The Journal of Microbiology (2010) Vol. 48, No. 4, pp. 419-425
DOI 10.1007/s12275-010-0071-4

Development of a Latex Agglutination Test for Norovirus Detection

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(Received February 23, 2010 / Accepted April 5, 2010)

Norovirus (NoV) is the leading cause of acute gastroenteritis worldwide. Currently, reverse transcription polymerase chain reaction (RT-PCR) is used commonly to detect NoVs in both clinical and environmental samples. However, RT-PCR requires expensive equipment and cannot be performed on site. In this study, a latex agglutination test (LAT) using antibody-labeled latex beads for detecting NoVs was developed. Two kinds of polyclonal antibodies, one generated from synthetic peptides and the other from *E. coli*-expressed NoV capsid proteins, were used to develop the LAT. Each of these polyclonal antibodies was immobilized on the surface of latex beads and tested for the ability to detect NoVs. Under optimized conditions, our LAT detected GII.4 NoV at concentrations as low as 3.3×10^5 RT-PCR units/ml in stool samples. The detection limit for the LAT was approximately 1.7×10^3 RT-PCR units. Forty-eight stool samples were tested for NoVs using this LAT. In comparison with an RT-PCR assay, the sensitivity and specificity of the LAT were 35% and 100%, respectively. With further optimization, this LAT used with appropriate antibodies could be applied for convenient detection of NoVs in clinical diagnosis and food monitoring.

***Keywords*:** polyclonal antibody, latex agglutination test (LAT), norovirus, rapid detection, sensitivity, specificity

Norovirus (NoV) is the most frequent etiological agent of acute gastroenteritis in all age groups worldwide, with an estimated over 10,000 cases per year in the United States alone (Lynch *et al.*, 2006). Recently, the incidence rate of NoVs has increased in Europe and Japan (Kroneman *et al.*, 2006; Sakon *et al.*, 2007). In South Korea, approximately 2,000 students in metropolitan Seoul were infected in 2006 (KCDC, 2007), this was the largest foodborne outbreak in the history of South Korea. NoV outbreaks can occur throughout the year but are more prevalent during winter (Hale *et al.*, 2000). Clinical symptoms include abdominal pain, diarrhea, vomiting, and nausea; the disease is rarely fatal in healthy individuals (Tan *et al.*, 2006).

NoV is an icosahedral, non-enveloped, positive-sense single-stranded RNA virus, 27-40 nm in size, belonging to the family *Caliciviridae* (Kapikian *et al.*, 1972; Ando *et al.*, 2000; Hardy, 2005). Based on the nucleic acid sequences of both the capsid and RdRp genes, NoVs can be classified into five distinct genogroups (GI-GV). Both GI and GII NoVs infect commonly humans; among these, the GII.4 genotype is the most frequently identified agent throughout the world (Green *et al.*, 2000; Fankhauser *et al.*, 2002).

NoV is highly infectious, with fewer than 10 viral particles being sufficient to infect and cause disease in humans (Lindesmith *et al.*, 2003). Typically, the virus is transmitted person to person, via contaminated food, water, or fomites (Parashar *et al.*, 2001). For these reasons, a rapid and sensitive detection method for clinical and environmental samples is important to identify and prevent NoV outbreaks. Molecular methods such as the reverse transcriptase-polymerase chain reaction (RT-PCR) are used widely to detect and identify NoVs (Jiang *et al.*, 1992; Ando *et al.*, 1995; Atmar and Estes, 2001; Katayama *et al.*, 2002; Loisy *et al.*, 2005; Dreier *et al.*, 2006). However, the RT-PCR assay is prone to cross-contamination and thus requires high levels of quality assurance and quality control; furthermore, the reaction can be inhibited by various agents such as humic acid and heavy metals (Loisy *et al.*, 2000; Dreier *et al.*, 2006). In addition, several procedures such as the concentration, purification, and nucleic acid extraction of samples, which require typically a great deal of time and a well-equipped laboratory, are required prior to RT-PCR (Katayama *et al.*, 2002).

A latex agglutination test (LAT) is a diagnostic method based on antibody-coated latex beads and is generally simple, rapid, specific, and inexpensive. Typically, the LAT provides test results within 10 min, and the labeled latex beads can be stable for several months (Slotved *et al.*, 2004). This test is easy to perform, field-portable, and can be performed without additional expensive equipment. Since 1956 when a LAT was used to detect rheumatoid arthritis (Plotz and Singer, 1956), it has been applied in the diagnosis of various pathogens, including *Staphylococcus aureus* (Smole *et al.*, 1998; Krishnan *et al.*, 2002), *Candida dubliniensis* (Marot-Leblond *et al.*, 2006), plant viruses (Bercks and Querfurth, 1971), rabies virus (Kasempimolporn *et al.*, 2000), herpes simplex virus (Halstead

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et al., 1987), rotavirus (Hughes *et al.*, 1984; Knisley *et al.*, 1986; Arya, 1988), adenovirus (Grandien *et al.*, 1987), human immunoeficiency virus (Riggin *et al.*, 1987), and influenza virus (Xu *et al.*, 2005; Chen *et al.*, 2007). Recently, a LAT was developed for the detection of coliphages and was used to monitor microbial contamination in water (Love and Sobsey, 2007). However, until now, LAT has not been applied to detect NoVs.

In this study, a LAT for NoVs detection was developed and characterized. Our LAT is a novel and inexpensive method for the rapid and sensitive detection of NoVs in various settings.

Materials and Methods

Preparation of clinical samples

The Korea Center for Disease Control and Prevention and the Seoul Metropolitan Research Institute of Public Health and Environment provided both NoV-positive and NoV-negative stool samples for use in this study. RT-PCR was conducted with the G2SKF/G2SKR primer set to determine the NoV concentrations in the clinical samples (Kojima *et al.*, 2002). Viral RNA was extracted using a QIAamp viral RNA Mini kit (QIAGEN, USA), following the manufacturer's protocol. The 50 μl of template from 150 μl of stool sample was extracted, which had been diluted 10-fold in phosphate-buffered saline (PBS, pH 7.4). The template was diluted 10-fold in distilled water prior to amplification. RT-PCR was performed using a QIAGEN OneStep RT-PCR kit. The RT-PCR reaction mixture (25 μl) contained 5 μl of 5× RT-PCR buffer, 1 μl of 10 mM dNTP mix, 1 μl of enzyme mix (reverse transcriptase and *Taq* polymerase), 50 pmol of primers (0.25 μl), 0.5 μl of RNAse inhibitor (40 U/μl), 14.5 μl of RNAse-free water, and 2.5 μl of insert RNA template. RT-PCR was performed using a thermal cycler (AB2720; Applied Biosystems, USA) under the following conditions: reverse transcription at 42°C for 60 min, initial denaturation at 95°C for 15 min, 40 amplification cycles (denaturation at 94°C for 30 sec, primer annealing at 50°C for 30 sec, and extension at 72°C for 30 sec), and a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis in a 1% agarose gel and visualized under UV light after ethidium bromide staining. The RT-PCR products were extracted using a QIAquick Gel Extraction kit. The identification of NoVs was confirmed by comparison with entries in the GenBank database using PubMed BLAST. The NoV-positive and NoV-negative stool samples were stored at -70°C until further analysis.

Production of polyclonal antibodies against GII NoVs

Two kinds of polyclonal antibodies were used. Antibodies were generated from synthetic peptides based on amino acid sequences of the NoV capsid protein. To produce an anti-peptide antibody, the amino acid sequences of NoV GII capsid genes obtained from both the Korea Center for Disease Control and Prevention in South Korea and GenBank were identified. Multiple sequence alignment analysis using the CLUSTAL W method (DNASTAR, USA) was performed. After identifying the conserved region of the capsid gene, the antigenicity (Kolaskar and Tongaonkar, 1990) and hydrophobicity (Kovacs *et al.*, 2006) of the identified peptide sequences were characterized. At least three conserved amino acid sequences with high antigenicity and hydrophobicity within the GII NoV capsid region were identified (Table 1). Two rabbits were inoculated with a mixture of these peptides to produce polyclonal anti-GII NoV antibodies. Enzyme-linked immunosorbent assay (ELISA) (Okame *et al.*, 2007) was performed to characterize the interactions between the polyclonal antibody and a mixture of peptides. The generated anti-peptide polyclonal antibody was diluted in phosphate buffer, and the optical density was measured using a spectrophotometer (ND-1000; NanoDrop Technologies, USA). In addition to anti-peptide antibodies, polyclonal antibodies were also generated using the entire NoV capsid protein. The protein was expressed using an *E. coli* expression system. Polyclonal antibodies were produced as described in our previous study (Park *et al.*, 2008). The protein concentrations of the antibodies as measured by spectrophotometry were estimated to be approximately 40-60 mg/ml.

Optimization of coating the latex beads with antibodies

The polyclonal antibodies were diluted to a final concentration of 4.54-5.39 μg/μl. Different volumes of antibodies (100, 50, and 25 μl) were added to 100 μl of 1% latex beads and mixed gently using a Dynal® sample mixer (MXIC1; Invitrogen, USA) for 2 h at room temperature. The mixture was centrifuged at 13,000×g for 3 min, and the supernatant was transferred to a new tube. The antibody concentration in the supernatant was measured by spectrophotometry. The amount of adsorbed antibody was estimated as the difference between the total amount of antibody and the remaining non-adsorbed antibody. As a negative control, the process was conducted without antibody. The amount of adsorbed antibody was estimated by subtracting the value of the negative control.

Preparation of latex beads coated with polyclonal antibody against GII NoVs

The sulfate-modified polystyrene latex beads (10% suspension; average diameter, 0.3 μm) were purchased from Sigma-Aldrich (USA). Pellets were collected by centrifugation at 13,000×g at 4°C for 5 min and then subjected to sonication (Power Sonic 520; Hwashin Co., Korea) for 1 min. The latex beads were then diluted serially in PBS and coated with the antibodies. One volume of antibody solution (1:10 dilution of the original antibody, 5-6 mg/ml) was added to two volumes of 1% latex beads and mixed using a Dynal® sample mixer (MXIC1; Invitrogen, USA) for 2 h at room temperature. The solution was centrifuged at 13,000×g for 3 min, and the supernatant, which contained non-adsorbed antibody, was carefully aspirated using a pipette. The pellet was re-suspended in various concentrations (0, 0.01, 0.1, and 1%) of bovine serum albumin (BSA) (Sigma, USA) in PBS by pipetting, followed by gentle mixing for 1 h at room temperature. Two volumes of blocking buffer were added for each initial volume of antibody. The blocked beads were then centrifuged at 13,000×g for 3 min, and the supernatant was removed carefully.

Table 1. Summary of synthesized NoV peptides used to produce the anti-peptide polyclonal antibody

Peptide number	Sequence of conserved amino acids	Length (residues)	NoV genogroup	Capsid region	Location[a]
1	LAGNAFTAGKI	11	GI, GII	S domain	5,409-5,441
2	VNPDTGRVLFE	11	GII	P1-2 domain	6,513-6,545
3	NGYFRFDSWVN	11	GII	P1-2 domain	6,615-6,647

[a] Nucleotide positions based on Lordsdale virus (GII, GenBank accession no. X86557).

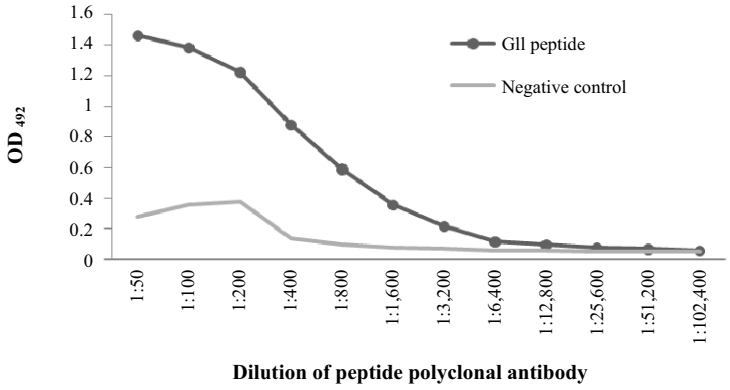

Fig. 1. Enzyme-linked immunosorbent assay (ELISA)-based quantification of synthesized viral peptide bound to the anti-peptide polyclonal antibody. Based on absorbance at 492 nm UV light. Serum from a non-inoculated rabbit was used as a negative control.

The washing and blocking steps were repeated twice. The final solution was re-suspended in PBS at a final concentration of 2% latex beads and was stored at 4°C until further use. As a control, unlabeled beads were prepared following the same procedure.

LAT with GII NoV-positive or -negative samples

A total of 48 stool samples with or without GII NoVs were tested for latex agglutination. A stool suspension (5 μl) was mixed with 2% antibody-labeled beads (5 μl) on a sterilized glass slide. After 1-5 min of mixing, latex agglutination was evaluated visually by eye. The appearance of obvious clumping was considered a positive result. As negative controls, NoV-negative stool samples inoculated with either poliovirus Sabin 1 (1×10^6 RT-PCR units) or adenovirus (1×10^6 PCR units) were used. The experiment was repeated at least three times for each condition. The sensitivity and specificity of the LAT were estimated compared with the RT-PCR assay results. A Chi-squared (χ^2) test was performed to determine the statistical significance of the association between the RT-PCR and LAT results. All statistical analyses were performed using SPSS 12.0 software.

Results

Production of anti-peptide polyclonal antibody against GII NoVs

The anti-peptide polyclonal antibody was highly reactive against a mixture of peptides. In an enzyme immunoassay, the optical density of the peptide plus the anti-peptide antibody was seven-fold the optical density of the negative control (Fig. 1). These results demonstrate that the anti-peptide polyclonal antibody was highly reactive against the conserved region of NoVs GII capsid protein.

Characterization of antibody adsorption to latex beads

Significant amounts of the antibodies, which depended on the antibody volume, were adsorbed onto the latex beads (Table 2). When 25 μl of polyclonal antibodies were mixed with 100 μl of latex beads, most of the antibodies attached to the beads (88.8-103.0%). The proportion of adsorbed antibodies decreased when either 100 or 50 μl of antibodies were mixed with 100 μl of latex beads. There was no significant difference in adsorption between the two different polyclonal antibodies.

Latex agglutination test (LAT)

A stool sample containing 1.33×10^7 RT-PCR units/ml of NoV strain GII.4 was serially diluted by two-fold and tested to determine the sensitivity of our LAT. Table 3 summarizes the sensitivity of the LAT using beads coated with the polyclonal antibody raised against NoVs GII capsid protein expressed in *E. coli*. The stool samples containing NoVs exhibited obvious agglutination, whereas the sample without NoVs showed no agglutination. In addition, when uncoated latex beads were used in the LAT, agglutination did not occur. These results indicate that the LAT is able to detect NoV GII.4. When the beads were blocked with BSA solution, the limit of detection increased, and the signs of agglutination were more clear at all concentrations (0.01-1%) of BSA in the blocking solution. The lower limit of detection was estimated to be 1.7×10^3 RT-PCR units in 5 μl of stool sample.

The results for the latex beads coated with the anti-peptide polyclonal antibody were generally similar to those with the anti-capsid protein polyclonal antibody (Table 4), except that the limit of detection was lower at certain BSA concentrations. The highest limit of detection, which was estimated at 3.3×10^3 RT-PCR units, occurred with 0.01% BSA in the LAT performed

Table 2. Total amount of polyclonal antibody adsorbed onto 100 μl of 1% latex beads

Antibody[a]	Volume of antibody (amount of antibody)		
	100 μl (500 μg)	50 μl (250 μg)	25 μl (125 μg)
Anti-peptide[b]	66.1±21.0 (12.4±3.6)[d]	105.8±9.7 (40.2±2.1)	116.3±10.4 (88.8±11.8)
Anti-capsid protein[c]	49.7±22.3 (10.4±4.1)	92.4±26.8 (39.4±8.8)	111.2±10.0 (103.0±5.1)

[a] Antibody concentrations ranged from 4.54-5.39 μg/μl.
[b] Polyclonal antibody generated from entire NoV capsid protein expressed in *E. coli*.
[c] Polyclonal antibody generated from synthetic amino acid sequences of conservative GII NoV capsid protein.
[d] The numerical value (μg) is the Mean±SD. Value in parentheses is the percentage of adsorbed antibodies. Experiments were conducted in triplicate.

Table 3. Summary of LAT results for NoV-positive and NoV-negative stool samples using the polyclonal antibody generated against the entire NoV capsid protein expressed in *E. coli*

Sample	Dilution	NoV concentration in stool (RT-PCR unit/5 μl)[c]	Concentration of BSA in blocking buffer (%)				Negative control[b]
			1	0.1	0.01	0	
NoV-positive stool samples[a] (GII.4)	1:10	6,650	3/3[g]	3/3	3/3	3/3	-[f]
	1:20	3,325	3/3	3/3	3/3	-	-
	1:40	1,663	2/3	1/3	1/3	-	-
	1:80	831	-	-	-	-	-
Poliovirus[d]	1:10 to 1:80 (5,000 to 625 RT-PCR unit/5 μl)		-	-	-	-	-
Adenovirus[e]	1:10 to 1:80 (5,000 to 625 PCR unit/5 μl)		-	-	-	-	-

[a] Stool suspension containing GII.4 NoV with an estimated concentration of 1.33×10^7 RT-PCR units per ml.
[b] Antibody-unlabeled latex bead.
[c] Calculated based on RT-PCR units and 5 μl was tested with sensitized latex beads.
[d] Poliovirus Sabin 1 was used as a negative control. Experiments were repeated three times.
[e] Adenovirus 40 was used as a negative control. Experiments were repeated twice.
[f] – indicates no agglutination observed within 5 min.
[g] + indicates that agglutination was observed within 5 min. The experiments were repeated three times. The first number indicates positive results out of three experiments.
Each experiment was performed with NoV-negative stool samples as a negative control. All experiments resulted in negative results.

with the anti-peptide polyclonal antibody.

LAT of various strains of GII NoVs

A total of 48 stool samples from the Korea Center for Disease Control and Prevention and the Seoul Metropolitan Research Institute of Public Health and Environment were tested using both LAT and RT-PCR (Table 5). Among tested 48 stool samples, 23 the samples were identified as GII NoVs by RT-PCR, whose concentrations ranged from 104 to 106 RT-PCR units/ml. Eight of 23 RT-PCR positive samples were also positive by LAT. All of 25 RT-PCR negative samples were also negative by LAT. Thus, the sensitivity and specificity of LAT relative to RT-PCR were 35% and 100%, respectively. The results of the LAT and RT-PCR were significantly different ($P<0.001$). There is no clear significance between viral concentration and LAT results ($P=0.392$ by Cochran-Mantel-Haenszel test). Based on the partial nucleic acid sequence of the capsid gene, 23 GII NoV strains tested in this study were GII.3 (7 samples), GII.4 (11 samples), GII.12 (2 samples), and GII.13 strains (3 samples). Five (45%) of the eleven GII.4 NoV samples were positive by LAT. None of the GII.13 samples were positive by LAT. The viral concentrations in tested stool samples ranged from 10^4 to 10^6 RT-PCR units/ml.

Discussion

Currently, RT-PCR is the primary analytical method for the detection of NoVs in clinical and environmental samples. The development of real-time RT-PCR techniques has reduced the time for analysis and has improved sensitivity, with the ability to detect as low as 10 copies of NoVs (Dreier *et al.*, 2006; Trujillo *et al.*, 2006). However, these molecular methods are time-consuming, require nucleic acid extraction steps, and must be analyzed in a laboratory by a skilled technician. The present study demonstrated that a NoV-specific LAT could be a useful alternative analytical method for NoVs. This method is rapid, inexpensive, and portable, and does not require expensive equipment or skilled technicians.

In this study, a LAT using a polyclonal antibody detected NoVs at levels as low as 3.3×10^5 RT-PCR units/ml. The agreement between the NoV results obtained by RT-PCR and LAT was 69%, and real-time RT-PCR is 10- to 100-fold more sensitive than conventional RT-PCR (Houde *et al.*, 2006). Although the limit of detection of our LAT is not as high as that of RT-PCR, it is sufficient for the detection of NoVs in clinical samples. The limit of detection of LAT appears to be similar to those of other antibody-based detection methods such as ELISA. In comparison with other previous immunological methods such as ELISA and the immune-chromato-

Table 4. Summary of LAT results for NoV-positive and NoV-negative stool samples with polyclonal antibody generated against 11 amino acid sequences of conserved GII NoV capsid protein

Sample	Dilution	NoV concentration in stool (RT-PCR unit/5 μl)[c]	Concentration of BSA in blocking buffer (%)				Negative control[b]
			1	0.1	0.01	0	
NoV-positive stool samples[a] (GII.4)	1:10	6,650	3/3[g]	3/3	3/3	3/3	-[f]
	1:20	3,325	1/3	2/3	3/3	2/3	-
	1:40	1,663	-	-	-	-	-
	1:80	831	-	-	-	-	-
Poliovirus[d]	1:10 to 1:80 (5,000 to 625 RT-PCR unit/5 μl)		-	-	-	-	-
Adenovirus[e]	1:10 to 1:80 (5,000 to 625 PCR unit/5 μl)		-	-	-	-	-

[a, b, c, d, e, f, g] see footnote of Table 3.

Table 5. Detection of NoVs in stool samples, using LAT and RT-PCR

LAT[a]	RT-PCR[b] (Number of samples)		
	+	–	Total
+	8	0	8[c]
–	15	25	40[d]
Total	23	25	48

+ indicates positive; – indicates negative.
[a] The latex beads were blocked with 0.01% BSA.
[b] All stool samples were diluted by 10% with PBS prior to viral RNA extraction.
[c] Positive by both LATs (with the anti-peptide antibody and the anti-capsid protein antibody).
[d] Negative by both LATs.

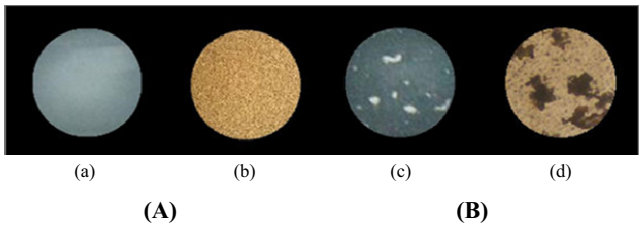

Fig. 2. GII.4 NoV detection using the LAT. (A) LAT with NoV-negative stool suspension. (a) By naked eye. (b) Under a microscope (magnification, ×4). (B) LAT with NoV-positive stool suspension. (c) By naked eye. (d) Under a microscope (magnification, ×4). 2% latex beads and 0-1% BSA blocking buffer were used in this experiment.

graphic test (Khamrin *et al.*, 2008; Takanashi *et al.*, 2008), the detection limit of LAT (10^6-10^7 genomic copies per gram of stool) was comparable. In our study, the specificity of the LAT for NoVs was 100%, which was comparable and even better that those from ELISA and the immune-chromatographic test (88-96%) (Rabenau *et al.*, 2003; de Bruin *et al.*, 2006; Okitsu-Negishi *et al.*, 2006; Okame *et al.*, 2007; Khamrin *et al.*, 2008; Takanashi *et al.*, 2008). However, the sensitivity of LAT was only 35%, which was lower than that previous ELISA (31-90%) and the immune-chromatographic test (70-79%). The LAT for various pathogens which had been evaluated previously showed good sensitivity (88-97%) and specificity (99-100%) comparing to the reference method (Chart, 1999; Akanmu *et al.*, 2001; Al-Yousif *et al.*, 2001), some LAT kit was used for epidemiology (Korycka, 2006). Although this study demonstrated the possibility of LAT for detecting NoVs, further study using additional antibodies and viral receptor should be evaluated in future.

Three major factors should be optimized for this LAT: size and type of latex beads, type of antibody, and blocking conditions. Typically, the size of the beads depends on the target pathogen. Small-diameter beads (0.22-0.8 μm) are used commonly for viruses such as influenza, rotavirus, coliphages, and rabies virus (Hughes *et al.*, 1984; Kasempimolporn *et al.*, 2000; Xu *et al.*, 2005; Chen *et al.*, 2007; Love and Sobsey, 2007). For bacterial pathogens, larger beads (>0.8 μm) are commonly used (Dey *et al.*, 2007). In our study, 0.3-μm latex beads were used to detect NoVs. In addition, different types of bead materials are selected for different pathogens. The most commonly used latex beads are made of either sulfate- or carboxylate-modified latex. In our study, the sulfate-modified variety was chosen because of its hydrophobicity and stability at high pH (Bagchi and Birnbaum, 1981; Brinkley, 1992).

In this study, two types of polyclonal antibodies against GII NoVs were generated. Based on immunological methods, our antibodies were sensitive and specific to NoVs. In this study, the LAT did not tested with various monoclonal antibodies. Without a doubt, a monoclonal antibody would be more specific to NoVs (Shiota *et al.*, 2007). However, it may be specific for only certain genotypes of NoVs rather than a broader group of GII NoVs (Knisley *et al.*, 1986; Arya, 1988; Xu *et al.*, 2005; Marot-Leblond *et al.*, 2006). In addition, the sensitivity is more important issues than specificity because of high specificity in our study. Part of our study focused on the GII.4 genotype of NoVs. As this genotype is highly infectious and is the most prevalent throughout the world (Fankhauser *et al.*, 2002), it is of great clinical concern. Although the LAT with the polyclonal antibody in this study showed low sensitivity, it was able to detect both GII.3 and GII.4. Given the genetic and antigenic diversity of NoVs, further studies will be required to test additional NoV genotypes.

In addition to the type of latex and the antibody, blocking conditions are critical for LAT optimization (Ortega-Vinuesa and Bastos-Gonzalez, 2001). The different concentrations of BSA and incubation times with the latex beads were examined to find conditions that prevent self-agglutination of the beads. BSA concentrations of 0.01-1% of were optimal for effective blocking. This is comparable to a previous study with influenza virus, in which 0.02% BSA buffer was optimal (Chen *et al.*, 2007). Optimal blocking conditions depend on both the target microorganism and the antibody. Under our optimized conditions, the beads coated with polyclonal antibody agglutinated with NoV-containing stool samples within 5 min. The rapid assay time makes this LAT a very attractive analytical tool for detecting NoVs in stool samples, compared with other diagnostic kits. (Takanashi *et al.*, 2008).

One limitation of this study is that the types and numbers of tested GII NoV samples were limited. Although the GII.4 NoV genotype is the most important and prevalent worldwide (Green *et al.*, 2000; Ramirez *et al.*, 2008), other genotypes including GI NoVs should also be considered. Previous studies have revealed a significant difference between the immune responses of GI and GII NoVs (Okame *et al.*, 2007). In addition, LAT was able to detect NoVs ranging from 10^4 to 10^6 RT-PCR units/ml in stool samples. Thus, the LAT detection limit should be taken into consideration as well as the types of virus, antibody, beads, and other LAT conditions.

In conclusion, a LAT for the detection of NoVs was developed and optimized. With some improvement, this technique may have great value in clinical diagnosis and food monitoring. Additional studies are required to further optimize the analytical capability of this technique and to field test it.

Acknowledgements

This research was in part supported by a grant (07192KFDA 030) from Korea Food & Drug Administration and Seoul R&BD program (10580).

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