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Detection of Noroviruses in Ready-To-Eat Foods by Using Carbohydrate-Coated Magnetic Beads▽

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This study used histo-blood group antigen-conjugated beads to detect norovirus (NoV) in contaminated strawberries, green onions, lettuce, and deli ham. In addition, multiple strains of NoV from genogroups I and II were recovered. This provides an effective protocol for food testing in the investigation of suspected NoV outbreaks.

Norovirus (NoV) is a leading cause of gastroenteritis outbreaks in humans. Food-borne outbreaks of NoV are commonly caused by shellfish grown in contaminated waters (2) or ready-to-eat food products contaminated by infected food handlers (4, 5, 7, 17, 21). Methods for the detection of NoV in foods need to be sensitive due to the low numbers of infectious particles present in foods (15) and the lack of a routine cell culture method for amplifying NoV in the laboratory (3, 22). Real-time reverse transcription (RT)-PCR methods are widely used for the detection of NoV RNA (11, 12). Since PCR amplification requires a small sample size and is sensitive to inhibition, steps are required to extract and concentrate viral particles from food samples prior to RNA purification. The complex and varied nature of food products presents a challenge for the development of extraction procedures.

NoV binds to histo-blood group antigens (HBGA), carbohydrates present on the surface of red blood cells, on the mucosal epithelium, and in bodily fluids (9, 18). The *Norovirus* genus is extremely diverse, and various NoV strains have been shown to recognize different patterns of HBGA (10, 24). Preliminary work has shown that single strains of NoV can be captured from water or buffer using porcine gastric mucin (23) or synthetic HBGA type H1 (1). This report presents a method to detect NoV contamination of foods based on the interaction of the virus with HBGA.

For each experiment, the surfaces of four replicate 25-g food samples were sterilized under UV light for 30 min. Three samples were inoculated with serially diluted NoV filtrate, and one was inoculated with phosphate-buffered saline (PBS). Inocula were allowed to dry for 30 min at room temperature. A minimum of three 10-fold serial dilutions of the NoV stock was tested for each data set. The inoculum level was quantified for each experiment and varied from 1 to 10^7 copies due to natural variability in titer between samples.

Two different types of magnetic beads were prepared by washing 50 μl of streptavidin-coated magnetic beads (Invitrogen, Carlsbad, CA, or Matrix MicroScience Ltd., Newmarket, United Kingdom) with sterile PBS, mixing them with 10 μg or 1 μg of multivalent biotinylated HBGA type A, B, H(2), or H(3) (Glycotech, Rockville, MD) for 30 min at room temperature, and washing them again with PBS. The beads were stored at 4°C until needed.

Samples were transferred to sterile Erlenmeyer flasks or stomacher bags containing 225 ml of citrate buffer (33 mM citric acid and 17 mM sodium citrate, pH 4), and 12.5 μl each of precoated A, B, H(2), and H(3) magnetic beads was added. The beads were concentrated from the sample using either the *i*CropTheBug (Filtaflex, Almonte, ON, Canada) or the Pathatrix (Matrix MicroScience Ltd.) automated extraction system. For the *i*CropTheBug system, Erlenmeyer flasks were mixed on an orbital shaker (Bel-Art Products, Pequannock, NJ) for 30 min at room temperature. The flasks were transferred to the IMBCOL (immunomagnetic bead collector), a powerful settling magnet, followed by 10 min on the IMBCON (immunomagnetic bead concentrator) vibrating table. The beads were collected using the IMBPIP (immunomagnetic bead pipette) and resuspended in 140 μl of PBS. For the Pathatrix system, the samples and beads were mixed by hand for 1 min and then added to the Pathatrix machine. The samples were recirculated for 30 min at room temperature, during which time the beads were collected on the magnet. The beads were washed with 100 ml PBS and collected in 140 μl PBS.

RNA was extracted from 140-μl samples using the QIAamp viral RNA kit as described in the manufacturer's instructions (Qiagen, Hilden, Germany). Real-time RT-PCRs were performed for genogroup I (GI) and GII norovirus as described previously, with no-template controls on each plate (11, 12). A standard curve was generated for GI and GII real-time RT-PCR assays, using RNA transcripts from a plasmid as described previously (16).

NoV detection in food. Tenfold serial dilutions of a GII.4 2006b/Den Haag NoV were concentrated using A-, B-, H(2)-, and H(3)-conjugated beads and either the *i*CropTheBug or Pathatrix system (Table 1). Detection was less sensitive for most food samples than with experiments in buffer. With the *i*CropTheBug system, the detection limit for NoV on green onions remained the same as in buffer (10^2 copies/250 ml). Virus was detected on lettuce and deli ham at a minimum input level of 10^3 copies/250 ml and on strawberries at 10^4

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TABLE 1. Detection of GII.4 2006b/DenHaag NoV in buffer and various food products using A-, B-, H(2)-, and H(3)-conjugated magnetic beads with two automated extraction systems[a]

System	Matrix	No. of positive replicates/total no. at input virus titer[b] of:			
		10^4	10^3	10^2	10^1
*i*CropTheBug	Buffer	3/3	2/3	1/3	—
	Lettuce	3/3	1/3	0/3	—
	Green onions	—	2/3	1/3	0/3
	Strawberries	1/3	0/3	0/3	—
	Deli ham	2/3	1/3	0/3	—
Pathatrix	Buffer	—	3/3	1/3	0/3
	Lettuce	—	1/3	1/3	1/3
	Green onions	—	1/3	1/3	1/3
	Strawberries	—	0/3	0/3	1/3
	Deli ham	—	0/3	0/3	0/3

[a] For each data set, the three viral additions were undiluted, diluted at 1/10, and diluted at 1/100.
[b] The input titer was calculated for each experiment and varied due to differences in the stock sample. The quantity of input virus has been rounded to the nearest factor of 10 copies/250 ml. —, dilution not tested.

TABLE 2. Detection of NoV strains in buffer using 50 μl of HBGA-conjugated magnetic beads with two automated extraction systems

System	Strain	No. of positive replicates/total no. at input virus titer[a] of:				
		10^4	10^3	10^2	10^1	10^0
*i*CropTheBug	GI.1	—	3/3	3/3	1/3	0/3
	GI.3b	—	—	1/3	1/3	1/3
	GI.13	3/3	3/3	—	1/3	0/3
	GII.2	3/3	1/3	2/3	0/3	—
	GII.3	3/3	3/3	3/3	1/3	0/3
	GII.4 2002	3/3	3/3	1/3	1/3	—
	GII.4 2004	3/3	2/3	—	2/3	0/3
	GII.4 2006a	3/3	3/3	2/3	—	0/3
	GII.4 2006b	3/3	2/3	1/3	—	—
Pathatrix	GI.1	3/3	3/3	3/3	0/3	—
	GI.3b	3/3	3/3	0/3	—	—
	GI.13	—	0/3	0/3	0/3	—
	GII.2	—	3/3	2/3	1/3	0/3
	GII.3	—	—	3/3	3/3	1/3
	GII.4 2002	3/3	3/3	1/3	1/3	—
	GII.4 2004	—	—	1/3	0/3	0/3
	GII.4 2006a	3/3	1/3	0/3	0/3	—
	GII.4 2006b	—	3/3	1/3	0/3	—

[a] The input titer was calculated for each experiment and varied due to differences in the stock sample. The quantity of input virus has been rounded to the nearest factor of 10 copies/250 ml. —, dilution not tested.

copies/250 ml. The Pathatrix system had a lower minimum limit of detection (10^1 copies/250 ml) for lettuce, green onions, and strawberries than the *i*CropTheBug system; however, detection using the Pathatrix system was less consistent, with only one of three samples positive over a wide range of NoV titers. Virus was not detected in any deli ham sample (10^1 to 10^3 copies/250 ml) by the Pathatrix system.

Detection of multiple NoV strains. The combination of A-, B-, H(2)-, and H(3)-conjugated beads was able to detect all nine NoV strains tested (Table 2). The *i*CropTheBug system detected the strains with a minimum level of detection in the range of 10^0 to 10^2 copies/250 ml. Detection with the Pathatrix system was more variable, with a minimum level of detection in the range of 10^0 to 10^3 copies/250 ml. One strain, GI.13, was not detected in the Pathatrix system (Table 2).

This study provides evidence that HBGA-magnetic-capture methods are fast and efficient for the concentration of NoV. The procedures successfully extracted NoV particles from several different food types. The procedures are compatible with detection of at least nine NoV strains covering two genogroups. Samples were processed, RNA was extracted, and real-time RT-PCRs were completed in less than 5 h from start to finish.

Methods used previously to concentrate NoV include ultracentrifugation (20), polyethylene glycol precipitation (8, 13), adsorption/elution (6), and immunomagnetic separation (14, 19). These methods can be time-consuming (polyethylene glycol precipitation and adsorption/elution) and strain specific (immunomagnetic separation). Other methods have been developed to detect NoV using HBGA-coupled magnetic beads (1, 23). Porcine gastric mucin and synthetic HBGA type H(1) conjugated to magnetic beads have been used to capture NoV from small-volume surface washings or from water samples (1, 23). The work presented here is the first to demonstrate the use of HBGA-conjugated beads for the extraction of multiple NoV strains. In addition, the reagents have been tested in automated concentration systems and with a variety of food products.

Both magnetic separation systems used in this study concentrated NoV from 250 ml to 140 μl. The Pathatrix system was efficient, allowing multiple samples to run simultaneously. However, it required the purchase of consumables for each reaction and the magnetic beads compatible with the system are a proprietary technology, resulting in a cost of $30 per sample. The *i*CropTheBug system was simple to use and compatible with multiple bead types, but each flask was processed individually. Our costs were approximately $8 per sample. The *i*CropTheBug system provided more consistent detection of NoV from food samples.

The methods developed here achieved detection within the range of the infectious dose for NoV and similar to the best methods for NoV detection from foods (15, 19). This was demonstrated for multiple strains and food types (lettuce, green onion, and strawberries). However, the percent recovery calculated for each extraction was highly variable (0.005% to 99%), and numerous examples of sporadic detection were observed at lower levels of inoculation. This suggests that our method is not suitable for quantifying the amount of NoV present in a sample. The different food matrices may have had a significant impact on the number of beads recovered from each sample (4).

Conclusion. The protocols developed here for the extraction and concentration of NoV from food matrices provide a rapid and effective tool for investigating the suspected contamination of food products. The standardized reagents and automated extraction systems involved are readily available for implementation in food testing laboratories. The complete validation of the method requires additional NoV strains and would ideally include the processing of naturally contaminated food samples. However, our data indicate that this system detects the most common GII.4 NoV as well as additional

strains from GI and GII. The HBGA-NoV interaction has the potential to be used in conjunction with multiple rapid testing platforms for further advancements in food testing technology.

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