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Comparison of an Enzyme Immunoassay with Electron Microscopic Procedures for Detecting Rotavirus

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The sensitivity and specificity of an enzyme immunoassay (Rotazyme), an on-grid immunoelectron microscopic procedure, and conventional negative stain electron microscopic techniques were compared. By using partially purified human rotavirus and simian rotavirus (SA-11) of known particle concentration, the enzyme immunoassay was essentially equivalent to the immunoelectron microscopic procedure and significantly more sensitive than conventional electron microscopic techniques. The level of sensitivity was approximately 10^6 particles per ml for simian rotavirus SA-11 and 10^7 particles per ml for human rotavirus. In an evaluation of 455 clinical samples by these techniques, a sensitivity of 98% and specificity of 92% were demonstrated. Samples negative by the immunoelectron microscopic procedure and positive by enzyme immunoassay could be confirmed by a blocking assay.

The enzyme immunoassay (EIA) principle is now used in a variety of diagnostic tests, and the simplicity and sensitivity of these procedures have greatly facilitated rapid viral diagnosis (5, 6, 8, 10, 11, 22, 29, 30, 33, 34, 38, 39; A. S. Rubenstein, K. Chau, C. Ling, M. Miller, F. Nehmadi, and L. Overby, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C6, p. 275). Rotavirus, a major cause of nonbacterial gastroenteritis (1, 9, 13, 18), cannot be detected by routine viral isolation techniques (4, 36, 37), and before immunoassay systems were shown to be effective in detecting this virus in clinical specimens, electron microscopy (EM) was the only method capable of providing a definitive diagnosis (1, 2, 9, 13, 18). Recently we developed a direct solid-phase enzyme immunoassay called Rotazyme (Rubenstein et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C6, p. 275) and an on-grid immunoelectron microscopic (IEM) technique (21) for detecting rotavirus. Both procedures involve the capturing of human rotavirus (HRV) antigen by antibodies raised against the simian rotavirus SA-11. In this paper we report results obtained from a comparative study of the Rotazyme EIA, the on-grid IEM technique, and conventional EM negative staining techniques. These data were collected to provide insight into the relative sensitivities of these methods for diagnosing HRV infection.

MATERIALS AND METHODS

Animal virus growth and purification. Simian rotavirus SA-11 (originally isolated by H. H. Malherbe) and Nebraska calf diarrhea virus (NCDV) were kindly provided by P. J. Middleton of the Hospital for Sick Children, Toronto, Canada. The virus was propagated in the BSC-1 line of African green monkey kidney cells (12), which were purchased from Flow Laboratories, Inc., McLean, Va. Cells were cultured in 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum. Confluent cell monolayers were infected with approximately 0.1 PFU per cell of either SA-11 (15) or NCDV (17). After adsorption of the virus for 1 h at 37°C, 1640 medium supplemented with 2% fetal bovine serum was added, and incubation was continued for an additional 36 to 48 h. When approximately 90% of the cells exhibited a cytopathic effect, the virus released into the medium was purified by a combination of differential and sucrose density gradient centrifugation as previously described (28). Virus bands were collected, pooled, and dialyzed overnight at 4°C against phosphate-buffered saline (PBS), pH 7.2. Resultant virus stocks were evaluated for purity and particle content by electron microscopy.

Immune sera. Hyperimmune sera were prepared by multiple site injection of rabbits with 100 μg/ml of purified NCDV or SA-11 virus mixed with an equal amount of complete Freund adjuvant. After a minimum of three additional biweekly booster immunizations, the rabbits were bled by heart puncture, and the sera were separated by centrifugation. These antisera were used in both EIA and IEM procedures.

Clinical specimens. A collection of 455 stool samples from children suffering from gastroenteritis was assembled from kind donations by P. J. Middleton; R. H. Yolken, Johns Hopkins University Hospital, Baltimore, Md; J. Vollet, University of Texas Health Science Center, Houston, Tex.; and R. Neagle, St. Jude Children's Research Hospital, Memphis, Tenn. All specimens were collected by cotton swab or in a

container and frozen until sufficient numbers were obtained for evaluation. These were then assayed either undiluted, when possible, or as a 10% crude mixture in PBS. Selected specimens were pooled, diluted to a 10% suspension in PBS, and partially purified by first filtering through four layers of cheesecloth and then clarifying the filtrate at 3,000 rpm for 10 min. This material was used as a source of HRV for EIA and EM titration-sensitivity experiments.

Standard EIA procedure. The EIA procedure was carried out as outlined in the instruction booklet included in the Rotazyme diagnostic kit. In brief, a bead that had been precoated with antirotavirus antibody (23) was incubated for 3 h at 45°C with 0.2 ml of diluted SA-11 or a 10% suspension of fecal sample. The bead was then washed four times with distilled water and further incubated for 1 h at 45°C with 0.2 ml of horseradish peroxidase-conjugated antibody to rotavirus. In positive samples, these incubations resulted in the formation on the bead of an antibody-rotavirus-antibody enzyme sandwich. After the formation of the complexes, the beads were washed six times with distilled water and incubated for 15 min at room temperature in 0.2 ml of *o*-phenylenediamine·2HCl substrate. The resulting reaction was stopped by adding 1 ml of N HCl. Since the intensity of color developed was proportional to the quantity of enzyme complex on a bead, the amount of viral antigen present in samples was determined spectrophotometrically at a wavelength of 492 nm. All data were obtained as optical density units at this wavelength.

Confirmatory EIA procedure. Clinical samples positive by EIA and negative by IEM techniques were tested by a confirmatory (blocking) EIA. Samples were treated as described in the standard EIA procedure except that immediately after being incubated in fecal sample and washed in distilled water, the beads were incubated for 16 to 18 h at room temperature in 0.2 ml of a 1:100 dilution of anti-NCDV hyperimmune or preimmune control serum. The beads were then washed four times with 5-ml volumes of distilled water and further treated with conjugate and enzyme substrate as described previously. A 50% or greater reduction in optical density after treatment with hyperimmune serum was considered positive and confirmatory for the presence of rotavirus antigen.

Conventional EM procedure. Partially purified HRV and SA-11 virus stocks were diluted and used directly, whereas crude stool specimens were first diluted and then clarified by low-speed centrifugation for 10 min at 1,500 rpm before EM examination. A drop of sample was applied to a Formvar carbon-backed 400-mesh copper specimen grid held in jeweler's forceps. After 60 s, the drop was removed by being blotted from the edge with Whatman filter paper, and particles adhering to the grid (non-specifically) were negatively stained by the immediate application of a drop of 2% phosphotungstic acid. After 30 to 60 s, phosphotungstic stain was removed by being blotted from the edge of the grid until dry. Grids were prepared in duplicate and examined in the electron microscope at magnifications ranging from 20,000 to 45,000×. The average number of particles per five grid openings was determined and recorded for comparison with IEM and EIA techniques.

IEM procedure. The IEM technique has previously been reported (21). Briefly, 400-mesh Formvar carbon-backed copper specimen grids were sensitized with anti-SA-11 hyperimmune serum diluted 1:2,000 with PBS. Grids were floated Formvar side down for 15 min on droplets of antiserum and then placed on three successive droplets of PBS for 60 s each and blotted dry. Antibody-sensitized grids were next incubated for 15 min on droplets of partially purified HRV and SA-11 virus or clarified fecal extract, again washed on three droplets of PBS, negatively stained for 30 to 60 s with 2% phosphotungstic acid, and blotted dry. The average number of particles per five grid openings was determined as in the conventional EM method. Initially, grids were prepared in duplicate. However, results after this procedure was used were highly consistent, and this practice was not followed with ca. the last 150 clinical samples examined.

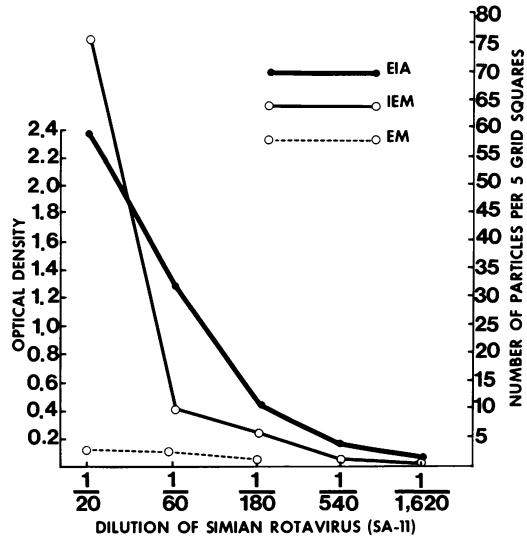

FIG. 1. Dose-response plot comparing sensitivity of EIA, on-grid IEM, and conventional EM methods for detecting SA-11 rotavirus.

Virus particle-counting procedure. Partially purified HRV and SA-11 virus stocks used in EIA and EM titration-sensitivity experiments were quantitated by sensitive thin section (19) and direct-grid (20) sedimentation particle-counting techniques. Stocks of HRV and SA-11 virus contained 10^{10} and 10^{9} particles per ml, respectively.

RESULTS

The EIA and EM techniques used in this study were first evaluated by the performance of assays on known concentrations of partially purified and serially diluted HRV and SA-11 virus samples. A dose-response plot showing results obtained when serially diluted SA-11 virus was assayed by the EIA, on-grid IEM, and conventional EM procedures is depicted in Fig. 1. A similar plot of HRV is shown in Fig. 2. After a careful search of the surface of five grid openings, neither virus could be detected at dilutions higher than 1/180 with the conventional

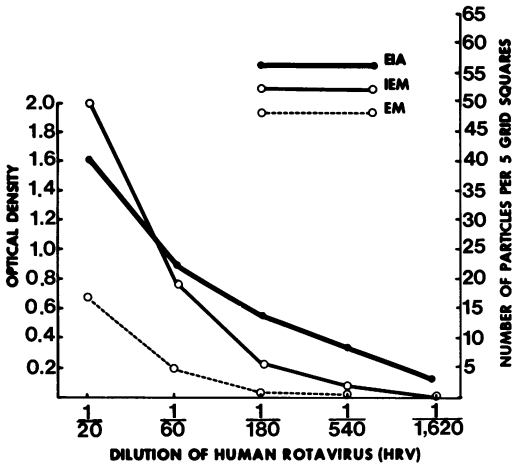

FIG. 2. Dose-response plot comparing sensitivity of EIA, on-grid IEM, and conventional EM methods for detecting HRV.

EM method. However, both HRV and SA-11 viruses were detectable in EIA and IEM assays in which the viruses were diluted 1/1620. HRV and SA-11 virus stocks were found by the EM particle-counting methods (10, 20) to contain 10^{10} and 10^9 virus particles per ml, respectively. Therefore, it was calculated that the EIA and IEM methods used in this study were capable of detecting as few as 10^7 HRV and 10^6 SA-11 virus particles per ml. The appearance of a typical field of rotavirus particles adhering to an antibody-sensitized grid is shown in Fig. 3.

After a demonstration of the sensitivity of the EIA and IEM tests devised, their suitability for clinical testing was evaluated by assaying 455 stool samples obtained from children suffering from diarrheal diseases. The results of these assays are summarized in Fig. 4. Rotavirus particles could generally be found in EIA-positive samples within 5 min of searching with the on-grid IEM technique. The vast majority of particles were single shelled (Fig. 3 and 5b), but double-shelled particles (Fig. 5a) were occasionally detected. Exclusively double-shelled populations were never observed. Viral capsomeres (Fig. 5c) were sometimes found in the absence of complete single- or double-shelled particles. Of the 197 specimens positive by IEM, 193 were also positive by EIA, which corresponds to a sensitivity of 98%. Of the 258 samples negative by IEM, 238 were negative by EIA, yielding a specificity of 92%.

As shown in Fig. 4, a number of specimens positive by EIA were negative by IEM. For this

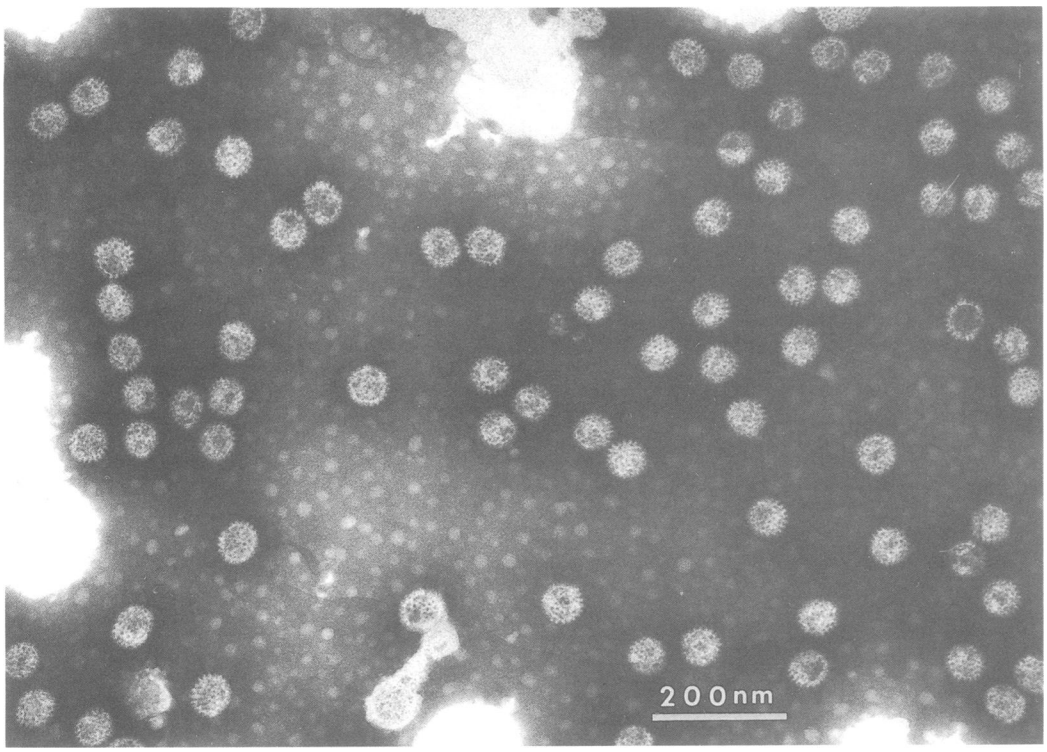

FIG. 3. Typical field of negatively stained SA-11 rotavirus particles attached to an antibody-sensitized microscope grid.

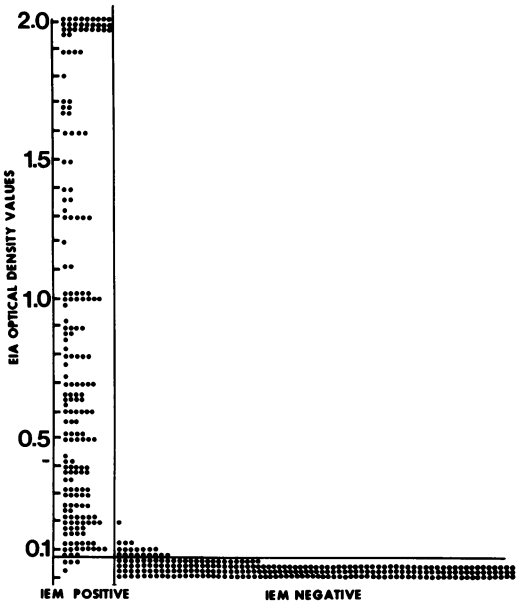

FIG. 4. Scattergram showing results derived when 455 human stool samples were tested for the presence of rotavirus by the EIA and on-grid IEM procedures. The horizontal line corresponds to an EIA cutoff of 0.07 optical density units.

TABLE 1. Confirmatory assay for rotavirus

Sample no.	Control (optical density units)	Blocking assay (optical density units)	Percent blocking[a]
1	2.000	0.493	75
2	0.749	0.249	70
3	0.705	0.070	90
4	0.583	0.088	93
5	0.465	0.195	58
6	0.410	0.032	92
7	0.407	0.111	73
8	0.377	0.039	90
9	0.326	0.056	83
10	0.285	0.085	70
11	0.255	0.093	64
12	0.209[b]	0.084	60
13	0.173[b]	0.035	80
14	0.163[b]	0.050	69
15	0.095[b]	0.033	65
16	0.094[b]	0.034	64
17	0.083[b]	0.023	72
18	0.081[b]	0.022	73

[a] Specimen was considered positive if 50% or more inhibition was obtained after incubation with anti-NCDV serum.

[b] Specimens were negative for rotavirus by the on-grid IEM method.

reason, a confirmatory (blocking) EIA was used to determine whether these results represented false-positive reactions or whether the EIA test might be slightly more sensitive than the IEM technique. Table 1 shows the degree of inhibition found when EIA-positive and IEM-negative specimens were tested. In all tests, 50% or greater inhibition was obtained, which further indicates the high specificity of the EIA used.

DISCUSSION

The need for rapid and reliable viral diagnostic procedures has led to the development of a number of sensitive immunoassays for rotavirus. The success of the EIA (Rotazyme) and IEM techniques used in this study may be attributed to (i) the ease with which SA-11 virus can be grown and purified from tissue culture (6, 15, 16), (ii) the serological cross-reactivity among rotavirus of different species (32, 35, 38), (iii) the ability of plastic beads (26) and plastic-coated EM grids (7) to adsorb immunoglobulins, and (iv) the knowledge that infected human feces contain copious quantities of incomplete rotavirus particles lacking outer shells. The latter is essential since the common antigenic determinants with which heterologous antibodies combine reside on the inner shells of rotavirus particles (35).

There has always been controversy with regard to the sensitivity and reliability of the conventional EM-negative staining method (14). Factors which may affect the reliability of this method include virus size, concentration and purity, wetting properties of grids used, reagents used, time devoted to searching in samples, and operator skill. It has been shown that the concentration of fecal samples by ultracentrifugation only slightly improves the sensitivity of viral diagnosis by this method (27). Moreover, if the particles have a high propensity for spontaneous aggregation (24), they may be inadvertently removed from grids during the blotting procedure. For these reasons, most microscopists would agree that a minimum of 10^8 particles per ml of sample are necessary to permit the reliable detection of virus in a reasonable period of time with this method. In this study, conventional EM was three times more sensitive in detecting HRV than SA-11, further demonstrating the inconsistency of the technique.

The results of this study show that the direct EIA and on-grid IEM techniques are approximately equivalent in sensitivity and reliability. Compared with the conventional EM method, both techniques were about nine times more sensitive in detecting purified SA-11 and three times more sensitive in detecting HRV in crude stool samples. Based on the evaluation of 455 clinical stool specimens and knowledge of the sensitivity of the techniques used, we estimated that most rotavirus-positive fecal samples contain a minimum of 10^8 virus particles per ml of a 10% suspension. Thus, both the EIA and on-grid

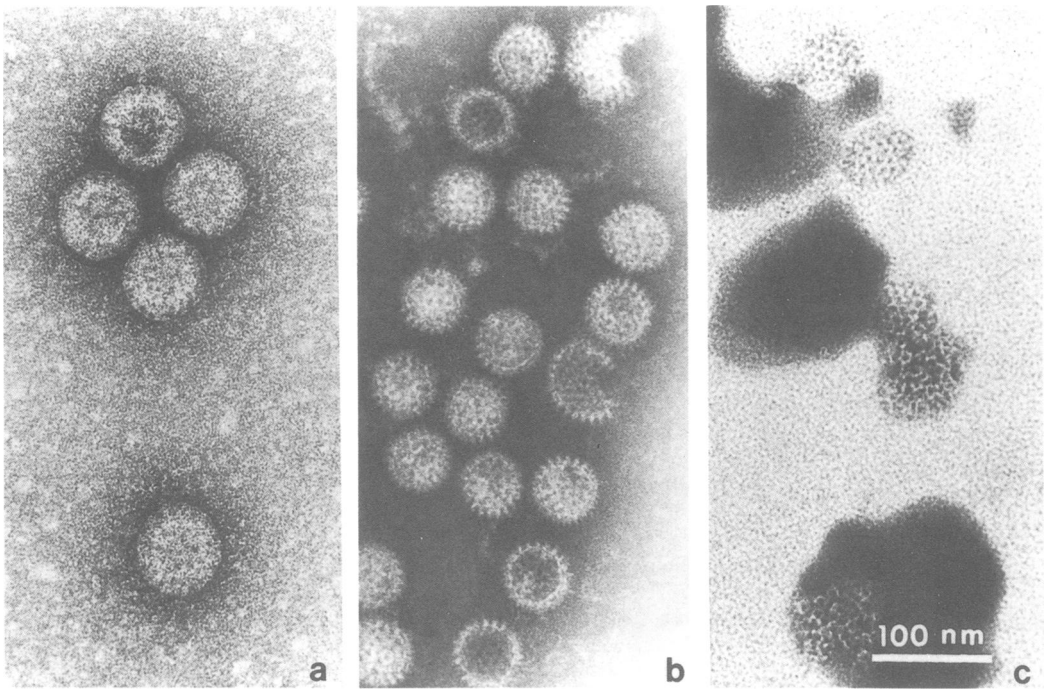

FIG. 5. Rotavirus particles detected in feces of children suffering from gastroenteritis. (a) Complete double-shelled particles. (b) Incomplete single-shelled particles. (c) Viral debris (capsomeres). Most positive specimens contained predominantly single-shelled particles.

IEM techniques appear to be quite adequate for making accurate clinical diagnoses.

It has recently been reported that pretreatment of EM grids with staphylococcal protein A before antibody incubation improves serological trapping of plant viruses (31) and rotavirus (25). The authors pointed out that the concentration of protein A used was extremely critical and varies for different viruses. Moreover, 18 h of incubation of rotavirus specimens with protein A-antibody-sensitized grids was necessary to maximize the trapping of particles. In the present study, the complete procedure for sensitizing grids and trapping virus particles required only ca. 35 min. The high sensitivity and specificity obtained by both the IEM and EIA methods used in this study and the high degree of correlation demonstrated when results from the two tests were compared suggest that little would have been gained by lengthening the IEM test to include incorporation of protein A into the grid-sensitization process.

Approximately 8% of clinical samples negative by the IEM method were positive by EIA. All of these specimens were borderline or low positives but could be confirmed by the EIA blocking assay. The occurrence of false-positive results with EIA and radioimmunoassay tests and the desirability of the use of confirmatory blocking assays has been advocated by others (3, 30). The failure to detect virus by IEM in these EIA-positive samples can partially be explained by the exclusive observation of viral debris (Fig. 5c) and no intact virus particles in a few samples. Although such debris may be antigenically detectable by EIA, it could have easily been overlooked by EM in other specimens. It is also possible that some of the EIA-positive samples not tested in the blocking assay were false-positives. False EIA-positive samples due to the presence of *Staphylococcus* organisms in fecal samples have been reported (3). Cultured *Staphylococcus* containing protein A gave a strong reaction with EIA, but when mixed with normal fecal extract, this reaction did not develop (unpublished data).

It is of interest that a few samples positive by IEM were negative in the EIA test. The reasons for these discrepancies are not clear but may have been caused by inhibitors present in fecal samples that interfere with the first EIA binding step. Another possibility is that the common antigenic determinants on observed virus particles were somehow blocked by the antibodies of the patients.

Previous comparative studies between EM and immunoassay tests have generally been based on indirect EIA and conventional EM-negative staining procedures (3). Although the sensitivity of these EIA tests was good for

research purposes, the specificity often approached unacceptable levels. The high degree of specificity demonstrated in this study is due to the use of a direct EIA system. The on-grid IEM technique has the desirable feature of direct virus visualization, but it in no way precludes use of a more practical EIA such as Rotazyme in clinical diagnoses.

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LITERATURE CITED

1. **Bishop, R. F., G. P. Davidson, I. H. Holmes, and B. J. Ruck.** 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet **ii**:1281–1283.
2. **Bishop, R. F., G. P. Davidson, I. H. Holmes, and B. J. Ruck.** 1974. Detection of a new virus by electronmicroscopy of fecal extracts from children with acute gastroenteritis. Lancet **ii**:149–151.
3. **Brandt, C. D., H. W. Kim, W. J. Rodriquez, L. Thomas, R. H. Yolken, J. O. Arrobio, A. Z. Kapikian, R. H. Parrott, and R. M. Chanock.** 1981. Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. J. Clin. Microbiol. **13**:976–981.
4. **Bryden, A. S., H. A. Davies, M. E. Thouless, and T. H. Flewett.** 1977. Diagnosis of rotavirus infection by cell culture. J. Med. Microbiol. **10**:121–125.
5. **Clementi, M., P. Pauri, P. Bagnarelli, G. Carloni, and L. Calegari.** 1981. Diagnosis of human rotavirus infections: comparison of an electrophoretic method, a modified complement fixation test and electron microscopy for rotavirus detection. Arch. Virol. **67**:341–344.
6. **Cukor, G., M. K. Berry, and N. R. Blacklow.** 1978. Simplified radioimmunoassay for detection of human rotavirus in stools. J. Infect. Dis. **138**:906–910.
7. **Derrick, K. S.** 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology **56**:652–653.
8. **Ellens, D. J., and P. W. De Leeuw.** 1977. Enzyme-linked immunosorbent assay for diagnosis of rotavirus infections in calves. J. Clin. Microbiol. **6**:530–532.
9. **Flewett, T. H., A. S. Bryden, H. Davies, G. N. Woode, J. C. Bridger, and J. M. Derrick.** 1974. Relationship between virus from acute gastroenteritis of children and newborn calves. Lancet **ii**:61–63.
10. **Grauballe, P. C., B. F. Vestergaard, A. Meyling, and J. Genner.** 1981. Optimized enzyme-linked immunosorbent assay for detection of human and bovine rotavirus in stools: comparison with electron-microscopy, immunoelectro-osmophoresis and fluorescent antibody techniques. J. Med. Virol. **7**:29–40.
11. **Gravelle, M., P. H. Dorsett, O. Gutensson, and A. C. Levy.** 1977. Detection of antibody to rubella by ELISA. J. Infect. Dis. **176**(Suppl.):300–303.
12. **Hopps, H. E., A. Bernheim, A. Nisalak, J. H. Tjio, and J. E. Smadel.** 1963. Biological characteristics of a continuous kidney cell line derived from the African green monkey. J. Immunol. **91**:416–424.
13. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. J. Rodriquez, W. L. Cline, R. H. Parrott, and R. M. Chanock.** 1974. Reovirus like agent in stools: association with infantile diarrhea and development of serological tests. Science **185**:1049–1053.
14. **Madeley, C. R.** 1979. Viruses in stools. J. Clin. Pathol. **32**:1–10.
15. **Malherbe, H. H., and M. Strickland-Cholmley.** 1967. Simian virus SA-11 and the related O agent. Arch. Gesamte. Virusforsch. **22**:235–245.
16. **Matsuno, S., S. Inouye, and R. Kono.** 1977. Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera. J. Clin. Microbiol. **5**:1–4.
17. **Mebus, C. A., N. R. Underdahl, M. B. Rhodes, and M. J. Twiehaus.** 1969. Calf diarrhea (scours) reproduced with a virus from a field outbreak. Univ. Nebr. Agric. Exp. Station Res. Bull. **233**:1–16.
18. **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.
19. **Miller, M. F., P. T. Allen, and L. Dmochowski.** 1973. Quantitative studies of oncornaviruses in thin sections. J. Gen. Virol. **21**:57–68.
20. **Miller, M. F., and E. J. Rdzok.** 1981. Improved virus particle counting by direct sedimentation onto EM grids, p.404–405. *In* G. W. Bailey (ed.), 39th Annual Proceedings of the Electron Microscope Society of America. Claitors Publishing Division, Baton Rouge, La.
21. **Miller, M. F., and A. S. Rubenstein.** 1980. A sensitive on-grid immunoelectron microscopic procedure for diagnosis of rotavirus infection, p. 506–507. *In* G. W. Bailey (ed.), 38th Annual Proceedings of the Electron Microscope Society of America. Claitors Publishing Division, Baton Rouge, La.
22. **Miranda, Q. R., G. D. Bailey, A. S. Fraser, and H. J. Tenesco.** 1977. Solid phase enzyme immunoassay for herpes simplex. J. Infect. Dis. **136**(Suppl):304–310.
23. **Mushahwar, I. K., and L. R. Overby.** 1981. An enzyme immunoassay for hepatitis B e-antigen and antibody. J. Virol. Methods **3**:89–97.
24. **Narang, H. K., and A. A. Codd.** 1981. Frequency of preclumped virus in routine fecal specimens from patients with acute nonbacterial gastroenteritis. J. Clin. Microbiol. **13**:982–988.
25. **Nicolaieff, A., G. Obert, and M. H. V. Regenmortel.** 1980. Detection of rotavirus by serological trapping on antibody-coated electron microscope grids. J. Clin. Microbiol. **12**:101–104.
26. **Oreskes, I., and J. M. Singer.** 1964. Influence of pH, ionic strength and human gamma globulin concentration on stability of polystyrene latex particle suspensions. Proc. Soc. Exp. Biol. Med. **115**:753–755.
27. **Rice, S. J., and A. D. Phillips.** 1980. Rapid preparation of faecal specimens for detection of viral particles by electron microscopy. Med. Lab. Sci. **37**:371–372.
28. **Rubenstein, A. S., M. Gravell, and G. Darlington.**, 1972. Protein kinase in the enveloped herpes simplex virions. Virology **50**:287–290.
29. **Sanekata, T., Y. Yoshida, and H. Okada.** 1981. Detection of rotavirus in faeces by latex agglutination. J. Immunol. Methods **41**:377–385.
30. **Sarkkinen, H. K., H. Toukko, and P. E. Halonen.** 1980. Comparison of enzyme immunoassay and radioimmunoassay for detection of human rotaviruses and adenoviruses from stool specimens. J. Virol. Methods **1**:331–341.
31. **Shukla, D. D., and K. H. Gough.** 1979. The use of protein A, from *Staphylococcus aureus* in immune electron microscopy for detecting plant virus particles. J. Gen. Virol. **45**:533–536.
32. **Thouless, M. E., A. S. Bryden, T. H. Flewett, G. N. Woode, J. C. Bridger, D. R. Snodgrass, and J. A. Herring.** 1977. Serological relationship between rotaviruses from different species as studied by complement fixation and neutralization. Arch. Virol. **53**:287–294.
33. **Vandervelde, E. M., B. J. Cohn, and V. E. Cossart.** 1977. An enzyme-linked immunosorbant assay for hepatitis B surface antigen. J. Clin. Pathol. **30**:714–716.
34. **Voller, A., D. Bidwell, and A. Bartlett.** 1976. Microplate enzyme immunoassays for immunodiagnosis of viral infections, p. 506–512. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
35. **Woode, G. N., J. C. Bridger, J. M. Jones, T. H. Flewett, A. S. Bryden, H. A. Davies, and G. B. White.** 1976. Morphological and antigenic relationships between viruses (rotavir-

uses) from acute gastroenteritis of children, calves, piglets, mice, and foals. Infect. Immun. **14**:804–810.

36. **Wyatt, R. G., W. D. James, and E. H. Bohl.** 1980. Human rotavirus type 2: cultivation in vitro. Science **207**:189–191.
37. **Wyatt, R. G., A. Z. Kapikian, T. S. Thornhill, M. M. Sereno, H. W. Kim, and R. M. Chanock.** 1974. In vitro cultivation in human fetal intestinal organ culture of a reovirus-like agent associated with nonbacterial gastroenteritis in infants and children. J. Infect. Dis. **130**:523–528.
38. **Yolken, R. H., B. Barbour, R. G. Wyatt, A. R. Kalica, A. Z. Kapikian, and R. M. Chanock.** 1978. Enzyme-linked immunosorbent assay for identification of rotavirus from different animal species. Science **201**:259–262.
39. **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 reovirus-like agent of infantile gastroenteritis. Lancet **ii**:263–266.