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Evaluation of a rapid immunochromatographic assay for the detection of rotavirus, norovirus and adenovirus from children hospitalized with acute watery diarrhoea

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SUMMARY

We evaluated the IP-Triple I immunochromatographic rapid test for the detection of rotavirus, norovirus and adenovirus using stool samples from children with diarrhoea. The detection of norovirus and adenovirus was poor compared to polymerase chain reaction assays. However, high sensitivity (92%) and specificity (99%) were obtained for the detection of rotavirus.

Introduction

Diarrhoeal illnesses are a leading cause of morbidity and mortality in children throughout the world. Globally, an estimated 1.5 million children die from these illnesses each year (1). Many viral, bacterial and protozoan agents have been associated with diarrhoeal illnesses in children. However, viruses such as rotavirus, norovirus and adenovirus have been established as major causes of paediatric diarrhoea in both developed and developing country settings. Rotavirus and norovirus in particular are associated with over 40% of all diarrhoeal episodes in developing countries (2,3). Adenovirus subgenus F (types 40 and 41) has also been confirmed as an important cause of diarrhoea in young children (4).

Materials and Methods

Rapid identification of aetiological agents of outbreaks is essential so that appropriate treatment and control procedures can be implemented. However, diagnostic capacity is lacking in most resource-limited countries and hence there is the potential application of rapid diagnostic tests (RDTs) for outbreak investigations and disease surveillance in these settings. In this study we evaluated a one-step rapid immunochromatographic assay (IP-Triple I, ImmunoProbe Co. Ltd, Japan) for the simultaneous detection of rotavirus, norovirus and adenovirus from stool samples.

A total of 199 stool samples collected from children aged <5 years admitted for acute gastroenteritis at Goroka Provincial Hospital, Papua New Guinea were included in this study. All samples were collected within 48 hours of hospitalization, transported to the laboratory at 4°C and then stored at -80°C until required for analysis. The commercial IP-Triple I

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assay was conducted on all 199 samples according to the manufacturer's instructions. Stool samples (semi-solid – 50 mg; liquid – 50 µl) were added to 800 µl of extraction buffer, mixed thoroughly and then left on the bench for 3 minutes. The sample (80 µl) was then added to the sample well. Following a 15-minute incubation at room temperature the samples were analysed independently by two experienced researchers. The presence of a visible red/violet line on the control line indicated a valid result; a red/violet line on test line 1 indicated a positive result for norovirus; a black line on test line T indicated a positive result for rotavirus; and a red/violet line on test line 2 indicated a positive result for adenovirus.

Nucleic acids were extracted using the Qiagen DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Eluates were tested for rotavirus, norovirus (G1 and G2) and adenovirus (40 and 41) using a range of real-time polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) assays (5,6) as reported in Soli et al. (7). For the RNA viruses, norovirus and rotavirus, the QuantiTect Real-Time RT-PCR Mastermix (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. For adenovirus (a DNA virus) the QuantiTect Real-Time PCR Mastermix (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. Positive and negative controls were included with each run to ensure the validity of results and all samples were independently tested in duplicate to ensure the repeatability of results.

The diagnostic accuracy of the IP-Triple I test was determined by comparing the results with real-time PCR/RT-PCR results. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated in Excel (Microsoft Corporation, Redmond, WA, USA), as described by the Diagnostics Evaluation Expert Panel of TDR (Tropical Diseases Research – the Special Programme for Research and Training on Tropical Diseases, World Health Organization) (8), but using the Wilson score method to calculate confidence intervals (9).

Results and Discussion

Rotavirus was detected in approximately one-quarter of all samples: the IP-Triple I detected rotavirus in 49/199 samples, with 51/199 samples positive by real-time RT-PCR. The sensitivity (92%), specificity (99%), PPV (0.96) and NPV (0.97) of the IP-Triple I were high for rotavirus detection (Table 1).

Detection rates of norovirus (RDT: 3/199; PCR: 19/199) and adenovirus (RDT: 6/199; PCR: 23/199) were considerably lower than for rotavirus. The IP-Triple I assay detected norovirus in 1.5% of samples, whereas norovirus G1 and norovirus G2 were detected by PCR in 3.5% and 6.0% of samples, respectively. The samples were subsequently tested by additional norovirus G1 and norovirus G2 real-time RT-PCR assays to confirm the results (10). The additional assays showed a high degree of correlation with the original real-time RT-PCR assays (data not shown), but poor correlation with the RDT. The IP-Triple I detected adenovirus in 3.0% of samples compared to 11.6% by real-time PCR. The IP-Triple I lacks sensitivity and has low PPVs for norovirus and adenovirus (Table 1), suggesting that the assay is unsuitable for the detection of norovirus and adenovirus in diarrhoeal specimens in Papua New Guinea. Future genotyping studies on Papua New Guinean norovirus and adenovirus strains may provide evidence for why the RDT did not correlate with the real-time PCR/RT-PCR results.

In this study we have shown the utility of a rapid immunochromatographic assay for the detection of rotavirus. Similarly, evaluation of other RDTs for the detection of rotavirus have reported high sensitivity (88-97%) and specificity (93-100%) (11). In comparison, a recent evaluation of three commercially available enzyme immunoassay kits, which are commonly used in rotavirus surveillance networks, reported sensitivities of 75-83% and specificities of 100% (12). The World Health Organization has recommended that rotavirus vaccination be included in all national immunization programs (13) and thus there will be an increased need for disease surveillance to monitor vaccine efficacy. As such the IP-Triple I test and other RDTs should be further evaluated to determine their utility for such surveillance activities, and also potentially for use in rotavirus outbreak investigations.

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TABLE 1

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF THE IP-TRIPLE I ASSAY FOR THE DETECTION OF ROTAVIRUS, NOROVIRUS AND ADENOVIRUS WHEN COMPARED TO REAL-TIME PCR/RT-PCR

Virus	Sensitivity*	Specificity*	PPV†	NPV†
Rotavirus	92.2 (81.5-96.9)	98.6 (95.2-99.6)	0.959 (0.863-0.989)	0.973 (0.933-0.990)
Norovirus	10.5 (2.9-31.4)	99.4 (96.9-99.9)	0.667 (0.208-0.939)	0.913 (0.864-0.946)
Adenovirus	6.3 (1.1-28.3)	97.3 (93.8-98.8)	0.167 (0.030-0.564)	0.922 (0.876-0.952)

RT-PCR = reverse transcription-polymerase chain reaction
PPV = positive predictive value
NPV = negative predictive value
*Percentage (95% confidence interval)
†Value (95% confidence interval) RT-PCR = reverse transcription-polymerase chain reaction

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REFERENCES

1 **Wardlaw T, Salama P, Brocklehurst C, Chopra M, Mason E.** Diarrhoea: why children are still dying and what can be done. *Lancet* 2010;375:870-872.
2 **Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD.** Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis* 2008;14:1224-1231.
3 **Widdowson MA, Steele D, Vojdani J, Wecker J, Parashar U.** Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. *J Infect Dis* 2009;200(Suppl 1):S1-S8.
4 **Uhnoo I, Wadell G, Svensson L, Johansson ME.** Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J Clin Microbiol* 1984;20:365-372.
5 **Logan C, O'Leary JJ, O'Sullivan N.** Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. *J Clin Microbiol* 2006;44:3189-3195.
6 **van Maarseveen NM, Wessels E, de Brouwer CS, Vossen AC, Claas EC.** Diagnosis of viral gastroenteritis by simultaneous detection of adenovirus group F, astrovirus, rotavirus group A, norovirus genogroups I and II, and sapovirus in two internally controlled multiplex real-time PCR assays. *J Clin Virol* 2010;49:205-210.
7 **Soli KW, Maure T, Kas MP, Bande G, Bebes S, Luang-Suarkia D, Siba PM, Morita A, Umezaki M, Greenhill AR, Horwood PF.** Detection of enteric viral and bacterial pathogens associated with paediatric diarrhoea in Goroka, Papua New Guinea. *Int J Infect Dis* 2013, in press.
8 **Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R, O'Brien R, Perkins M, Cunningham J, Matsoso P, Nathanson CM, Olliaro P, Peeling RW, Ramsay A; TDR Diagnostics Evaluation Expert Panel (WHO/TDR).** Evaluation of diagnostic tests for infectious diseases: general principles. *Nat Rev Microbiol* 2006;4(12 Suppl):S21-S31.
9 **Newcombe RG.** Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 1998;17:857-872.
10 **Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K.** Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol* 2003;41:1548-1557.
11 **Khamrin P, Tran DN, Chan-it W, Thongprachum A, Okitsu S, Maneekarn N, Ushijima H.** Comparison of the rapid methods for screening of group A rotavirus in stool samples. *J Trop Pediatr* 2011;57:375-377.
12 **Gautam R, Lyde F, Esona MD, Quaye O, Bowen**

MD. Comparison of Premier™ Rotaclone®, ProSpecT™, and RIDASCREEN® rotavirus enzyme immunoassay kits for detection of rotavirus antigen in stool specimens. *J Clin Virol* 2013;58:292-294.

13 **World Health Organization.** Global use of rotavirus vaccines recommended: vaccines can protect millions of children from diarrhoeal disease. Geneva: World Health Organization, 2009. www.who.int/mediacentre/news/releases/2009/rotavirus_vaccines_20090605/en/