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Field Evaluation of a Rota- and Adenovirus Immunochromatographic Assay Using Stool Samples from Children with Acute Diarrhea in Ghana▽

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We evaluated the Rida Quick rotavirus/adenovirus Combi rapid immunochromatographic test (ICT) under field conditions with Ghanaian children with acute diarrhea. Compared to PCR results, sensitivities and specificities were 75% and 95% for rotavirus and 22% and 84% for adenovirus. In resource-poor settings, ICTs may help to overcome difficulties in the diagnosis of rotavirus infection.

Diarrhea is a major cause of childhood morbidity and mortality in socioeconomically developing countries. Annually, more than 1 billion episodes of diarrhea occur among children under 5 years of age, causing approximately 2.5 million deaths, particularly in tropical regions (8, 10, 16). Acute childhood diarrhea is commonly caused by viruses, notably rotavirus (11). However, in underdeveloped regions, data on the prevalence of enteropathogenic viruses are sparse, since laborious and expensive virological testing is usually not performed (3). In this regard, inexpensive and easy-to-perform microbiological tools for viral detection are needed to (a) avoid unnecessary and potentially harmful antibiotic treatment and (b) improve the knowledge on the epidemiology of childhood diarrhea. The latter is becoming increasingly important considering the recent introduction of two rotavirus vaccines. Especially in resource-poor countries where respective data are widely lacking, solid information on prevalence, morbidity, and mortality are prerequisites for successful vaccine implementation (4, 14).

Several "point-of-care" immunochromatographic tests (ICTs) for viral enteropathogens such as rota-, adeno-, or noroviruses are commercially available (7). Only a few studies have analyzed their validity under tropical field conditions. The aim of the present study was to evaluate the performance of an ICT for rota- and adenovirus among Ghanaian children with acute diarrhea, setting the results of specific PCR assays as the reference.

The stool samples were derived from 238 children with acute diarrhea who were enrolled in a comprehensive clinicoepidemiological study on childhood diarrhea in Ghana between November 2005 and January 2006. Further study details, such as patient recruitment and frequencies of the detected parasitic and bacterial enteric pathogens, are described elsewhere (K. Reither, R. Ignatius, T. Weitzel, A. Seidu-Korkor, L. Anyidoho, E. Saad, A. Djie-Maletz, P. Ziniel, F. Amoo-Sakyi, F. Danikuu, S. Danour, R. N. Otchwemah, E. Schreier, U. Bienzle, K. Stark, and F. P. Mockenhaupt, submitted for publication). The patients had a mean age of 10 months (range: 15 days to 11 years). Samples were taken and processed by trained health workers at the Bulpeila Health Centre in Tamale, Northern Ghana. One fresh aliquot was used to perform the Rida Quick rotavirus/adenovirus Combi test (R-Biopharm AG, Darmstadt, Germany), a lateral-flow immunochromatographic assay, which uses labeled monoclonal antibodies against surface antigens of rota- and adenoviruses. The test had a dipstick format, was easy to handle, and did not require extensive laboratory work experience. The kits were stored at 4°C and the assays performed according to the manufacturer's instructions. Briefly, each stool sample was mixed with extraction buffer before the test strip was applied to the supernatant in a separate tube. After an incubation of five minutes at room temperature, the test results, including an internal quality control, were visible. In total, the test took less than 20 min. Another aliquot of each stool sample was immediately frozen at −20°C. After shipment on dry ice to Germany, specimens were tested at the Robert Koch Institute in Berlin by nested PCR for the presence of rota- or adenoviruses as described previously (9, 12). Both methods, ICT and PCR, detected only rotavirus group A but all adenovirus types. Samples were defined as positive for rota- or adenovirus if the respective viral nucleic acid was detected by PCR. For data analysis, StatView statistical software (SAS Institute Inc., Cary, NY) was used.

By PCR, rota- and adenoviruses were detected in 133 (55.9%) and 67 (28.2%) samples, respectively (Table 1). The rotavirus results of ICT and PCR were concordant in 84.5% (95% confidence interval [CI], 79.9% to 89.1%) of cases. Five false-positive results occurred, and 32 rotavirus-positive samples were missed by the ICT. The respective sensitivity and

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TABLE 1. Diagnostic characteristics of Rida Quick rotavirus/adenovirus Combi test[a]

Pathogen and PCR result	No. of specimens		% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
	ICT positive	ICT negative				
Rotavirus			75 (69–83)	95 (91–99)	95 (91–99)	76 (68–84)
Positive	101	32				
Negative	5	100				
Adenovirus			22 (12–32)	84 (79–90)	36 (21–50)	74 (68–84)
Positive	15	52				
Negative	27	144				

[a] Sensitivities and specificities are in comparison to those of PCR. PPV, positive predictive value; NPV, negative predictive value.

specificity values for rotavirus detection by the ICT were 75% and 95%, yielding positive and negative predictive values of 95% and 76%, respectively, within our study population. For adenovirus, concordance between ICT and PCR results was observed for 66.8% (95% CI, 60.8% to 72.8%) of samples. The sensitivity, specificity, and positive and negative predictive values of the ICT for adenovirus were 22%, 84%, 36%, and 74%, respectively. The ICT provided the correct diagnosis for 7 of 32 patients with dual infections. In the remaining cases, either rotavirus ($n = 2$), adenovirus ($n = 15$), or both infectious agents ($n = 8$) were missed by the ICT.

In contrast to classical detection methods for viral stool pathogens, such as electron microscopy, enzyme immunoassay (EIA) or PCR, ICTs can be performed without elaborate laboratory equipment and are therefore suitable for field use in tropical regions. Studies of rotavirus ICTs which were mainly done under laboratory conditions in industrialized countries reported sensitivities of 94% to 100% and specificities of 96% to 100% compared with the results of methods such as latex agglutination, electron microscopy, or EIA (5, 6, 13, 15). At 75%, our study's sensitivity for rotavirus detection was lower. Reasons could be climate conditions in Ghana or the handling of tests and samples by study personnel without extensive laboratory experience. Alternatively, the observed lower sensitivity could result from the applied gold standard, since PCR is by far the most sensitive method for the detection of rotaviruses (1). Only one other study evaluated the reliability of a rotavirus ICT under field conditions in a developing country. In accordance with our results, it reported sensitivity and specificity values of 70.5% and 95%, respectively, compared with PCR results (2).

Data on the performance of adenovirus stool ICTs have not been published yet. In our study, both the sensitivity and specificity of the adenovirus ICT were poor. The reasons for the unsatisfactory results remained uncertain. Again, false-negative results could stem from the high sensitivity of the PCR. However, PCR possibly overestimates the true burden of acute adenovirus infection, since patients might shed low virus numbers for weeks to months after enteric or respiratory adenovirus infections. The unsatisfactory performance of the adenovirus component of the assay evaluated may also have local, e.g., climatic, reasons. Nevertheless, for a setting such as the study area, its results are hardly informative.

For the detection of rotavirus under the conditions of an African health center, the evaluated ICT proved to be a practical, rapid, and sufficiently reliable tool. Realistically, however, even if a retail price of, e.g., EUR 1 would be possible, in developing countries such devices might suffer low acceptance by the individual patient and by the health system. Nevertheless, rotavirus ICTs could easily be applied in epidemiological studies to provide baseline data on the usefulness and success of rotavirus vaccination programs and to reduce the amount of unnecessary antibiotic treatment.

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