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ORIGINAL ARTICLE

Evaluation of Immunochromatographic Tests for Detection of a Wide Variety of Group A Rotavirus Genotypes and Adenovirus

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SUMMARY

Background: **Viral gastroenteritis is one of the most common illnesses in humans worldwide, and different viral agents have been shown to be associated with the disease. Among these, rotaviruses and adenoviruses are the responsible causative agents of acute gastroenteritis and causing numerous outbreaks. Therefore, a simple and rapid diagnostic tool, such as an immunochromatographic (IC) test, is required for rapid diagnosis, especially during an outbreak of these pathogens.**
Methods: **The efficiency of two commercial IC kits were evaluated for simultaneous detections of rotavirus and adenovirus in clinical stool specimens by a single test kit.**
Results: **The data demonstrated that both IC test kits could detect either adenovirus or rotavirus positive alone, as well as mixed infections of both viruses in a single stool specimen. In addition, a wide variety of rotavirus genotypes, including G1-P[8]-I1, G2-P[4]-I2, G3-P[8]-I2, G8-P[8]-I2, and G9-P[8]-I1 could be detected by both IC kits. The detection limit of the kits for the detection of rotavirus and adenovirus were comparable to those of real-time PCR at 10^5 copies/mL.**
Conclusions: **These two IC test kits could be used as an alternative choice for rapid screening of rotavirus and adenovirus in the stool specimens, especially during the seasonal outbreak of acute gastroenteritis.**
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INTRODUCTION

Viral gastroenteritis is one of the most common illnesses in humans worldwide, and different viral agents such as rotavirus, norovirus, and adenovirus have been described as associated with the disease. Rotavirus is a major etiological agent of acute gastroenteritis in infants and young children worldwide. Globally, rotavirus has been estimated to cause the deaths of 150,000 - 200,000 children annually and more than 85% of those deaths occurring in low income countries [1,2]. The most com-

mon genotypes of rotavirus infection in humans are G1P[8], G2P[4], G3P[8], and G9P[8], of which G1P[8] accounts for approximately 30% of all human rotavirus strains [3-6]. Numerous surveillances showed that different G and P genotypes are predominant in particular areas and the genotypes have changed overtime. Recently, the increased detections of rotavirus strains bearing unusual combinations of human rotavirus genotype, such as G8P[8], have been reported as the causative agent of several acute gastroenteritis outbreaks [7-9]. Adenovirus has been reported to be associated with a broad range of clinical illness, including gastroenteritis, respiratory illness, and keratoconjunctivitis [10-12]. Enteric adenovirus (AdV-40 and AdV-41) can spread by person to person direct contact and by fecal-oral-route, and is found to be associated with acute gastroenteritis, particularly, in young children. The overall detection rates for rotavirus and adenovirus in children admitted to the hospitals with acute gastroenteritis range from approximately 5 to 60% and less than 1 to 40%, respectively [6,13,14], and co-infections are often detected, especially in immunocompromised patients [15].

For laboratory diagnosis of rotavirus and enteric adenovirus infections, several diagnostic methods are available. These include ELISA, immunochromatographic (IC) assay, loop mediated isothermal amplification (LAMP), PCR, Reverse Transcription-PCR, real-time PCR, and nucleic acid sequence analysis. The PCR-based methods are the gold standard for the detection of these viruses in clinical stool specimens. However, the method requires a well-trained staff and expensive equipment. Therefore, a rapid, simplified, and inexpensive test for screening of these viruses in clinical specimens is required. In this study, we evaluated the efficiency of two commercial IC kits developed for the detections of rotavirus and adenovirus simultaneously in a single strip test.

MATERIALS AND METHODS

Fecal specimens included in this study were obtained from out-patients who visited the hospitals or private clinics with acute gastroenteritis in Japan from November 2016 to May 2017. The 39 representative specimens of known rotavirus positive (n = 32), adenovirus positive (n = 5), and mixed-infection between rotavirus and adenovirus (n = 2), as determined by RT-PCR/PCR and nucleotide sequencing, were included. The fecal specimens were kept at -30°C until tested. The study was conducted with the approval of the ethical committee, Nihon University School of Medicine, Tokyo, Japan (No. 29-9-0).

To evaluate the sensitivity for rotavirus and adenovirus detection, all 39 specimens were tested for rotavirus and adenovirus antigens by two commercial IC kits available in Japan (Quick Chaser-Rota/Adeno and Rapid Testa Rota-Adeno II). All IC tests were performed according to the manufacturer's instruction. Both IC kits were developed for simultaneous detection of rotavirus and adenovirus antigens in human stool specimens. The IC strips were membrane coated with gold colloid conjugated with mouse monoclonal antibodies against human adenovirus and rotavirus. The adenovirus test line was coated with mouse monoclonal anti-human adenovirus antibody, while the rotavirus test line was coated with mouse monoclonal anti-human rotavirus antibody. The control line was coated with rabbit anti-mouse immunoglobulin antibodies. The result was considered positive for rotavirus when the bands appeared at "Rota" and "Control" positions. Similarly, the result was considered positive for adenovirus when the bands appeared at "Adeno" and "Control" positions. In case of mixed-infections of rotavirus and adenovirus, three bands appeared at "Rota", "Adeno", and "Control" positions. The tested specimens were further characterized for viral genotypes by multiplex PCR or nucleotide sequencing as described previously [16-19] and the virus copy numbers in the specimens were determined by real-time PCR [20].

RESULTS

Out of 39 rotavirus and adenovirus positive specimens, as tested by RT-PCR and PCR, 32 and 5 were positive for rotavirus and adenovirus alone, respectively, and 2 were mixed infections of rotavirus and adenovirus. Rotavirus genotypes of VP7, VP4, and VP6 genes as determined by multiplex PCR or nucleotide sequencing revealed a wide variety of rotavirus genotype combinations. Among these, 5 combination patterns were identified, G1-P[8]-I1 (n = 4), G2-P[4]-I2 (n = 7), G3-P[8]-I2 (n = 2), G8-P[8]-I2 (n = 14), and G9-P[8]-I1 (n = 5). In addition, 2 rotavirus strains, G2-P[4]-I2 and G8-P[8]-I2, were found to be mixed-infection with adenovirus. To assess the sensitivities of Quick Chaser-Rota/Adeno and Rapid Testa Rota-Adeno II IC kits, a panel of all 39 stool specimens was tested by these two kits and the results were compared with those of the conventional RT-PCR and PCR as shown in Table 1. Overall, the Quick Chaser-Rota/Adeno kit and Rapid Testa Rota-Adeno II kit had the same sensitivity for the detection of rotavirus and adenovirus in stool specimens. For rotavirus detection, among 32 stool specimens that were solely infected with rotavirus, 31 could be detected by both Quick Chaser-Rota/Adeno and Rapid Testa Rota-Adeno II IC kits, while one was negative. For adenovirus detection, 4 out of 5 adenovirus infected specimens tested positive by both IC kits, while one was negative. In addition, 2 mixed infections of rotavirus and adenovirus stool specimens were positive by these two kits. When looking at the detection of rotavirus genotypes, these kits could detect a wide variety of rotavirus genotypes, including G1-P[8]-I1, G2-P[4]-I2, G3-P[8]-I2, G8-P[8]-I2, and G9-P[8]-I1. It was of note that one specimen which contained G1-P[8]-I1 genotype remained negative by these two IC kits. The data indicated that the sensitivities of

Table 1. Immunochromatographic tests for the detection of rotavirus and adenovirus in clinical stool specimens.

Viruses	Number of specimens tested (n = 39)	Quick Chaser-Rota/Adeno (positive/negative)		Rapid Testa Rota-Adeno II (positive/negative)	
		Rotavirus	Adenovirus	Rotavirus	Adenovirus
Rotavirus					
G1-P[8]-I1	4	3/4	0/4	3/4	0/4
G2-P[4]-I2	7	7/7	0/7	7/7	0/7
G3-P[8]-I2	2	2/2	0/2	2/2	0/2
G8-P[8]-I2	14	14/14	0/14	14/14	0/14
G9-P[8]-I1	5	5/5	0/5	5/5	0/5
Adenovirus	5	0/5	4/5	0/5	4/5
Rotavirus + Adenovirus					
G2-P[4]-I2 + Adenovirus	1	1/1	1/1	1/1	1/1
G8-P[8]-I2 + Adenovirus	1	1/1	1/1	1/1	1/1

Table 2. The concentration of virus copies/mL of rotavirus and adenovirus, and the results of two immunochromatographic kits tested in this study.

Sample code	Positive virus	Virus titer (copies/mL)	Immunochromatographic test	
			Quick Chaser-Rota/Adeno	Rapid Testa Rota-Adeno II
7519947	Rotavirus	1.39×10^6	Positive	Positive
7263277	Rotavirus	9.04×10^5	Positive	Positive
7139534	Rotavirus	1.72×10^5	Positive	Positive
15386	Rotavirus	1.02×10^5	Negative	Negative
7517936	Adenovirus	3.33×10^9	Positive	Positive
15429	Adenovirus	1.90×10^7	Positive	Positive
7246757	Adenovirus	5.79×10^5	Negative	Negative
7516720	Rotavirus/Adenovirus	6.11×10^7 / 2.11×10^7	Positive / Positive	Positive / Positive

these two IC kits for the detection of rotavirus and adenovirus were comparable with those of RT-PCR and PCR.

In order to determine the detection limits of the kits, 4 rotavirus and 4 adenovirus positive stool specimens, and one rotavirus-adenovirus co-infected stool specimen were selected and tested for viral loads by real-time PCR. The selection of rotavirus and adenovirus positive specimens included in this experiment was based on the intensity of the IC bands from low to high intensity. The viral loads of rotavirus and adenovirus in each specimen is shown in relation of the results tested by the two IC kits (Table 2). The lower limit for rotavirus detection of both IC kits was 1.72×10^5 copies/mL. The results of both IC kits turned negative when the rotavirus titer decreased to 1.02×10^5 copies/mL. For adenovirus, the detection limit of both IC kits was at least 1.97×10^7 copies/mL, the tests turned negative when the adenovirus titer decreased to 5.79×10^5 copies/mL.

DISCUSSION

Acute gastroenteritis is still responsible for high levels of morbidity and mortality in the general population, particularly in the at-risk groups, such as infants and young children, the elderly, and the immunocompromised patients. Among these, rotaviruses and adenoviruses are responsible for the causative agents of acute gastroenteritis and numerous outbreaks of nonbacterial gastroenteritis in several settings such as hospitals, day care centers, and restaurants [21-23]. Therefore, a simple and rapid diagnostic tool such as the immunochromatographic (IC) test is required, especially during an

outbreak of these pathogens. The advantages of the IC assay are the speed, cost effectiveness, and the ease of use at the primary care unit and private clinic without the need of special laboratory equipment. Currently, several rapid IC kits for rotavirus and adenovirus detections are commercially available. However, those commercially available IC kits have mainly been developed for the detection of a single virus [24,25]. Few reports have evaluated the sensitivity of commercialized IC kits that were developed for dual detections of rotavirus and adenovirus simultaneously in a single kit. The study in Ghana demonstrated that the sensitivity of the IC kit for rotavirus detection was higher than that of adenovirus (75% vs. 22%) [26]. In addition, evaluation of the IC kit for the detection of both rotavirus and adenovirus in stool specimens collected from patients with acute gastroenteritis in Korea also demonstrated that the sensitivity of the kit for rotavirus detection was higher than that of adenovirus (100% vs. 71.4%) [27]. Our study also revealed that the sensitivity of the Quick Chaser-Rota/Adeno and Rapid Testa Rota-Adeno II for rotavirus detection was higher than adenovirus (97% vs. 86%) and several rotavirus genotypes could be detected.

For the evaluation of the sensitivity for adenovirus detections, only 5 adenovirus positive specimens were tested, which may reflect a low statistical power for this evaluation. In addition, the other limitations of the study are i) relatively small number of the clinical specimens of rotavirus and adenovirus positive were included in this study, ii) the limits of detection have been done by comparing the viral concentrations that were determined by real-time PCR in the original selected positive specimens rather than making a serial dilution of the original specimens. In order to clarify these points, additional testing with a large number of clinical specimens including the specimens that are positive for other viral enteropathogens as well as negative stool samples may need to be performed to evaluate cross reactivity of the viruses. Moreover, serial dilutions of the original stool positive specimens should be performed in order to determine the true limit of viral detection.

CONCLUSION

Our data demonstrated that the IC test kit is an attractive diagnostic tool as it is less time consuming, easy to use, and the test can be performed at the bedside. The dual rotavirus and adenovirus IC test kits could be used as alternative choice for rapid screening of rotavirus and adenovirus in stool specimens, especially during the seasonal outbreaks of acute gastroenteritis.

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Declaration of Interest:
No conflict of interest is declared.

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