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SHORT COMMUNICATION

Assessing the Effectiveness of Fast-Track Diagnostic Kit for Detecting Viral Gastroenteritis Agents

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SUMMARY

Background: **Viral gastroenteritis is a significant global health concern. An effective, rapid, and easy-to-use diagnostic tool is essential for screening causative viruses.**
Methods: **Forty-eight samples, known to be infected with one of the following viruses: norovirus, group A rotavirus, astrovirus, adenovirus, and sapovirus determined by reverse transcription-PCR and nucleotide sequencing, were evaluated by the Fast Track Diagnostics (FTD) viral gastroenteritis assay.**
Results: **The assay demonstrated 100% specificity for all viruses and matched the RT-PCR sensitivity for norovirus GI, classic human astrovirus, adenovirus, and sapovirus. It identified norovirus GII and rotavirus with 87.5% and 85.7% sensitivity, respectively. However, its sensitivity for detecting novel human astrovirus MLB and VA was lower, at 35%.**
Conclusions: **The FTD viral gastroenteritis assay can effectively screen simultaneously for norovirus GI, GII, group A rotavirus, adenovirus, and sapovirus in clinical settings. The study also suggests that improved detection methods are necessary for novel astrovirus strains.**
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Fast Track Diagnostics kit, acute viral gastroenteritis, Japan

LIST OF ABBREVIATIONS

FTD - Fast Track Diagnostics
RVA - Group A rotavirus
NoV - Norovirus
AdV - Adenovirus
SaV - Sapovirus
AGE - Acute gastroenteritis
RT-PCR - Reverse transcription- Polymerase chain reaction

INTRODUCTION

Viral gastroenteritis is the most common disease affecting all ages, especially children under 5. It remains a significant contributor to global morbidity and mortality. Numerous viruses have been identified as causative agents of the disease. Notably, group A rotavirus (RVA), norovirus (NoV), adenovirus (AdV), and sapovirus (SaV) are the primary agents frequently found in most cases of the illness [1]. Rotavirus, a member of the *Reoviridae* family, comprises at least ten groups designated as A to J. RVA is a significant pathogen causing diarrhea in humans, with a notable impact on young children [2]. NoV and SaV are members of the *Caliciviridae* family and are recognized as a significant etiological agent of both outbreaks and sporadic cases of acute gastroenteritis (AGE) across various age groups. A distinctive feature of NoV is the considerable amino acid sequence variation in the VP1 region, leading to the classification of ten genogroups (GI-GX). Among these genogroups, GI and GII are the most prevalent and are associated with human diseases. They can be further divided into nine genotypes (GI.1-GI.9) and 26 genotypes (GII.1-GII.14, GII.16-GII.27), respectively [3]. Currently, SaV is divided into 19 genogroups (GI to GXIX). Among these, GI, GII, GIV, and GV infect humans, with GI subdivided into seven genotypes (GI.1 to GI.7) and GII into eight genotypes (GII.1 to GII.8) being the most predominant genotype [4]. AstV is one of the common causes of viral gastroenteritis, following norovirus and sapovirus. At present, numerous genotypes of the classic HAstV (HAstV1 to -V8), as well as novel MLB HAstV genotypes (MLB1 to -3) and VA HAstV genotypes (VA1 to -5), have been identified [5]. AdV, a member of the *Adenoviridae* family, is widely acknowledged as a significant pathogen capable of infecting individuals across all age groups, with a particularly high incidence among children [6].

There has been a consistent need for the development of efficient and reliable diagnostic methods for identifying primary AGE viruses, including NoV, RVA, AstV, AdV, and SaV. While various laboratory techniques are available for the detection of these viruses, PCR-based methods are widely regarded as the gold standard due to their sensitivity and specificity [7,8]. In this study, we evaluated the effectiveness of the FTD Viral GE, a multiplex real-time PCR kit from Fast Track Diagnostics, Luxembourg, using samples previously confirmed to be infected with NoV, RVA, AstV AdV, and SaV, by reverse transcription (RT)-PCR and nucleotide sequencing.

MATERIALS AND METHODS

The samples were collected from pediatric outpatients in Japan diagnosed clinically with AGE by pediatricians in six prefectures: Hokkaido, Tokyo, Shizuoka, Kyoto, Osaka, and Saga, in which 35 samples were mono-infection including NoV: 7 (GI: 1 and GII: 6), RVA: 5, AstV: 15, AdV: 2, and SaV: 6, 13 samples were co-infection including NoV GI and GII: 1, NoV GII and RAV: 1, NoV GII and AstV: 8, AstV and AdV: 1, AstV and SaV: 1, and RVA and SaV: 1. All samples were stored at -30℃ until use. The study received approval from the Ethics Committee of the Nihon University School of Medicine in Tokyo, Japan (29-9-0, 29-9-1, 29-9-2).

The viral RNA was extracted from 140 µL of a 10% fecal suspension using the QIAamp viral RNA Mini kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's instructions. Subsequently, the RNA underwent multiplex real-time PCR analysis utilizing the FTD viral GE kit to detect NoV GI, NoV GII, AstV, RVA, AdV, and SaV [9,10]. The assay employed three tubes, each containing one set of primers and probe mix targeting different viruses: NoroPP for NoV GI and NoV GII, ARA PP for AstV, RV, and AdV, and Sapo PP for SaV. Briefly, 10 µL of RNA extract was mixed with 15 µL of each of the three master mixes of the FTD viral solutions in a final volume of 25 µL. The assay was performed on a QuantStudio5 Real-Time PCR System (Applied Biosystems, MA, USA) with the following thermal cycling conditions: 42℃ for 15 minutes, 94℃ for 3 minutes, 40 cycles of 94℃ for 8 seconds, and 60℃ for 34 seconds. In addition, a positive control and a negative control were included for each assay.

RESULTS AND DISCUSSION

Of the 48 samples positive with NoV, AstV, RVA, AdV, and SaV determined by RT-PCR and sequence analysis, 35 were single infections, and 13 showed co-infection (Table 1 and 2). It is worth noting that no false positive results were reported across all the tested viruses, indicating the high specificity of this kit (100%). Among the NoV samples, 17 were identified, with one belonging to NoV GI, specifically genotype 6 (GI.6), and 15 samples belonging to GII. This included five samples with single infections of genotype 2 (GII.2) and 1 sample of genotype 3 (GII.3), along with nine samples of genotype 4 (GII.4) co-infections with AstV (8 samples) and RVA (1 sample). Additionally, one sample exhibited mixed NoV GI and GII infections (GI.2 and GII.2). Regarding AstV, out of the 25 samples analyzed, 15 were single infections, while 10 showed co-infection. Classic HAstV accounted for 11 samples comprising HAstV1 (8 samples), HAstV2 (1 sample), and HAstV3 (2 samples). Additionally, novel MLB AstV was 12 (including MLB1: 6, MLB2: 4, and MLB3: 2), along with novel VA2 in 2 cases. Out of 7 RVA samples, 5 were single infections, comprising G1 (2 samples) and G2, G8, and G9 (1 sample each), 2 were co-infection in which one sample with NoV GII.4 (G4 and NoV GII.4) and the other with SaV (G2 and SaV GI.2). Among the 3 AdV samples, 2 HAdV-F41 were single infections, and the remaining one, HAdV-C5 was co-infection with

Table 1. Detection of single infection of norovirus, rotavirus, astrovirus, adenovirus, and sapovirus in clinical stool samples using FTD viral gastroenteritis kit.

Virus	Sample tested	Noro PP		ARA PP			Sapo PP	FTD positive/negative					
		NoV GI	NoV GII	AstV	RVA	AdV	SaV	NoV GI	NoV GII	AstV	RVA	AdV	SaV
NoV GI								**1/0**					
NoV GI.6	**1**	**1**						**1/0**					
NoV GII									**5/1**				
NoV GII.2	**5**		**4**						**4/1**				
NoV GII.3	**1**		**1**						**1/0**				
RVA											**5/0**		
G1	**2**				**2**						**2/0**		
G2	**1**				**1**						**1/0**		
G8	**1**				**1**						**1/0**		
G9	**1**				**1**						**1/0**		
AstV										**10/5**			
HAstV1	**5**			**5**						**5/0**			
HAstV2	**1**			**1**						**1/0**			
HAstV3	**2**			**2**						**2/0**			
MLB1	**2**			**1**						**1/1**			
MLB2	**2**			**1**						**1/1**			
MLB3	**1**			**0**						**0/1**			
VA2	**2**			**0**						**0/2**			
AdV												**2/0**	
AdV-F41	**2**					**2**						**2/0**	
SaV													**6/0**
GI.1	**2**						**2**						**2/0**
GII.1	**1**						**1**						**1/0**
GII.3	**2**						**2**						**2/0**
GII.8	**1**						**1**						**1/0**
Total	**35**							**1/0**	**5/1**	**9/6**	**5/0**	**2/0**	**6/0**

HAstV1. In the case of 7 SaV samples, 6 were single infection, comprising GI.1 (2 samples), GII.1 (1 sample), GII.3 (2 samples), and GII.8 (1 sample). The remaining sample (GI.2) presented a co-infection with RVA G2. To assess the sensitivities of the FTD viral GE kit, all samples were tested by utilizing FTD viral GE assay, and the result was compared with those of the conventional RT-PCR, as shown in Tables 1 and 2. In total, the FTD viral GE kit successfully detected all 13 instances of single and co-infection involving NoV GI (2 samples), AdV (3 samples), and SaV (8 samples), exhibiting comparable sensitivity to that of the RT-PCR assay. For detecting NoV GII, out of 16 samples tested, including six single infections and ten co-infections, 14 were positive, yielding a sensitivity of 87.5%, and the remaining two samples (one NoV GII.2 single infection and one co-infection of NoV GII.4 and RVA) tested negative. The sensitivity of the test was calculated at 85.7% for RVA, seven rotavirus samples underwent testing, encompassing RVA strains G1 (2 samples), G2 (2 samples), G4 (1 sample), G8 (1 sample), and G9 (1 sample). Among these, six tested positive for rotavirus using the FTD viral GE kit, while one sample of G4 tested negative. For the 25 AstV samples, as presented in Table 3, the FTD viral GE kit successfully identified all 11 classic HAstV strains (HAstV1: 8, HAstV2: 1, and HAstV3: 2). However, it failed to detect 7 out of 12 novel HAstV MLB strains and all 2 novel VA2 strains. It should be noted that FTD viral GE kit could detect several genotypes of classic HAstV, including HAstV1, HAstV2, and HAstV3. However, the kit's sensitivity was lower for novel HAstV MLB and VA, accounting

Table 2. Detection of co-infection of norovirus, rotavirus, astrovirus, adenovirus, and sapovirus in clinical stool samples using FTD viral gastroenteritis kit.

Virus	Sample tested	Noro PP		ARA PP			Sapo PP	FTD positive/negative					
		NoV GI	NoV GII	AstV	RVA	AdV	SaV	NoV GI	NoV GII	AstV	RVA	AdV	SaV
NoV GI + NoV GII								1/0	1/0				
NoV GI.2 + GII.2	1	1	1					1/0	1/0				
NoV GII + RVA									0/1		0/1		
NoV GII.4 + RVA G4	1		0		0				0/1		0/1		
NoV GII + AstV									8/0	6/2			
NoV GII.4 + HAstV1	2		2	2					2/0	2/0			
NoV GII.4 + MBL1	3		3	1					3/0	1/2			
NoV GII.4 + MBL2	2		2	2					2/0	2/0			
NoV GII.4 + MBL3	1		1	1					1/0	1/0			
AstV + AdV										1/0		1/0	
HAstV1 + AdV-C5	1			1		1				1/0		1/0	
AstV + SaV										0/1			1/0
MBL1 + SaV GI.1	1			0			1			0/1			1/0
RVA + SaV											1/0		1/0
RVA G2 + SaV GI.2					1		1				1/0		1/0
Total	13							1/0	9/1	7/3	1/1	1/0	2/0

Table 3. Evaluation of the FTD viral gastroenteritis assay for the detection of astrovirus types.

Astrovirus types	RT-PCR	FTD viral gastroenteritis kit		
		positive	negative	% detected by FTD viral GE
Classic HAstV				
HAstV1	8	8	0	100
HAstV2	1	1	0	100
HAstV3	2	2	0	100
Total	11	11		100
Novel MLB				
MLB1	6	2	4	33.3
MLB2	4	3	1	75
MLB3	2	0	2	00
Novel VA				
VA2	2	0	2	00
Total	14	5	9	35.7%

for 35.7%. This inefficiency may be due to genetic differences between classical and non-classical astroviruses, potentially affecting the conserved regions targeted by the primers and probes. These findings are slightly different from those of the previous studies, which observed a high sensitivity of the FTD Viral GE assay for detecting NoV, SaV, and AstV, but lower sensitivity for AdV and RVA [10]. Additionally, perfect agreement was noted for RVA, SaV, and NoV, substantial agreement for AdV, and moderate agreement for AstV [11]. This study assessed the FTD viral GE kit for detecting NoV GI and GII, AstV, RVA, AdV, and SaV. The kit

showed high specificity and identified effectively single and co-infections, with performance comparable to conventional RT-PCR. While the kit was effective for classic AstV genotypes, a reduced sensitivity for novel AstV MLB and VA strains was noted. These findings highlight the kit's utility for screening primary causative viruses in clinical samples but suggest that future research should focus on improving its sensitivity and specificity for all viral strains.

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Declaration of Interest:
All authors have no conflicts of interest to declare.

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