Evaluation of Two Enzyme Immunoassays for Detection of Human Rotaviruses in Fecal Specimens

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The two assays evaluated in this study (the Ridascreen rotavirus and the Pathfinder rotavirus) exhibited comparable sensitivities (100%) but highly divergent positive predictive values (93.74 and 57.7%, respectively) when compared on 393 specimens. This difference should be considered when using these tests on collectives with an unknown or low prevalence.

Enzyme immunoassays (EIAs) have replaced electron microscopy (EM) as the standard method for the detection of rotaviruses in stool samples in the 1980s (1, 3–6, 8–13, 15–18). Typically, these assays are applied on stool specimens from children younger than 2 years with diarrheal disease. Since the past few years, however, we have observed an increasing tendency in clinical practice to include antigen detection assays in pretransplantation evaluation protocols. Therefore, in the present study we examined the impact of this strategy on the predictive values of two representative commercial test kits for rotavirus antigen detection in stool samples: the Ridascreen rotavirus (R-Biopharm, Darmstadt, Germany) as an automated test in microtiter plate format and the Pathfinder rotavirus (Kallestadt, Austin, Tex.) as a manually performed test designed for small series or single specimens. EM served as a supportive method for defining a formal “gold standard.”

As a high-risk collective for our study, 192 stool samples were acquired from the pediatric unit of the university hospital of Münster during the rotavirus season in 1997 to 1998, which were either submitted with the clinical diagnosis of enteritis or macroscopically liquid (mostly both). As a low-prevalence collective, 201 formed stools from healthy adults were randomly selected from a series of routine examinations from the Institute of Public Health, North Rhine-Westphalia. Both EIAs were performed exactly as specified by the manufacturers. Specimens exhibiting borderline results were retested once, and the latter result was used for evaluation. EM was performed by

standard methods with negative staining after concentration of virus particles at $100,000 \times g$ (7). Specimens without clearly recognizable virus particles were considered negative after an examination time of 20 min at $\times 50,000$ magnification. To define a formal gold standard, all samples which had positive results in at least two of the three methods were regarded as true positive.

An overview of the results is shown in Table 1. A total of 15 samples in this study were considered true positive; 14 of these were recognized by EM (sensitivity, 93.9%), and none of the negative samples was wrongly considered positive (specificity, 100% [Table 1]). The Pathfinder test recognized all positive samples as positive (sensitivity, 100%) but gave 11 false-positive results (specificity, 79.1% [Table 1]), 1 of which emerged after retesting a primarily borderline specimen (specimen 1302 [Table 2]). The Ridascreen test also recognized all true-positive specimens, but it gave one false positive result (sensitivity, 100%; specificity, 99.73%). A detailed analysis of the 11 “false-positive” pathfinder results (Fig. 1; Table 2) showed that the majority of these (8 of 11) were not caused by optical densities (ODs) near the cutoff; thus, specificity could not have been substantially improved by increasing the cutoff. Eight of the false-positive Pathfinder results were obtained in the adult group with formed stools. The positive and negative predictive values (PPV and NPV, respectively) were 100 and 99.73% for EM, 93.74 and 100% for the Ridascreen rotavirus, and 57.7 and 100% for the Pathfinder rotavirus. These numbers were

TABLE 1. Summary of results

Test	No. of results that were:				Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
	Concordantly positive	Concordantly negative	False negative	False positive				
EM	14	378	1	0	93.3	100.00	99.73	100.00
Pathfinder rotavirus	15	367	0	11	100.00	79.10	100.00	57.70
Ridascreen rotavirus	15	377	0	1	100.00	99.73	100.00	93.74

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TABLE 2. Raw data of the 11 discordant positive results obtained with the Pathfinder test^a

Sample ID	Qual. 1	Qual. 2	OD 1	OD 2	Cutoff 1	Cutoff 2
1113	Positive	Negative	0.104	0.028	0.089	0.086
1302	Positive	Borderline	0.321	0.093	0.089	0.086
1315	Positive	Positive	0.444	0.132	0.089	0.086
2939	Positive	Negative	0.162	0.059	0.086	0.088
2940	Positive	Negative	0.814	0.480	0.086	0.088
4919	Positive	Positive	0.090	0.182	0.088	0.086
5065	Positive	Positive	0.428	0.818	0.090	0.086
5848	Borderline	Positive	0.086	0.162	0.090	0.087
98002812	Positive	Positive	0.366	0.659	0.087	0.085
98002955	Positive	Positive	0.153	0.518	0.085	0.085
98005321	Positive	Positive	0.149	0.985	0.087	0.086

^a Numbers in the column headers indicate the number of the test (i.e., 1 = first test, 2 = second test); Qual., qualitative result.

calculated using the rotavirus prevalence in the entire specimen collective (3.82%; 15 of 393). If the evaluation was restricted to the "classical" patient subset for rotavirus tests (children younger than 2 years [$n = 124$]), the rotavirus prevalence increased to 11.29% (14 positives of 124 samples) and, consequently, the PPV of the Pathfinder rotavirus test improved to 82.35%. This was an effect not only of higher prevalence but also of an improved specificity itself (now only 3 false positives in 110 true negatives [97.27%]). Because the Pathfinder test is a manually performed test, we suspected that the relatively large number of false-positive results was due to a lack of care during the washing steps. We therefore retested all false-positive samples, taking special care with the washing steps. As shown in Table 2, retesting did not improve the performance significantly (9 of 11 false positives still remained positive).

In summary, it turned out that the EIAs were equivalent with respect to their sensitivities and were comparable or even

slightly superior to EM. This is in concordance with the majority of publications covering this subject (4, 5, 8, 12, 13, 15, 17, 18). Some investigators have postulated that EIAs are probably a better standard method for detection of rotaviruses than is EM due to the low sensitivity of the latter (2, 5); however, in these studies EM was performed without ultracentrifugation of samples, which has been demonstrated to be an essential step in achieving maximum sensitivity (7). Rabenau et al. (14) have also evaluated the Ridascreen rotavirus test along with a panel of other assays and shown that this test, after adjustment of the cutoff value, exhibited superior sensitivity and specificity values for the detection of rotaviruses, which is in accordance with our study. In contrast, the false-positive results of the Pathfinder rotavirus assay could not be erased by increasing the cutoff value (Table 2; Fig. 1). Thus, in view of the broad interassay variability seen on retesting of the specimens giving false-positive results with the Pathfinder rotavirus assay, the most probable explanation for these false-positive results is the manual test format, which cannot guarantee highly reproducible performance compared with an automated test system.

We conclude that the two tests evaluated here exhibit comparable performance when used in a "classical" patient collective. However, if specimens are obtained from an "atypical" patient collective, PPVs can drop dramatically and the minor-looking differences in specificity may become vital. This study clarifies that tests originally designed for confirmation of a strong clinical suspect cannot be recommended for screening purposes. However, if testing of individual patients from "atypical" collectives is explicitly requested, it is crucial to use the more specific tests (e.g., the Ridascreen rotavirus in the setting of this study).

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REFERENCES

- Beards, G. M., A. D. Campbell, N. R. Cottrell, J. S. Peiris, N. Rees, R. C. Sanders, J. A. Shirley, H. C. Wood, and T. H. Flewett. 1984. Enzyme-linked immunosorbent assays based on polyclonal and monoclonal antibodies for rotavirus detection. *J. Clin. Microbiol.* **19**:248–254.
- Birch, C. J., N. I. Lehmann, A. J. Hawker, J. A. Marshall, and I. D. Gust. 1979. Comparison of electron microscopy, enzyme-linked immunosorbent assay, solid-phase radioimmunoassay, and indirect immunofluorescence for detection of human rotavirus antigen in faeces. *J. Clin. Pathol.* **32**:700–705.
- Brandt, C. D., H. W. Kim, W. J. Rodriguez, 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.
- Cheung, E. Y., S. I. Hnatko, H. Gunning, and R. Thomas. 1990. Evaluation of combined commercial enzyme-linked immunosorbent assay for detection of rota and adenoviruses for automation. *J. Virol. Methods* **30**:333–337.
- Dennehy, P. H., D. R. Gauntlett, and S. E. Spangenberg. 1990. Choice of reference assay for the detection of rotavirus in fecal specimens: electron microscopy versus enzyme immunoassay. *J. Clin. Microbiol.* **28**:1280–1283.
- Gerna, G., A. Sarasini, B. S. Coulson, M. Parea, M. Torsellini, E. Arbustini, and M. Battaglia. 1988. Comparative sensitivities of solid-phase immune electron microscopy and enzyme-linked immunosorbent assay for serotyping of human rotavirus strains with neutralizing monoclonal antibodies. *J. Clin. Microbiol.* **26**:1383–1387.
- Hammond, G. W., G. S. Ahluwalia, F. G. Barker, G. Horsman, and P. R. Hazelton. 1982. Comparison of direct and indirect enzyme immunoassays with direct ultracentrifugation before electron microscopy for detection of rotaviruses. *J. Clin. Microbiol.* **16**:53–59.
- Julkunen, I., J. Savolainen, A. Hautanen, and T. Hovi. 1985. Detection of rotavirus in faecal specimens by enzyme immunoassay, latex agglutination and electron microscopy. *Scand. J. Infect. Dis.* **17**:245–249.
- Keswick, B. H., T. W. Hejkal, H. L. DuPont, and L. K. Pickering. 1983.

FIG. 1. Plot of the relative ODs of the pathfinder rotavirus and the ridascreen rotavirus EIA. The ODs are normalized by division with the cutoff of the respective individual run.

- Evaluation of a commercial enzyme immunoassay kit for rotavirus detection. *Diagn. Microbiol. Infect. Dis.* **1**:111–115.
10. **Knisley, C. V., A. 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.
  11. **Martin, A. L., and E. A. Follett.** 1987. An assessment of the sensitivity of three methods for the detection of rotavirus. *J. Virol. Methods* **16**:39–44.
  12. **Moosai, R. B., R. Alcock, T. M. Bell, F. R. Laidler, J. S. Peiris, A. P. Wyn-Jones, and C. R. Madeley.** 1985. Detection of rotavirus by a latex agglutination test, Rotalax: comparison with electron microscopy, immunofluorescence, polyacrylamide gel electrophoresis, and enzyme linked immunosorbent assay. *J. Clin. Pathol.* **38**:694–700.
  13. **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.
  14. **Rabenau, H., B. Knoll, R. Allwinn, H. W. Doerr, and B. Weber.** 1998. Improvement of the specificity of enzyme immunoassays for the detection of rotavirus and adenovirus in fecal specimens. *Intervirology* **41**:55–62.
  15. **Selb, B., H. G. Baumeister, G. Maass, and H. W. Doerr.** 1985. Detection of human rotavirus by nucleic acid analysis in comparison to enzyme-linked immunoassay and electron microscopy. *Eur. J. Clin. Microbiol.* **4**:41–45.
  16. **Svensson, L., M. Grandien, and C. A. Pettersson.** 1983. Comparison of solid-phase immune electron microscopy by use of protein A with direct electron microscopy and enzyme-linked immunosorbent assay for detection of rotavirus in stool. *J. Clin. Microbiol.* **18**:1244–1249.
  17. **Thomas, E. E., M. L. Puterman, E. Kawano, and M. Curran.** 1988. Evaluation of seven immunoassays for detection of rotavirus in pediatric stool samples. *J. Clin. Microbiol.* **26**:1189–1193.
  18. **Wall, R. A., B. J. Mellars, P. Luton, and S. Boulding.** 1982. Comparison of ELISA, SPACE, and electron microscopy for the routine diagnosis of rotavirus infection. *J. Clin. Pathol.* **35**:104–106.