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Optimum Diagnostic Assay and Clinical Specimen for Routine Rotavirus Surveillance[▽]

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Rates of detection of rotavirus were compared by diagnostic assay and specimen type. For bulk stools, rates of detection by reverse transcriptase PCR (RT-PCR) and enzyme immunoassay (EIA) were similar, but 18% of healthy controls tested positive by RT-PCR. Testing of bulk stools by EIA appears to be optimum for rotavirus surveillance.

Rotaviruses are the leading cause of severe acute gastroenteritis (AGE) in infants and young children worldwide (7). The most widely used method of diagnosis of rotavirus infection is antigen detection in fecal specimens using one of several commercial enzyme immunoassays (EIA). Reverse transcriptase PCR (RT-PCR) has been shown to increase the rate of detection of rotavirus in clinical specimens from patients with AGE 10 to 20% over that by EIA (11, 12). Two types of fecal specimens used for the diagnosis of rotavirus are bulk or whole-stool specimens (collected from a diaper or a collection cup) and rectal swabs. Data comparing the rates of detection of rotavirus in these specimens by using different assays are needed in order to find a balance between diagnostic practicality and the most clinically meaningful rates of detection.

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We examined existing bulk stool and rectal swab samples collected from 1 March to 30 June 1999 as part of an active surveillance study conducted at the Cincinnati Children's Hospital Medical Center (CCHMC) (2). In brief, children below the age of 5 years with AGE presenting to the CCHMC emergency department were enrolled if they met study criteria (2). During this period, 165 children provided either a bulk stool specimen or a rectal swab. Sixty bulk specimens were randomly selected, and then each was matched with a swab specimen on the basis of the children's ages (±3 months) and the dates of collection (±2 weeks).

To evaluate the rate of rotavirus detection in healthy children, an existing set of bulk stool specimens from nonhospitalized children in the same community who were free of symptoms of diarrhea or vomiting for at least 14 days prior to specimen collection was tested. The specimens from healthy children were randomly selected at a 1:2 frequency to match those of the children with AGE on age (±3 months) and date of collection (±2 weeks).

Specimens were tested for rotavirus antigen by a Rotaclone commercial microplate EIA (Meridian Diagnostics) and for rotavirus RNA by conventional RT-PCR with VP6 primers at the Centers for Disease Control and Prevention (CDC). For RT-PCR, briefly, RNA was extracted from stool specimens by a guanidine-silica-based method and was analyzed by RT-PCR for the rotavirus VP6 gene by using a commercial one-step kit (Qiagen) (4, 6). Differences were evaluated for statistical significance ($P < 0.05$) by a chi-square test for the assay and specimen type and by a Wilcoxon rank-sum test for the severity score and age.

In total, 145 specimens were analyzed. Fifty-seven bulk stools and 60 rectal swabs were from children with AGE, and 28 bulk stools were from healthy children. For children with AGE from whom bulk samples were obtained, illness, determined by a modified 17-point Vesikari score (14), was similar to that of patients from whom rectal swabs were obtained ($P = 0.60$). The mean age of children classified with severe disease was 16 months (95% confidence interval, 10 to 22 months), with no age difference by specimen type ($P = 0.57$).

The results of RT-PCR and EIA testing are displayed in Table 1. The rate of detection of rotavirus in children with AGE was significantly greater with bulk stool specimens than with rectal swabs by EIA (49% versus 27%; $P = 0.01$). There was no significant difference by assay when bulk specimens were evaluated (49% versus 53% for EIA and RT-PCR, respectively; $P = 0.71$). No stools from healthy controls were positive by EIA, but five (18%) were positive by RT-PCR. Therefore, the difference in the rate of detection of rotavirus in bulk stool specimens between children with AGE and healthy controls was greater by the EIA (49 versus 0%, respectively; $P < 0.01$) than by RT-PCR (53 versus 18%, respectively; $P < 0.01$), although the rates were significantly different by both methods.

Our results confirm that RT-PCR is more sensitive than EIA for the detection of rotavirus in fecal specimens, but they also indicate that the high sensitivity of RT-PCR limits its utility for routine rotavirus surveillance. Whereas none of the healthy

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TABLE 1. Rates of detection of rotavirus by RT-PCR and EIA testing[a]

Comparison	Total no. of samples	No. (%) of samples positive by:	
		RT-PCR	EIA
By specimen type (for AGE patients)			
Bulk stool	57	30 (53)	28 (49)[b]
Rectal swab	60	25 (42)	16 (27)
By patient type (bulk stool specimens)			
AGE patients	57	30 (53)	28 (49)
Healthy children	28	5 (18)	0 (0)

[a] Bulk stool and rectal swab specimens from children with AGE, and bulk stools from healthy controls, were tested.

[b] $P = 0.01$ for comparison of bulk stool and rectal swab specimens by the chi-square test.

controls evaluated were positive by EIA testing, RT-PCR detected a viral genome in about 18% of controls. Contamination of the RT-PCR assay is unlikely, since internal negative controls in each assay batch were monitored, and all were negative. Viral shedding from current asymptomatic infections or resolving previous infections is a more likely explanation. Detection of rotavirus in children without AGE is well documented (1, 3), and extended excretion, detectable by RT-PCR, from severe rotavirus infections is also known to occur (8). The lower sensitivity of the EIA than of RT-PCR apparently allows the EIA to distinguish between the higher level of viral shedding that likely occurs with acute rotavirus illness and the lower level of shedding from an asymptomatic infection or a resolving infection, making EIA results easier to interpret in terms of their clinical significance.

Our results also suggest that a bulk or whole-stool specimen is superior to a rectal swab specimen for rotavirus detection, especially with the EIA. By EIA testing, the rate of detection of rotavirus in bulk stools was significantly higher than that in rectal swabs. The smaller amount of fecal material contained in swab specimens likely resulted in reduced sensitivity of detection by EIA. This hypothesis is supported by the observation that RT-PCR, which can detect smaller amounts of virus, increased the detection rate of rotavirus in rectal swab specimens by 15%, from 27% to 42%.

We did not obtain both a rectal swab and a bulk stool specimen from the same child for this evaluation, and while patients were matched, it is possible that children with bulk stool and rectal swab specimens were not directly comparable. The specimens used in this evaluation had been stored 8 years before the testing for this study was conducted. While rotavirus is durable, the freeze-thaw cycles after collection may have resulted in a reduction of the amount of detectable virus. The tests we compared in this study were all performed in 2007, but it remains possible that detection of antigen by EIA was affected more than detection of RNA by RT-PCR, because the amount of antigen necessary for detection by EIA is larger than the amount of RNA needed for detection by RT-PCR.

In conclusion, the findings of our study support the recommendations in the WHO generic protocol for hospital- and community-based surveillance of rotavirus disease (13) that bulk stool specimens should be obtained from children with AGE and should be tested by EIA for purposes of routine surveillance.

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