscience	5	0	1.5 h	48	4.06	Wash apparatus (optional), spectrophotometer
Pathfinder	MAb EIA	Kallestad	5	5	1.5 h	50	4.00	Vortex, wash apparatus, spectrophotometer
IDL assay	PAb EIA	International Diagnostic Laboratories	15	15	2.25 h	32 or 96	1.83	Vortex, wash apparatus, spectrophotometer
Rotazyme II	PAb EIA	Abbott	5	5	2.5 h	50	3.60	Water bath, wash apparatus, spectrophotometer
Wellcozyme	PAb EIA	Wellcome	15	15	1.75 h	96	2.88	Centrifuge, water bath, wash apparatus, spectrophotometer
Rotastat	LA	International Diagnostic Laboratories	15	0	3–4 min	25	2.76	Vortex, centrifuge
Meritec	LA	Meridian	5	0	5 min	30	3.50	Rotator, high-intensity light
Virogen	LA	Wampole	15	0	2 min	50	4.15	Centrifuge
Wellcome latex	LA	Wellcome	15	0	2 min	25	4.08	Vortex, centrifuge

[a] See footnote *b* of Table 1.
[b] Based on manufacturers' quoted prices of November 1987.

allows the differentiation of nonspecific reactions from true positives in these tests, but this control is not included in Rotazyme II.

Other factors related to test performance, such as speed, expense, and simplicity, also need to be evaluated. Table 2 summarizes performance time, cost per test, and equipment needs for the nine tests evaluated in this study. Latex agglutination tests are more rapid than EIAs and do not require expensive equipment. Hence the latex agglutination tests with the highest sensitivity and specificity are suitable for use in the emergency room, physician's office, or small hospital laboratory where speed and simplicity are needed. The monoclonal antibody-based EIAs are the most costly but have the advantage of rapid performance times and highest sensitivity. Polyclonal antibody-based tests are the least expensive but most time consuming.

The choice of the rotavirus assay will depend to a great extent on the requirements of the individual laboratory. Reference laboratories may wish to select tests with the highest sensitivity and specificity, such as the monoclonal antibody-based EIAs or, if cost is an issue, a polyclonal antibody-based EIA with high sensitivity and specificity. Latex agglutination assays are best utilized in situations where a small number of specimens are to be tested, since latex assays cannot be automated, and where simplicity and speed are of importance.

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LITERATURE CITED

1. **Banatvala, J. E., B. Totterdell, I. L. Chrystie, and G. N. Woode.** 1975. In vitro detection of human rotaviruses. Lancet **ii**:821.
2. **Bradburne, A. F., J. D. Almeida, P. S. Gardner, R. B. Moosai, A. A. Nash, and R. R. A. Coombs.** 1979. A solid phase system (SPACE) for the detection and quantification of rotavirus in faeces. J. Gen. Virol. **44**:615–623.
3. **Brandt, C. D., C. W. Arndt, G. L. Evans, H. W. Kim, E. P. Stallings, W. J. Rodriguez, and R. H. Parrott.** 1987. Evaluation of a latex test for rotavirus detection. J. Clin. Microbiol. **25**: 1800–1802.
4. **Brandt, C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries, E. P. Stallings, C. Lewis, A. J. Miles, R. M. Chanock, A. Z. Kapikian, and R. H. Parrott.** 1983. Pediatric viral gastroenteritis during eight years of study. J. Clin. Microbiol. **18**:71–78.
5. **Chernesky, M., S. Casticiano, J. Mahony, and D. DeLong.** 1985. Examination of the Rotazyme II enzyme immunoassay for the diagnosis of rotavirus gastroenteritis. J. Clin. Microbiol. **22**:462–464.
6. **Cromien, J. L., C. A. Himmelreich, R. I. Glass, and G. A. Storch.** 1987. Evaluation of new commercial enzyme immunoassay for rotavirus detection. J. Clin. Microbiol. **25**:2359–2362.
7. **Dennehy, P. H., and G. Peter.** 1985. Risk factors associated with nosocomial rotavirus infection. Am. J. Dis. Child. **139**:935–939.
8. **Doern, G. V., J. E. Herrmann, P. Henderson, D. Stobbs-Walro, D. M. Perron, and N. R. Blacklow.** 1986. Detection of rotavirus with a new polyclonal antibody enzyme immunoassay (Rotazyme II) and a commercial latex agglutination test (Rotalex): comparison with a monoclonal antibody enzyme immunoassay. J. Clin. Microbiol. **23**:226–229.
9. **Flewett, T. H., H. Davies, A. S. Bryden, and M. J. Robertson.** 1974. Diagnostic electron microscopy of the faeces. II. Acute gastroenteritis associated with reovirus-like particles. J. Clin. Pathol. **27**:608–614.
10. **Gerna, G., A. Sarasini, N. Passarani, M. Torsellini, M. Parea, and M. Battaglia.** 1987. Comparative evaluation of a commercial enzyme-linked immunoassay and solid-phase immune electron microscopy for rotavirus detection in stool specimens. J. Clin. Microbiol. **25**:1137–1139.
11. **Gilchrist, M. J. R., T. S. Bretl, K. Moultney, D. R. Knowlton, and R. L. Ward.** 1987. Comparison of seven kits for detection of rotavirus in fecal specimens with a sensitive, specific enzyme immunoassay. Diagn. Microbiol. Infect. Dis. **8**:221–228.
12. **Grauballe, P. C., B. F. Vestergaard, A. Meyling, and J. Genner.** 1981. Optimized enzyme-linked immunosorbent assay for detection of human and bovine rotaviruses in stools: comparison with electron-microscopy, immunoelectro-osmophoresis, and fluorescent antibody techniques. J. Med. Virol. **7**:29–40.
13. **Halvorsrud, J., and I. Orstavik.** 1980. An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scand. J. Infect. Dis. **12**:161–164.
14. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by

direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol. **16**:473–477.

15. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriquez, D. A. Sack, R. M. Chanock, and R. H. Parrott.** 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N. Engl. J. Med. **294**: 965–972.
16. **Keswick, B. H., L. K. Pickering, H. L. DuPont, and W. E. Woodward.** 1983. Prevalence of rotavirus in children in day care centers. J. Pediatr. **103**:85–86.
17. **Knisley, C. V., J. Bednarz-Prashad, and L. K. Pickering.** 1986. Detection of rotavirus in stool specimens with monoclonal and polyclonal antibody-based assay systems. J. Clin. Microbiol. **23**:897–900.
18. **Krause, P. J., J. S. Hyams, P. J. Middleton, V. C. Herson, and J. Flores.** 1983. Unreliability of Rotazyme ELISA test in neonates. J. Pediatr. **103**:259–262.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Marrie, T. J., S. H. S. Lee, R. S. Faulkner, J. Ethier, and C. H. Young.** 1982. Rotavirus infection in a geriatric population. Arch. Intern. Med. **142**:313–316.
21. **Matsuno, S., and S. Nagayoshi.** 1978. Quantitative estimation of infantile gastroenteritis virus antigens in stools by immune adherence hemagglutination test. J. Clin. Microbiol. **7**:310–311.
22. **Middleton, P. J., M. D. Holdaway, M. Petore, M. T. Szymanski, and J. S. Tam.** 1977. Solid-phase radioimmunoassay for the detection of rotavirus. Infect. Immun. **16**:439–444.
23. **Middleton, P. J., M. Petric, C. M. Hewitt, M. T. Szymanski, and J. S. Tam.** 1976. Counter immunoelectroosmophoresis for the detection of infantile gastroenteritis virus (orbi group) antigen and antibody. J. Clin. Pathol. **29**:191–197.
24. **Middleton, P. J., M. T. Szymanski, G. D. Abbott, R. Bortolussi, and J. R. Hamilton.** 1974. Orbivirus acute gastroenteritis of infancy. Lancet **i**:1241–1244.
25. **Miotti, P. G., J. Eiden, and R. H. Yolken.** 1985. Comparative efficacy of commercial immunoassays for the diagnosis of rotavirus gastroenteritis during the course of infection. J. Clin. Microbiol. **22**:693–698.
26. **Morinet, F., F. Ferchal, R. Colimon, and Y. Perol.** 1984. Comparison of six methods for detecting human rotavirus in stools. Eur. J. Clin. Microbiol. **3**:136–140.
27. **Pacini, D. L., M. T. Brady, C. T. Budde, M. J. Connell, V. V. Hamparian, and J. H. Hughes.** 1988. Polyacrylamide gel electrophoresis of RNA compared with polyclonal- and monoclonal-antibody-based enzyme immunoassays for rotavirus. J. Clin. Microbiol. **26**:194–197.
28. **Pai, C. H., M. S. Shahrabadi, and B. Ince.** 1985. Rapid diagnosis of rotavirus gastroenteritis by a commercial latex agglutination test. J. Clin. Microbiol. **22**:846–850.
29. **Peterson, M. W., R. S. Spendlove, and R. A. Smart.** 1976. Detection of neonatal calf diarrhea virus, infant reovirus-like diarrhea virus, and a coronavirus using the fluorescent virus precipitin test. J. Clin. Microbiol. **3**:376–377.
30. **Pickering, L. K., and B. H. Keswick.** 1986. Rotaviruses and norwalk viruses, p. 301–327. *In* S. Specter and G. J. Lancz (ed.), Clinical virology manual. Elsevier Science Publishing, Inc., New York.
31. **Rodriquez, W. J., H. W. Kim, C. D. Brandt, A. B. Fletcher, and R. H. Parrott.** 1983. Rotavirus: a cause of nosocomial infection in the nursery. J. Pediatr. **101**:274–277.
32. **Rubenstein, A. S., and M. F. Miller.** 1982. Comparison of an enzyme immunoassay with electron microscopic procedures for detecting rotavirus. J. Clin. Microbiol. **15**:938–944.
33. **Ryder, R. W., J. E. McGowan, Jr., M. H. Hatch, and E. L. Palmer.** 1977. Reovirus-like agent as a cause of nosocomial diarrhea in infants. J. Pediatr. **90**:698–702.
34. **Sambourg, M., A. Goudeau, C. Courant, G. Pinon, and F. Denis.** 1985. 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. J. Clin. Microbiol. **21**:622–625.
35. **Sanekata, T., Y. Yoshida, and K. Oda.** 1979. Detection of rotavirus from faeces by reversed passive hemagglutination method. J. Clin. Pathol. **32**:963.
36. **Sanekata, T., Y. Yoshida, and H. Okada.** 1981. Detection of rotavirus in feces by latex agglutination. J. Immunol. Methods **41**:377–385.
37. **Sato, K., Y. Inaba, T. Shinozaki, R. Fujii, and M. Matsumoto.** 1981. Isolation of human rotaviruses in cell cultures. Arch. Virol. **69**:155–160.
38. **Skaug, K., K. J. Figenschau, and I. Orstavik.** 1983. A rotavirus staphylococcal co-agglutination test. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **91**:175–178.
39. **Wyatt, R. G., W. D. James, E. H. Bohl, K. W. Theil, L. J. Saif, A. R. Kalica, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1980. Human rotavirus type 2: cultivation in vitro. Science **207**:189–191.
40. **Yolken, R. H., H. W. Kim, T. Clem, R. G. Wyatt, A. R. Kalica, R. M. Chanock, and A. Z. Kapikian.** 1977. Enzyme-linked immunosorbent assay (ELISA) for detection of human reo-like agent of infantile gastroenteritis. Lancet **ii**:263–267.
41. **Yolken, R. H., and F. J. Leister.** 1981. Evaluation of enzyme immunoassays for the detection of human rotavirus. J. Infect. Dis. **144**:379.
42. **Yolken, R. H., and P. J. Stopa.** 1979. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotaviruses. J. Clin. Microbiol. **10**:703–707.