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ORIGINAL ARTICLE

Evaluation of Immunochromatographic Test for Rapid Detection of Norovirus, Rotavirus, and Adenovirus in Stool Samples

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SUMMARY

Background: **Viral enteric infections are illnesses caused by several types of viruses that primarily affect the gastrointestinal system. Globally, three major viruses associated with gastroenteritis, including norovirus (NoV), rotavirus A (RVA), and human adenovirus (HAdV), have been widely recognized. Accordingly, a rapid and sensitive diagnostic tool, such as immunochromatographic (IC) test, for diarrheal virus detection is required and helpful for rapid diagnosis.**
Methods: **The IP-Triple I IC test kit for simultaneous triple virus detections of NoV, RVA, and HAdV was evaluated for its efficacy by using stool specimens that were known positive for these viruses by real-time RT-PCR or PCR, which was used as the gold standard method.**
Results: **The results revealed that the IP-Triple I IC test kit exhibited a very high level of specificity (100%) for NoV, RVA, and HAdV detections, while the sensitivity was slightly different for these three viruses. The sensitivity of detection for RVA was 86.7%, whereas those for NoV and HAdV were 70.6% and 76.2%, respectively. This IP-Triple I IC kit could detect common antigens of a wide range of NoV, RVA, and HAdV genotypes (NoV GII.2, GII.4, GII.6, GII.7, GII.10, GII.17, RVA G1P[8], G2P[4], G3P[8], G8P[8], G9P[8], HAdV-C1, -C2, -C5, -A12, -F40, and -F41).**
Conclusions: **Our results revealed that the IP-Triple I IC test kit is highly effective for simultaneous, direct detection of common antigens of several genotypes of NoV, RVA, and HAdV in stool specimens and useful for screening and rapid diagnosis of diarrheal viruses during the seasonal outbreak.**
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INTRODUCTION

Viral gastroenteritis is a common illness that affects individuals of all age groups and poses particular risks to young children and the elderly in many countries worldwide [1,2]. Gastroenteritis is the inflammation of the

stomach and intestine, causing a broad spectrum of symptoms affecting the gastrointestinal tract, which includes abdominal pain, nausea, vomiting, and diarrhea [1,2]. Among acute infectious nonbacterial gastroenteritis, norovirus (NoV), rotavirus (RV), and human adenovirus (HAdV) are recognized as the major causes of the disease [1].

Norovirus (NoV) is an enteric virus belonging to the *Caliciviridae* family. It is a non-enveloped, single-stranded positive-sense RNA virus and is presently distinguished into at least ten genogroups (GI-GX) [3]. Within each genogroup, they are further subdivided into numerous genotypes. Notably, NoV genogroup I (GI), GII, GIV, GVIII, and GIX have been recognized as the causative agents of human disease [3]. Among these, NoV GII.4 is the most predominant genotype that has been detected worldwide. The GII.4 Sydney variant has been detected as the major variant circulating around the world since its emergence in 2012 [4].

Rotavirus (RV) is a non-enveloped, double-stranded RNA virus of the *Sedoreoviridae* family and is classified into nine species (A, B, C, D, F, G, H, I, and J). Among these, rotavirus A (RVA) is the major virus that is responsible for the disease in humans, especially in children under five years of age [5-7]. The RVA G1P[8], G2P[4], G3P[8], and G9P[8] genotypes are found to be the most predominant genotypes circulating worldwide [7].

Human adenovirus (HAdV) is a non-enveloped, double-stranded DNA virus belonging to the *Adenoviridae* family [8]. These are currently categorized into ten major species (A - I), and over 100 genotypes have been identified [9]. HAdV is responsible for a broad spectrum of illnesses, including the upper or lower respiratory tract disease, conjunctivitis, and gastrointestinal tract disease. The HAdV infections are more prevalent among young children and immunocompromised patients. Among the HAdV species, HAdV-F40 and -F41 are the enteric viral pathogens that are associated with diarrheal illness in children worldwide [10-12].

When outbreaks of diarrhea occur, the rapid diagnosis is essential to ensure the administration of appropriate treatment and further infection control [13]. Among different diagnostic tools, immunochromatographic (IC) test strip is a simple and less time-consuming method that requires minimal equipment. Nowadays, numerous IC tests have been developed for identifying enteric viruses in stool samples [14-17]. In this study, we investigated the sensitivity and specificity of the IP-Triple I IC test kit for simultaneous detection of NoV, RVA, and HAdV on a single test strip.

MATERIALS AND METHODS

Among a total of 98 fecal specimens included in this study, 34 and 15 were positive for NoV and RVA, respectively, as tested by real-time RT-PCR, 21 were positive for HAdV by PCR, while 28 were negative for NoV, RVA, and HAdV by real-time RT-PCR/PCR. Among these, seven different genotypes of 34 NoV strains had been previously identified as GII.2[P16] (n = 6), GII.3[P12] (n = 1), GII.4[P16] (n = 14), GII.6[P7] (n = 5), GII.7[P7] (n = 4), GII.10[P16] (n = 2), and GII.17 [P17] (n = 2). For RVA, G1[P8] (n = 3), G2[P4] (n = 1), G3[P8] (n = 4), G8[P8] (n = 4), and G9[P8] (n = 3) were included in this study. In addition, HAdV-A12 (n = 1), -C1 (n = 3), -C2 (n = 3), -C5 (n = 4), -F40 (n = 4), and -F41 (n = 6) were tested by using this IP-Triple I IC test kit. The specimens were collected from pediatric patients aged less than five years old who were admitted with acute gastroenteritis to five hospitals in Chiang Mai, Thailand (Maharaj Nakorn Chiang Mai Hospital, Sriphat Medical Center, Sanpatong Hospital, Sansai Hospital, and Rajavej Chiang Mai Hospital), from 2018 to 2023.

All 98 samples were tested for NoV, RVA, and HAdV antigens by using the IP-Triple I IC kit (Immuno Probe Co., Ltd., Japan) following the manufacturer's guideline (Figure 1). In brief, 50 µL of stool were added into 800 µL of extraction buffer and mixed thoroughly. After 3 minutes, 80 µL of the supernatant were added into the sample well of the test cassette. The result of the test was read and interpreted within 15 minutes, based on the appearance of the lines on the test strip. The strip consisted of test (T) zone and control (C) zone as shown in Figure 2. The separate lines that appeared on the T zone indicated a positive result for HAdV, RVA, and NoV, whereas the line that appeared on the C zone indicated that the kit worked properly as shown in Figure 2A. The first line on the right side represented a positive result for NoV (Figure 2B), the second line represented a positive result for RVA (Figure 2C), and the third line represented a positive result for HAdV (Figure 2D). The result was considered negative, when a line appeared only on C zone without the line on T zone (Figure 2E).

To evaluate the sensitivity and specificity of the IP-Triple I IC kit, the results obtained from immunochromatographic assay were compared with those of the real-time reverse transcription polymerase chain reaction (real-time RT-PCR) for NoV and RVA, whereas those of HAdV were compared with those of conventional PCR [12,18,19].

RESULTS

Among the 98 stool samples included in this study, 34, 15, and 21 were positive for NoV GII, RVA by real-time RT-PCR, and HAdV by PCR, respectively, whereas 28 samples were negative for these three viruses by real-time RT-PCR or conventional PCR methods (Table 1). Among 34 NoV samples positive by real-time RT-PCR, 24 were positive by IP-Triple I IC kit (Table 1). This kit could detect NoV GII common antigen of several NoV genotypes, including GII.2[P16], GII.4[P16], GII.6[P7], GII.7[P7], GII.10[P16], and GII.17[P17]. For

Table 1. Detections of norovirus, rotavirus, and adenovirus by IP-Triple I immunochromatographic kit and real-time RT-PCR/PCR (gold standard method).

Viruses	Number of specimens tested [a]	IP-Triple I		Real-time RT-PCR		PCR	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Norovirus	34	24 (70.6)	10 (29.4)	34 (100)	0 (0)	N/A	N/A
Rotavirus	15	13 (86.7)	2 (13.3)	15 (100)	0 (0)	N/A	N/A
Adenovirus	21	16 (76.2)	5 (23.8)	N/A	N/A	21 (100)	0 (0)
Negative	28	-	-	-	-	-	-

[a] - These specimens were tested positive for norovirus (n = 34), rotavirus (n = 15), and adenovirus (n = 21), and 28 were negative for norovirus, rotavirus, and adenovirus by real-time RT-PCR/PCR.
N/A - not applicable; norovirus and rotavirus were detected by real-time PCR; adenoviruses were detected by PCR.

Table 2. Norovirus, rotavirus, and adenovirus genotypes and Ct-values as tested by real-time RT-PCR of the stool samples that were negative by IP-Triple I immunochromatographic kit.

Virus	Sample ID	Genotype	Ct-value
Norovirus	CMH-S023-20	GII.4[P16]	32.38
	CMH-ST001-20	GII.4[P16]	22.59
	CMH-R006-20	GII.17 [P17]	24.10
	CMH-S016-21	GII.3[P12]	33.10
	CMH-S082-21	GII.2[P16]	28.77
	CMH-S141-21	GII.6[P7]	31.80
	CMH-ST002-21	GII.6[P7]	18.18
	CMH-ST029-21	GII.2[P16]	28.80
	CMH-R015-21	GII.6[P7]	21.03
	CMH-R026-21	GII.7[P7]	31.49
Rotavirus	CMH-ST053-21	G1P[8]	33.00
	CMH-SS037-21	G1P[8]	34.95
Adenovirus	CMH-S112-21	HAdV-C2	N/A
	CMH-ST015-21	HAdV-C5	N/A
	CMH-ST016-21	HAdV-C5	N/A
	CMH-SS009-23	HAdV-C5	N/A
	CMH-ST030-19	HAdV-F40	N/A

N/A - not applicable; adenoviruses were detected by PCR.

Table 3. Sensitivity, specificity, and agreement of IP-Triple I immunochromatographic test for norovirus, rotavirus, and adenovirus detections in comparison with these of the gold standard methods.

	Viruses		
	Norovirus	Rotavirus	Adenovirus
Sensitivity (%)	70.6	86.7	76.2
Specificity (%)	100	100	100
Agreement (%)	89.8	98.0	94.9

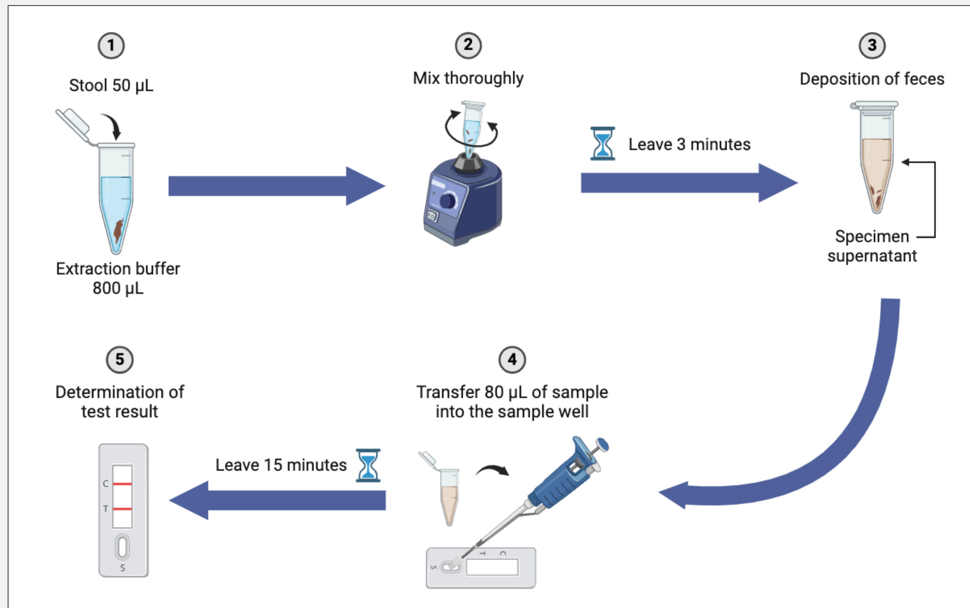

Figure 1. Illustration of the experimental workflow for immunochromatographic test.

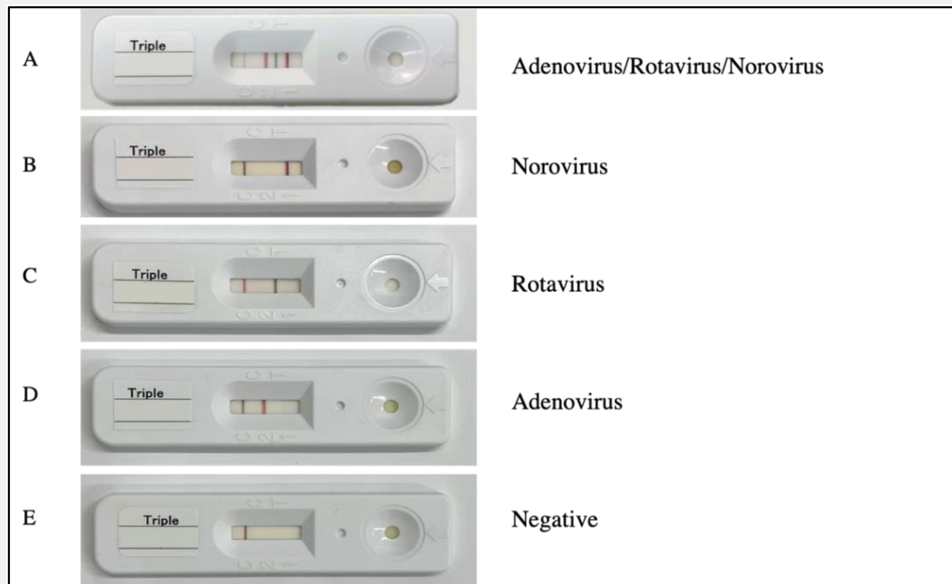

Figure 2. Detection of norovirus, rotavirus, and adenovirus in a stool sample, using the triplex immunochromatographic test (IP-Triple I).

The test presents positive results for all three viruses: norovirus, rotavirus, and adenovirus (A). The test line on the right side shows a positive result for norovirus (B). The test line on the middle shows a positive result for rotavirus (C). The test line on the left side shows a positive result for adenovirus (D). The control line on the left side is a control, which indicates that the kit works properly; if only this line appears, the sample is considered negative for all three viruses (E).

the remaining 10 samples that were negative by IP-Triple I, they belonged to GII.2[P16], GII.3[P12], GII.4 [P16], GII.6[P7], and GII.7[P7] genotypes (data not shown). It was observed that the NoV samples that were negative by IC kit showed cycle threshold (Ct) values over 20, as tested by real-time RT-PCR, with the exception of one sample (GII.6[P7]) that showed a Ct-value < 20 (Table 2). This observation demonstrated that low NoV viral load in stool samples could affect the results of IC kit testing.

For RVA detection, among 15 RVA samples positive by real-time RT-PCR, 13 were positive by IP-Triple I IC kit (Table 1). Out of these, the RVA common antigen of five RVA genotypes were detected by this IC kit, including G1P[8], G2P[4], G3P[8], G8P[8], and G9P [8]. However, this IP-Triple I IC kit could not detect the RVA common antigen of two samples of RVA G1[P8] genotype (data not shown). By real-time RT-PCR assay, these two samples had Ct-values higher than 33 (Table 2). The results suggest that the IC kit was unable to detect the RVA antigen in some samples with low levels of viral load.

For HAdV detection, out of 21 samples that were positive for HAdV by conventional PCR, 16 were positive for HAdV by the IP-Triple I IC kit (Table 1). This IC kit was capable of detecting HAdV common antigen of several HAdV genotypes, including HAdV-A12, -C1, -C2, -C5, -F40, and -F41 (data not shown). The remaining five samples that were negative by the IC test belonged to the HAdV-C2, -C5, and -F40 genotypes (Table 2).

Altogether, the sensitivity, specificity, and agreement of the IP-Triple I IC kit for detecting NoV, RVA, and HAdV were evaluated in comparison with those of the gold standard methods as shown in Table 3. The data showed that this IP-Triple I IC kit provided relatively high sensitivity for RVA at 86.7%, followed by those of HAdV at 76.2% and NoV at 70.6%. The specificity of the IP-Triple I IC kit was 100% for all three viruses. In addition, the agreement of the results, as tested by IP-Triple I IC kit and the gold standard methods (real-time PCR/PCR), was good, with very high agreement rates for detecting NoV, RVA, and HAdV at 89.8%, 98%, and 94.9%, respectively.

DISCUSSION

Viral gastroenteritis is an illness that is found globally and in severe cases can lead to significant morbidity and mortality [20]. The IC tests are essential for on-site rapid diagnosis of viral gastroenteritis, especially during the outbreaks in the areas with limited access to the centralized facilities, enabling timely, appropriate treatment and infection control. Currently, most of the commercially available IC kits have been developed for detecting a single or dual gastroenteritis viruses [15,16,21,22]. In this study, we evaluated the efficacy of the IP-Triple I IC test kit, which was developed for rapid and simultaneous detection of three gastroenteritis viruses, including NoV, RVA, and HAdV, in a single test. This IP-Triple I IC test provides direct detection of three common enteric viruses, including NoV, RVA, and HAdV, in stool samples. Moreover, this test is easy to use, does not require specialized equipment or highly trained staff, and is suitable for application in various healthcare settings and small laboratories. The data from the present study demonstrated that IP-Triple I IC kit provides high specificity for the detection of all three viruses (NoV, RVA, and HAdV) at 100%. In addition, the sensitivity of detections for RVA (86.7%), HAdV (76.2%), and NoV (70.6%) are acceptable levels for rapid diagnosis. Furthermore, the IP-Triple I IC test kit was able to detect the common antigen of several different genotypes of RVA (G1P[8], G2P[4], G3P[8], G8P [8], and G9P[8]), NoV (GII.2, GII.4, GII.6, GII.7, GII.10, and GII.17), and HAdV (HAdV-A12, -C1, -C2, -C5, -F40, and -F41). However, it was observed that some stool samples that contained low viral load of RVA G1P[8], NoV GII.2[P16], GII.3[P12], GII.4[P16], GII.6[P7], GII.7[P7], GII.17[P17], and HAdV-C2, -C5, -F40 could not be detected by this IP-Triple I IC kit, resulting in false negative results. This is the limitation of the IP-Triple I IC kit. To clarify this point, additional testing with a large number of samples and with known viral copy numbers may need to be performed. In case of inconclusive results, the test needs to be confirmed by more sensitive and specific methods such as PCR or real-time RT-PCR. Altogether, even though the current version of IP-Triple I IC kit showed high specificity for NoV, RVA, and HAdV, the sensitivity of detection for NoV and HAdV require a little more improvement.

In conclusion, the findings of this research indicate that the IP-Triple I IC test kit designed for the rapid and simultaneous detection of NoV, RVA, and HAdV, represents an alternative method for direct detection of these viruses in stool samples. However, for the purposes of epidemiological surveillance and identification of virus genotypes, employing amplification of specific gene by PCR and sequencing techniques is required.

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Ethical Approval:
The study was conducted with the approval of the institutional Ethics Committee of the Faculty of Medicine, Chiang Mai University (MIC-2567-0359).

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

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