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Research Note

Rapid Detection of Norovirus from Fresh Lettuce Using Immunomagnetic Separation and a Quantum Dots Assay

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ABSTRACT

Current molecular methods that include PCR have been used to detect norovirus in many food samples. However, the protocols require removing PCR inhibitors and incorporate time-consuming concentration steps to separate virus from analyte for rapid and sensitive detection of norovirus. We developed an immunomagnetic separation (IMS) and a quantum dots (QDs) assay to detect norovirus eluted from fresh lettuce with Tris buffer containing 1% beef extract (pH 9.5). IMS facilitated viral precipitation with a 10-min incubation, whereas virus concentration using polyethylene glycol (PEG) requires more than 3 h and an additional high-speed centrifugation step to precipitate virus before reverse transcription PCR (RT-PCR) analysis. The fluorescence intensity of QDs was detected qualitatively on norovirus dilutions of 10^{-1} to 10^{-3} in a stool suspension (100 RT-PCR units/ml). The results suggest that a fluorescence assay based on IMS and QDs is valid for detecting norovirus qualitatively according to fluorescent signal intensity within the same virus detection limit produced by IMS–RT-PCR and PEG–RT-PCR.

Noroviruses (NoVs; genus *Norovirus,* family *Caliciviridae*) are nonenveloped single-stranded RNA viruses and a leading cause of sporadic and epidemic gastrointestinal disease in humans via the fecal-oral route *(4, 12).* NoV has been isolated from contaminated water and food such as shellfish, cold cooked ham, commercial ice, and ready-to-eat food products, including fruit salad, celery, melons, frozen raspberries, and lettuce *(5, 7, 9, 11, 20, 25, 26).*

Highly purified and concentrated target virus is needed for rapid and repeatable nucleic acid amplification methods. However, conventional molecular methods that include PCR are susceptible to inhibitors such as polysaccharides and humic acids, which abundantly occur in food samples and may result in low sensitivity of the method *(8).* Another problem with virus detection is the low level of virus in most food samples. Current virus detection methods are highly dependent on a time-consuming concentration step using polyethylene glycol (PEG) 8000, zirconium chloride, and freon to compensate for low levels of virus *(6, 15, 28).* Magnetic beads coated with specific antibodies that capture foodborne pathogens are also efficient for detecting virus in large volumes of food and environmental samples *(21, 29).* Immunomagnetic separation (IMS) has been effective for concentrating *Escherichia coli* O157:H7 *(27), Cryptosporidium (23),* hepatitis A virus (HAV) *(1),* and adenovirus *(13)* without the need for centrifugation. Compared with current PCR-based detection methods, fluorescence assays based on quantum dots (QDs) have been widely used for faster and easier detection of biological target molecules enriched through IMS *(31, 32).* By changing antibodies, these biosensors have the potential to simultaneously detect any virus using the fluorescence intensity of the multicolored QDs, which are excited at the same single wavelength and emit different colors *(2, 10, 14, 19).* A new method to detect NoV using IMS and QDs was developed in this study.

MATERIALS AND METHODS

Virus. NoVs (GI-1, GI-12, GII-3, GII-4, GII-6, and GII-8) and hepatitis E virus (HEV) were obtained from Gwangju Health and Environment Research Institute and had been isolated from patient fecal samples. HAV strain HM-175/18f (VR-1402) was obtained from the American Type Culture Collection (Manassas, VA) *(18)* and was propagated in fetal rhesus kidney (FRhK-4) cells.

Virus extraction and concentration. Virus extraction and concentration were conducted by a procedure modified from a method previously described by Kingsley and Richards *(17).* Fifteen grams of crisphead lettuce was inoculated with 30 μl of NoV-containing stool suspension (100 RT-PCR units/ml) by spotting in 10 places and then allowed to dry for 30 min in a laminar flow hood. The dried lettuce was then homogenized in

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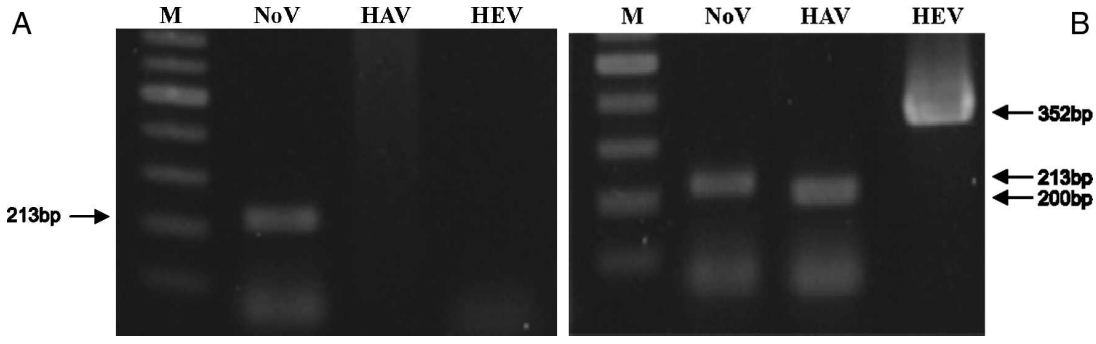

FIGURE 1. *Enhanced RT-PCR assay for detecting norovirus (NoV) among other enteric viruses using immunomagnetic separation (IMS). The amplified region B (213 bp) of NoV was detected in only the IMS eluent fraction (A). Three amplified genes, NoV region B (213 bp), hepatitis A virus (HAV) VP1-P2A (200 bp), and hepatitis E virus (HEV) RdRp (352 bp), were detected in the IMS washout fraction (B). M, 100-bp marker.*

60 ml of Tris elution buffer (100 mM Tris-HCl, 50 mM glycine, and 1% beef extract, pH 9.5) at 20°C with a homogenizer (Omni Macro Homogenizer, Kennesaw, GA) on the high setting for 2 min and then centrifuged at 15,000 × *g* at 4°C. NoV in the supernatant was mixed with an equal volume of concentration buffer (16% PEG 6000 [Sigma Chemical Co., St. Louis, MO] and 0.525 M NaCl), incubated for 3 h on ice, and centrifuged at 10,000 × *g* for 30 min at 4°C to concentrate the mixture *(22)*. The virus was then resuspended in 300 μl of RNase-free water before reverse transcription (RT) PCR. NoV in the supernatant from the artificially contaminated lettuce was transferred to 50 μl of protein G magnetic beads for IMS, linked with 4 μg of NoV antibody (rabbit polyclonal anti-human NoV antibody, 0.5 mg/ml; Abcam, Cambridge, UK), and incubated for 10 min at room temperature with rotation. The beads were washed twice with 200 μl of phosphate-buffered saline (PBS) and then resuspended in 300 μl of PBS before confirming the presence of NoV using RT-PCR.

RT-PCR. Viral RNA was prepared from 300 μl of IMS and PEG eluant using Trizol-LS reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total cDNA was synthesized in a volume of 21 μl, including 9.5 μl of the extracted RNA, 2 μl of random primer (Takara Biological, Shiga, Japan), 4 μl of 5× reaction buffer (Beams Biological, Seongnam, Kyunggi, Korea), 2 μl of 50 mM dithiothreitol (Beams), 2 μl of the deoxynucleoside triphosphate mixture (Takara), 0.5 μl of RNase inhibitor (Takara), and 1 μl of Moloney murine leukemia virus reverse transcriptase (200 U/μl; Beams). The reaction was incubated for 60 min at 37°C for cDNA synthesis, 10 min at 70°C for inactivation of reverse transcriptase, and 5 min at 8°C for chilling. PCR was performed to verify the viruses using a pre-mix PCR kit (GeneAll, Seoul, Korea) in a total volume of 20 μl, which included 1 μl of forward primer (10 pmol/μl), 1 μl of reverse primer (10 pmol/μl), 1 μl of cDNA, and 17 μl of DNase- and RNase-free water. We used NoV (MON 431 and 433 *(24, 30)*), HAV (VP1_F: TGG TTT GCC ATC AAC ACT GAG G, VP1_R: ACC CAA GGA GTA TCA ACG GCA AG; this study), and HEV (MJ-2) primer sets for the PCR analysis *(33)*. The partial NoV sequence for region B was amplified using MON 431 and MON 433 primers for genogroup II. These primers amplify a small region within the 3′ end of the open reading frame 1 portion of the genome *(16, 30)*. NoV PCR amplification was carried out at 95°C for 10 min, followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 60°C, and extension for 30 s at 72°C. The HAV PCR amplification was carried out at 95°C for 5 min, followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 57°C, and extension for 30 s at 72°C. The HEV PCR amplification was carried out at 95°C for 5 min, followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 61°C, and extension for 30 s at 72°C.

Detecting NoV with a fluorescent QD probe. The qualitative NoV detection system with a fluorescent QD probe was prepared as follows. Ten micrograms of monoclonal anti-NoV antibody (0.1 mg/ml, mouse monoclonal anti-human NoV antibody; Abcam) was added to 50 μl of Dynabeads conjugated with protein G and incubated for 10 min at room temperature. The beads were then washed twice with 200 μl of PBS containing 0.02% Tween 20 and incubated with 100 μl of a serial 10-fold dilution of NoV that ranged from 10^{-1} to 10^{-3} PFU/ml at room temperature for 10 min with rotation. After washing three times with 200 μl of PBS, the beads were incubated with 10 μg of polyclonal NoV antibody (rabbit polyclonal anti-NoV antibody, 0.5 mg/ml; Abcam) in 200 μl of PBS containing 0.02% Tween 20 for 10 min at room temperature and then washed three times with 200 μl of PBS. After washing, 4 μl of Qdot-conjugated secondary antibody (525 goat $F(ab')_2$ anti-rabbit IgG conjugate (H + L); Invitrogen) in 200 μl of PBS containing 0.02% Tween 20 was added immediately to the beads for 10 min at room temperature; the beads were then washed three times with 200 μl of PBS. Fluorescence intensity of the QDs was recorded with the excitation wavelength fixed at 360 nm on a Spectra Max GEMINI-XS spectrofluorometer (Molecular Devices Corp, Sunnyvale, CA).

Statistical analyses. All samples were analyzed in triplicate and represented three individual experiments to determine assay consistency. An analysis of variance was used to calculate the standard errors. Significant differences between the means were determined by Tukey's honestly significantly different test. All statistical analyses were carried out using SPSS software version 12.0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Optimization of the specific NoV detection method. Specific detection of NoV from virus mixtures containing HAV, HEV, and NoV was evaluated by IMS and RT-PCR. During development of the IMS–RT-PCR protocol, the optimal amount of Dynabeads for binding virus in 1-ml samples was determined to be 1.5 mg (data not shown). The RT-PCR results revealed that the IMS method successfully produced a 213-bp amplicon from 10-fold diluted NoV in a stool sample (100 RT-PCR units/ml) in both washout fractions harvested after washing the magnetic beads twice

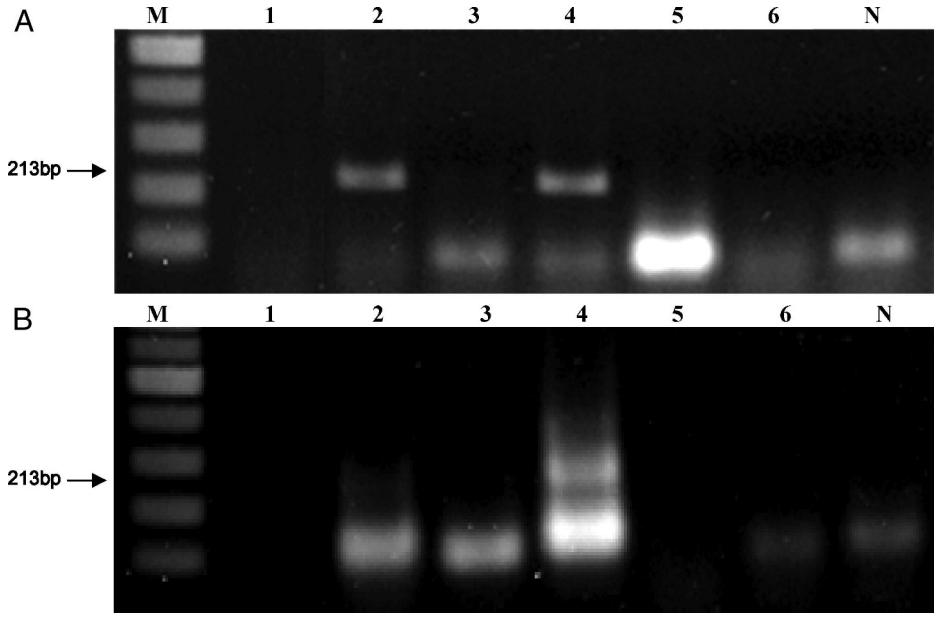

FIGURE 2. *Determination of cross-reactivity of the monoclonal norovirus (NoV) antibody (A) and polyclonal NoV antibody (B) to six subtypes of NoV in the immunomagnetic separation RT-PCR assay. M, 100-bp marker; lane 1, NoV GI-1; lane 2, NoV GI-12; lane 3, NoV GII-3; lane 4, NoV GII-4; lane 5, NoV GII-6; lane 6, NoV GII-8; N, negative control.*

with 200 μl of PBS and in the eluent fraction of the beads remaining after the washing step, whereas 200-bp HAV VP-1P2A amplicons and 352-bp HEV RdRp amplicons were detected only in washout fractions (Fig. 1). These results indicate that IMS is a specific assay for detecting NoV using a 10-min incubation with beads from a stool suspension containing NoV, HAV, and HEV. In a previous study, the IMS system using Dynabeads M280 beads coated with K3-2F2 antibody specifically captured 40.6% of the virus suspended in PBS (10^3 PFU/ml) *(3)*. In this study, NoV was found in the IMS washout fraction because of a number of factors, including the optimal binding capacity of the protein G magnetic beads with antibody, the type of antibody, and the incubation time between antibodies coated on the beads and virus. These results suggest that further studies are necessary to establish optimal IMS conditions by choosing magnetic beads with multivalent ligands to trap antibodies. We evaluated whether our antibodies cross-reacted with six NoV subtypes (GI-1, GI-12, GII-3, GII-4, GII-6, and GII-8) in the IMS–RT-PCR assay. Monoclonal antibody linked to magnetic beads reacted with NoV two subtypes (GI-12 and GII-4), and polyclonal antibody reacted with only one NoV subtype (GII-4), indicating that our detection system is specific to the NoV GII-4 subtype in this study (Fig. 2).

Comparison of two concentration methods for detecting NoV. We evaluated the effects of PEG and IMS on NoV concentration. Although IMS produced weaker NoV amplification than did PEG using RT-PCR, the two virus concentration methods produced the same detectable amplicons (213 bp) from lettuce samples artificially inoculated with 100-fold diluted NoV (Fig. 3B and 3C), which was the dilution endpoint of the positive NoV control in the stool sample (Fig. 3A). The use of IMS facilitated the separation of NoV through interactions between the ligand on the virion surface and the antibody, thus facilitating viral precipitation in a 10-min incubation, whereas the PEG treatment required a longer incubation time and additional high-speed centrifugation to precipitate virus before initiation of RT-PCR.

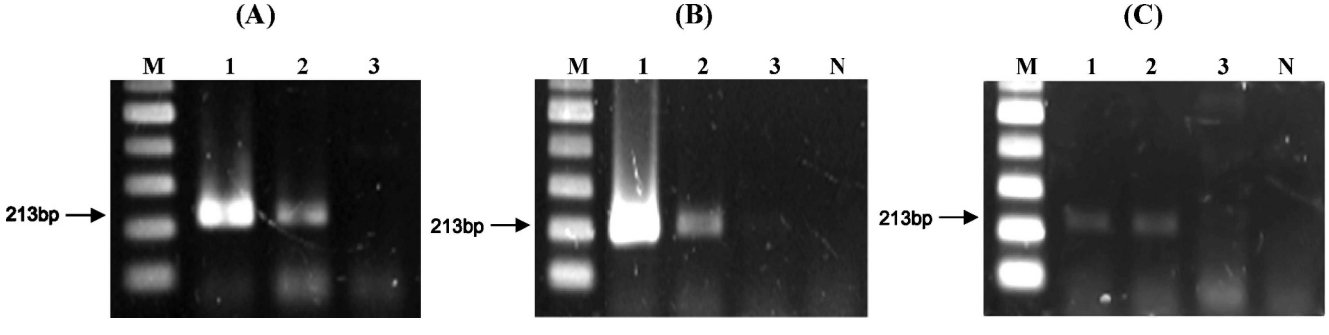

FIGURE 3. *Comparison of the two methods for concentrating norovirus (NoV) by RT-PCR assay. The limit of detection for the RT-PCR assay was assessed with 10-fold serial dilutions of stool sample (A). The amplified NoV RdRp gene (213 bp) was detected by RT-PCR after concentrating it using polyethylene glycol (PEG) (B) and immunomagnetic separation (IMS) (C). M, 100-bp marker; lane 1, 100 μl of 1:10 dilution; lane 2, 100 μl of 1:100 dilution; lane 3, 100 μl of 1:1,000 dilution; N, negative control.*

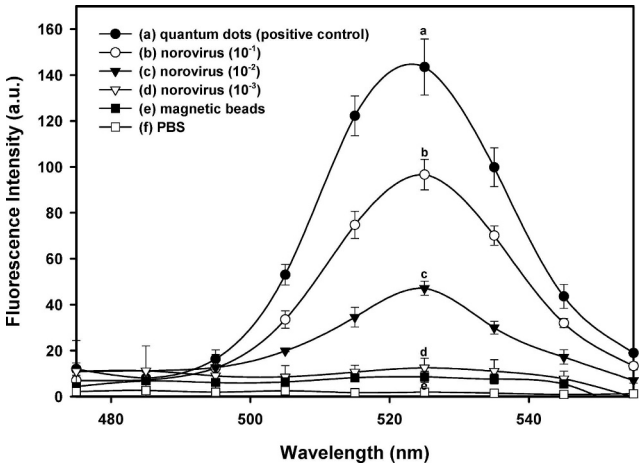

FIGURE 4. *Detection of norovirus (NoV) using a fluorescent quantum dot (QD) probe. (a) QDs (positive control). Ten-fold serially diluted NoV was prepared from a stool sample: (b) 1:10, (c) 1:100, and (d) 1:1,000, negative controls, (e) magnetic beads, and (f) PBS. The excitation and emission wavelengths were 360 and 525 nm, respectively.*

NoV detection limits using IMS and QDs. The NoV detection limit was the same for the PEG–RT-PCR and IMS–RT-PCR methods: a 100-fold dilution of the stool suspension (100 RT-PCR units/ml). The IMS-QD assay was performed to detect NoV more rapidly than can be done with the PEG–RT-PCR and IMS–RT-PCR assays. Our emission spectrum had good symmetry and relatively narrow spectral width, which provided sufficient spectral resolution to detect NoV qualitatively at the maximum emission intensity of 525 nm. We used QDs as a positive control and magnetic beads and PBS as negative controls. Fluorescence intensity decreased with NoV dilutions of 10^{-1} to 10^{-3} in the stool suspension (100 RT-PCR units/ml) (Fig. 4), indicating that this detection system was valid to detect NoV only qualitatively according to fluorescent signal intensity within the range of proposed virus detection limits by IMS–RT-PCR.

In conclusion, a fluorescence detection system using QDs and the IMS method produced enriched fluorescent signals in viral samples and allowed us to detect target virus from a food sample qualitatively within 2 h without the need for the PEG precipitation step. The IMS-QDs technology could serve as a universal sensor for any protein or virus after selecting specific antibodies. Different colored QDs with many emission wavelength maxima can be applied to simultaneously detect different foodborne viruses such as HAV, HEV, NoVs, astrovirus, and rotavirus.

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REFERENCES

1. Abd El Galil, K. H., M. A. El Sokkary, S. M. Kheira, A. M. Salazar, M. V. Yates, W. Chen, and A. Mulchandani. 2005. Real-time nucleic acid sequence–based amplification assay for detection of hepatitis A virus. *Appl. Environ. Microbiol.* 71:7113–7116.
2. Bailey, R. E., and S. Nie. 2003. Alloyed semiconductor quantum dots: tuning the optical properties without changing the particle size. *J. Am. Chem. Soc.* 125:7100–7106.
3. Bidawid, S., J. M. Farber, and S. A. Sattar. 2000. Rapid concentration and detection of hepatitis A virus from lettuce and strawberries. *J. Virol. Methods* 88:175–185.
4. Daniels, N. A., D. A. Bergmire-Sweat, K. J. Schwab, K. A. Hendricks, S. Reddy, S. M. Rowe, R. L. Fankhauser, S. S. Monroe, R. L. Atmar, R. I. Glass, and P. Mead. 2000. A foodborne outbreak of gastroenteritis associated with Norwalk-like viruses: first molecular traceback to deli sandwiches contaminated during preparation. *J. Infect. Dis.* 181:1467–1470.
5. David, S. T., L. McIntyre, L. MacDougall, D. Kelly, S. Liem, K. Schallié, A. McNabb, A. Houde, P. Mueller, P. Ward, Y. L. Trottier, and J. Brassard. 2007. An outbreak of norovirus caused by consumption of oysters from geographically dispersed harvest sites, British Columbia, Canada, 2004. *Foodborne Pathog. Dis.* 4:349–358.
6. D'Souza, D. H., and L. A. Jaykus. 2002. Zirconium hydroxide effectively immobilizes and concentrates human enteric viruses. *Lett. Appl. Microbiol.* 35:414–418.
7. Fino, V. R., and K. E. Kniel. 2008. Comparative recovery of foodborne viruses from fresh produce. *Foodborne Pathog. Dis.* 5: 819–825.
8. Flekna, G., W. Schneeweiss, F. J. Smulders, M. Wagner, and I. Hein. 2007. Real-time PCR method with statistical analysis to compare the potential of DNA isolation methods to remove PCR inhibitors from samples for diagnostic PCR. *Mol. Cell. Probes* 21:282–287.
9. Friedman, D. S., D. Heisey-Grove, F. Argyros, E. Berl, J. Nsubuga, T. Stiles, J. Fontana, R. S. Beard, S. Monroe, M. E. McGrath, H. Sutherby, R. C. Dicker, A. DeMaria, Jr., and B. T. Matyas. 2005. An outbreak of norovirus gastroenteritis associated with wedding cakes. *Epidemiol. Infect.* 133:1057–1063.
10. Gao, X., W. C. W. Chan, and S. Nie. 2002. Quantum-dot nanocrystals for ultrasensitive biological labeling and multicolor optical encoding. *J. Biomed. Opt.* 7:532–537.
11. Gaulin, C. D., D. Ramsay, P. Cardinal, and M. A. D'Halevyn. 1999. Epidemic of gastroenteritis of viral origin associated with eating imported raspberries. *Can. J. Public Health* 90:37–40.
12. Glass, R. I., J. Bresee, B. Jiang, J. Gentsch, T. Ando, R. Fankhauser, J. Noel, U. Parashar, B. Rosen, and S. S. Monroe. 2001. Gastroenteritis viruses: an overview. *Novartis Found. Symp.* 238:5–25.
13. Haramoto, E., M. Kitajima, H. Katayama, and S. Ohgaki. 2010. Real-time PCR detection of adenoviruses, polyomaviruses, and torque teno viruses in river water in Japan. *Water Res.* 44:1747–1752.
14. Ho, Y. P., M. C. Kung, S. Yang, and T. H. Wang. 2005. Multiplexed hybridization detection with multicolor colocalization of quantum dot nanoprobes. *Nano Lett.* 5:1693–1697.
15. Kim, D., S. R. Kim, K. S. Kwon, J. W. Lee, and M. J. Oh. 2008. Detection of hepatitis A virus from oyster by nested PCR using efficient extraction and concentration method. *J. Microbiol.* 46:436–440.
16. Kim, S. E., M. Y. Kim, D. G. Kim, Y. J. Song, H. J. Jeong, S. W. Lee, J. B. Lee, S. Y. Park, C. S. Song, S. J. Oh, H. S. Yoo, and I. S. Choi. 2008. Determination of fecal shedding rates and genotypes of swine hepatitis E virus (HEV) in Korea. *J. Vet. Med. Sci.* 70:1367–1371.
17. Kingsley, D. H., and G. P. Richards. 2001. Rapid and efficient extraction method for reverse transcription–PCR detection of hepatitis A and Norwalk-like viruses in shellfish. *Appl. Environ. Microbiol.* 67:4152–4157.
18. Lemon, S. M., P. C. Murphy, P. A. Shields, L. H. Ping, S. M. Feinstone, T. Cromeans, and R. W. Jansen. 1991. Antigenic and genetic variation in cytopathic hepatitis A virus variants arising

during persistent infection: evidence for genetic recombination. *J. Virol*. 65:2056–2065.
19. Li, Y., Y. T. Cu, and D. Luo. 2005. Multiplexed detection of pathogen DNA with DNA-based fluorescence nanobarcodes. *Nat. Biotechnol*. 23:885–889.
20. Malek, M., E. Barzilay, A. Kramer, B. Camp, L. A. Jaykus, B. Escudero-Abarca, G. Derrick, P. White, C. Gerba, C. Higgins, J. Vinjé, R. Glass, M. Lynch, and M. A. Widdowson. 2009. Outbreak of norovirus infection among river rafters associated with packaged delicatessen meat, Grand Canyon, 2005. *Clin. Infect. Dis*. 48:31–37.
21. Monceyron, C., and B. Grinde. 1994. Detection of hepatitis A virus in clinical and environmental samples by immunomagnetic separation and PCR. *J. Virol. Methods* 46:157–166.
22. Park, H., M. Kim, and G. Ko. 2010. Evaluation of various methods for recovering human norovirus and murine norovirus from vegetables and ham. *J. Food Prot*. 73:1651–1657.
23. Quintero-Betancourt, W., E. R. Peele, and J. B. Rose. 2002. *Cryptosporidium parvum* and *Cyclospora cayetanensis*: a review of laboratory methods for detection of these waterborne parasites. *J. Microbiol. Methods* 49:209–224.
24. Richards, G. P., M. A. Watson, R. L. Fankhauser, and S. S. Monroe. 2004. Genogroup I and II noroviruses detected in stool samples by real-time reverse transcription–PCR using highly degenerate universal primers. *Appl. Environ. Microbiol*. 70:7179–7184.
25. Schmid, D., H. P. Stüger, I. Lederer, A. M. Pichler, G. Kainz-Arnfelser, E. Schreier, and F. Allerberger. 2007. A foodborne norovirus outbreak due to manually prepared salad, Austria 2006. *Infection* 35:232–239.
26. Seymour, I. J., and H. Appleton. 2001. Foodborne viruses and fresh produce. *J. Appl. Microbiol*. 91:759–773.
27. Shelton, D. R., J. A. Higgins, J. A. S. Van Kessel, Y. A. Pachepsky, K. Belt, and J. S. Karns. 2004. Estimation of viable *Escherichia coli* O157 in surface waters using enrichment in conjunction with immunological detection. *J. Microbiol. Methods* 58:223–231.
28. Sobsey, M. D., R. J. Carrick, and H. R. Jensen. 1978. Improved methods for detecting enteric viruses in oysters. *Appl. Environ. Microbiol*. 36:121–128.
29. Tian, P., A. Engelbrektson, and R. Mandrell. 2008. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. *Appl. Environ. Microbiol*. 74:4271–4276.
30. Trujillo, A. A., K. A. McCaustland, D. P. Zheng, L. A. Hadley, G. Vaughn, S. M. Adams, T. Ando, R. I. Glass, and S. S. Monroe. 2006. Use of TaqMan real-time reverse transcription–PCR for rapid detection, quantification, and typing of norovirus. *J. Clin. Microbiol*. 44:1405–1412.
31. Wang, H., Y. Li, A. Wang, and M. Slavik. 2011. Rapid, sensitive, and simultaneous detection of three foodborne pathogens using magnetic nanobead-based immunoseparation and quantum dot–based multiplex immunoassay. *J. Food Prot*. 74:2039–2047.
32. Wang, L., C. S. Wu, X. Fan, and A. Mustapha. 2012. Detection of *Escherichia coli* O157:H7 and *Salmonella* in ground beef by a bead-free quantum dot–facilitated isolation method. *Int. J. Food Microbiol*. 156:83–87.
33. Zhai, L. J., X. Dai, and J. Meng. 2006. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. *Virus Res*. 120:57–69.