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**Capture and Concentration of Viral and Bacterial Foodborne Pathogens using
Apolipoprotein H**

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Abstract

The need for improved pathogen separation and concentration methods to reduce time-to-detection for foodborne pathogens is well recognized. Apolipoprotein H (ApoH) is an acute phase human plasma protein that has been previously shown to interact with viruses, lipopolysaccharides (LPS) and bacterial proteins. The purpose of this study was to determine if ApoH was capable of binding and efficiently capturing two representative human norovirus strains (GI.1 and GII.4), a cultivable surrogate, and four bacterial pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serovar Enteritidis, and *Staphylococcus aureus*). Experiments were carried out using an ApoH-conjugated magnetic bead-based capture followed by pathogen detection using nucleic acid amplification. For all three viruses studied, >10% capture efficiency (<1 Log₁₀ loss in RT-qPCR amplifiable units) was observed. The same capture efficiencies were observed for the bacterial pathogens tested, with the exception of *E. coli* O157:H7 (approximately 1% capture efficiency, or 2 Log₁₀ loss in CFU equivalents). The efficiency of the capture steps did not vary as a consequence of input target concentration or in the presence of an abundance of background microflora. A complementary plate-based capture assay showed that ApoH bound to a variety of human norovirus virus-like particles. ApoH has the potential to be a broadly reactive ligand for separating and concentrating representative foodborne pathogens, both bacteria and viruses.

Keywords

ApoH, detection, norovirus, bacteria, concentration

1.0 Introduction

Foodborne pathogens are responsible for approximately 9.4 million illnesses each year in the United States, with an estimated total illness cost of \$14.0 billion annually (Batz et al, 2012; Scharff, 2012). About 58% (5.5 million) of these illnesses are caused by human norovirus (Scallan et al, 2011). While the bacterial foodborne pathogens are responsible for fewer illnesses (3.6 million each year), they constitute the majority of serious outcomes (Scallan et al, 2011). Nontyphoidal *Salmonella* spp., the most common bacterial causative agent, is the leading cause of food-related hospitalizations (35%) and deaths (28%) (Centers for Disease & Prevention, 2013), while *Listeria monocytogenes* is the third leading cause of foodborne disease deaths (19%) (Scallan et al, 2011; Gandhi & Chikindas, 2007). Two other important foodborne pathogens are shiga toxin-producing *Escherichia coli* (STEC, for which *E. coli* O157:H7 is the most common serovar.), and *Staphylococcus aureus*, which causes self-limiting food intoxication associated with food handling and subsequent temperature abuse (Rangel et al, 2005; Kadariya et al, 2014).

Regulators and industry alike use microbiological testing as one tool to manage these foodborne disease agents. The inability to cultivate human norovirus *in vitro* means that separation and concentration of low levels of virus particles from food matrices is necessary prior to detection, which is done using molecular amplification methods. Conversely, it is possible to enrich low concentrations of most bacterial pathogens, however enrichment steps are time-consuming, often taking up to two days. Waiting for test results has many implications, both financial (e.g., storage and warehousing, reduced product shelf-life) and public health in nature. In fact, users of testing methods almost

universally recognize that the enrichment and/or concentration process is the single most limiting factor in the development of near real-time methods for foodborne pathogen detection (Dwivedi & Jaykus, 2011).

There is a need to develop methods that either shorten enrichment times, or allow bypass of enrichment steps. Separation and concentration of pathogens from the sample matrix prior to detection is necessary in order to achieve these goals. This so-called “pre-analytical sample processing” is of increasing interest to the detection sector and approaches have been reviewed elsewhere (Stevens & Jaykus, 2008; Dwivedi and Jaykus, 2011). The most commonly used methods for pathogen separation and concentration are immuno-magnetic separation (antibody-conjugated paramagnetic beads) or affinity magnetic separation (ligand-conjugated paramagnetic beads) (Fluit et al, 1993a; Fluit et al, 1993b; Liebana et al, 2009; Stevens & Jaykus, 2008). However, depending on the ligand, these methods have their disadvantages, and none is ideal (Dwivedi and Jaykus, 2011). For example, it is very difficult to find broadly reactive ligands for capture of all human norovirus genotypes (and for some bacteria as well); binding affinities can be poor; and cost and/or shelf-life can be problematic. No ligand has binding specificity to a comprehensive and diverse group of foodborne pathogens.

Apolipoprotein H (ApoH), also known as beta2-glycoprotein I, is an acute phase protein found circulating in human plasma (Bouma et al, 1999). ApoH has already been shown to interact directly with a number of different viruses such as hepatitis B, rotavirus and human immunodeficiency virus (Adlhoch et al, 2011; Stefas et al, 1997; Stefas et al,

2001). It also binds with high affinity to LPS on Gram-negative bacteria (Agar et al, 2011) and specific proteins on two Gram-positive pathogens, *S. aureus* (Zhang et al, 1999) and *Streptococcus pyogenes* (Nilsson et al, 2008). The broad range specificity of ApoH may provide a viable alternative to current organism-specific techniques (e.g., antibody capture) for the concentration and separation of viral and bacterial pathogens from food or environmental samples.

The purpose of this study was to determine whether ApoH-conjugated magnetic beads could be used to capture norovirus (representative human genotypes and the Tulane virus, a cultivable surrogate) and select Gram-negative (*E. coli* O157:H7 and *S. enterica* serovar Enteritidis) and Gram-positive (*L. monocytogenes* and *S. aureus*) bacteria from solution. The efficiency of this concentration and separation technique was also determined.

2.0 Materials and Methods

2.1 Virus Strains

The genogroup I, genotype 1 (GI.1) Norwalk virus (NV) (courtesy of Dr. Christine Moe, Emory University, Atlanta, GA) and a GII.4 New Orleans outbreak strain (courtesy of Dr. Shermalyn Greene, North Carolina State Laboratory of Public Health, Raleigh, NC) were obtained as stool samples and diluted 10-20% in phosphate buffered saline (PBS) (MP Biomedicals, Santa Ana, CA). Tulane virus was propagated in the LLC-MK2 cell line as previously described (Farkas et al, 2008) and recovered after three sequential freeze-thaw cycles. All virus stocks were aliquoted and stored at -80°C until used. Serial

dilutions of whole viruses were prepared in PBS or proprietary 1X Binding Buffer (ApoH Technologies, Villaneuve St Georges, France).

2.2 Bacterial cultures and plating

Stock cultures of *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 19115), *Salmonella enterica* serovar. Enteritidis (ME46) and *Staphylococcus aureus* (ATCC 25235) (all from the American Type Culture Collection, Manassas, VA) were propagated aerobically overnight at 37°C in Brain Heart Infusion (BHI) broth (Fisher Scientific, Waltham, MA) before experiments. Plating for enumeration was carried out using the pour plate technique on BHI agar. Plates were grown for 24-48 h at 37°C prior to counting colonies.

2.3 Magnetic Bead Binding to Pathogens

Magnetic bead capture of viruses was performed using the ApoH-CaptoVIR kit (ApoH Technologies), whereas bacteria were captured using both the ApoH-CaptoVIR and ApoH-CaptoBAC kits (ApoH Technologies). Serial dilutions of whole virus particles or bacteria were prepared in 1X PBS (Life Technologies, Carlsbad, CA). One hundred microliters of the serially diluted pathogen was added to 900µl of binding buffer prepared as per the manufacturer instructions (ApoH Technologies). Ten microliters of ApoH-coated magnetic beads (ApoH Technologies) were added to each of the pathogen/binding buffer suspensions and incubated with rotation at 4°C for 60 min.

2.4 Nucleic Acid Extraction

92 For the virus samples, beads were removed from the suspension using a magnetic rack
93 and were washed twice with PBS-0.05% Tween (PBS-T) before being resuspended in
94 2ml of easyMAG Lysis Buffer (bioMérieux SA, Marcy l'Etoile, France). Beads were
95 removed from the lysis buffer using a magnetic rack, and the buffer was processed for
96 RNA extraction using the NucliSENS® easyMAG automated system (bioMérieux), with
97 a final resuspension volume of 40µl. To determine input virus concentration, 100µl of
98 serially diluted virus suspension (described in section 2.1 above) was added to 1800µl of
99 easyMAG Lysis Buffer, which was then processed for nucleic acid extraction as above.
100 RNA was stored at -80°C until use.

101

102 For bacteria samples, beads were removed from suspension using a magnetic rack and the
103 supernatants were reserved for cultural enumeration. The beads were gently washed and
104 resuspended in 200µl of PBS. The PBS-bead mixture was added to 750µl Lysis Solution
105 in a ZR BashingBead Lysis Tube for DNA extraction using the ZR Fungal/Bacterial
106 DNA Miniprep kit (Genesee Scientific, San Diego, CA, USA) as per manufacturer
107 instructions. To determine input CFU for each bacterial concentration, 100µl of each
108 dilution mixed with 100µl of PBS went directly into the Lysis Solution for DNA
109 extraction. The DNA was reconstituted into 40µl of elution buffer and kept at -20°C until
110 use.

111

112 Samples containing both virus and bacteria were run in parallel, with one sample being
113 processed for viral RNA isolation and the other for bacterial DNA isolation. These
114 samples were prepared as above, however the binding buffer was reduced to 800µl to

accommodate the increase in input sample volume. Unlike the initial studies looking at bacteria capture alone, input bacteria concentrations for bacteria-virus co-capture remained constant at approximately 10^6 cfu/mL, while virus dilutions [10^{-1} , 10^{-2} and 10^{-3} , dilutions corresponding to approximately 10^4 , 10^3 and 10^2 input RT-qPCR amplifiable units (RT-qPCRU), respectively] were evaluated.

2.5 Detection and Quantification of Viral RNA

Detection of viral RNA was carried out by reverse transcriptase quantitative PCR (RT-qPCR) using the SuperScript[®] III Platinum[®] One-Step qRT-PCR kit (Life Technologies) with the primers and probes outlined in Table 1. The amplification temperature profile was as follows: 30 min at 50°C, 15 min at 95°C; 45 cycles of 15 s at 95°C, 30 s at 60°C. Thermal cycling was done using a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA). Standard curves were generated using 10-fold serial dilutions of RNA extracted from stock virus (GI.1 or GII.4), and RNA extracted from serially diluted cell culture lysate containing Tulane virus. Consistent with others (Tung et al, 2013), the cycle threshold (Ct) values were plotted against each respective dilution, and the highest dilution for which three positive signals were obtained from three separate runs was defined as one RT-qPCR unit (RT-qPCRU); this also corresponded to the lower limit of detection of the assay. RT-qPCR was performed on both sample ‘input’ and sample ‘output’ (virus captured by ApoH-coated beads). The capture efficiency was determined by using the following equation: [(RT-qPCRU corresponding to virus captured by ApoH-conjugated beads)/(RT-qPCRU corresponding to input virus)] x 100 (Tian et al, 2008).

2.6 Detection and Quantification of Bacteria

As has been previously reported for other ligands (Boulanger & Edelstein, 1995; Cullison & Jaykus, 2002), inconsistent counts were obtained when performing cultural enumeration on the ApoH bead-bound bacteria (data not shown). Therefore, capture efficiency for the bacterial work was determined based on loss to supernatant (evaluated using cultural methods) and/or by qPCR performed on DNA extracts corresponding to captured bacteria. For the former approach, input bacteria and the non-adherent bacteria in the discarded supernatant after bead capture were plated for enumeration on BHI agar. Percent capture was calculated using the following formula: $[(\text{total concentration of input bacteria} - \text{concentration of bacteria in supernatant}) / (\text{total concentration of input bacteria})] \times 100$ (Stevens & Jaykus, 2004).

Amplification of bacterial DNA was carried out using the primers and probes described in Table 1. The reagents used were the Platinum[®] Taq DNA Polymerase kit (Life Technologies) supplemented with a dNTP mix (Life Technologies). The amplification temperature profile was as follows: 10 min at 95°C; 40 cycles of 20 s at 95°C, 30 s at 56°C and 1 min at 72°C. The same PCR cycling conditions were used for all bacteria (Rodríguez-Lázaro et al, 2004), done using a Bio-Rad CFX96 Touch Real-Time PCR Detection System. Standard curves were generated using 10-fold serial dilutions of DNA extracted from untreated overnight bacterial cultures. The resulting bacterial Ct values were plotted against the input bacteria based off of serial dilution and plating, and analyzed using linear regression to determine the slope. To approximate the bacterial

counts, the experimental Ct values were converted to CFU equivalents (CFU-E) by application of the standard curves. Bacteria capture efficiency based on qPCR was calculated as follows: [(qPCR-based CFU-E corresponding to bacteria captured by ApoH-conjugated beads)/(CFU-E corresponding to input bacteria] x 100 (Kramer et al, 2009).

2.7 Virus-Like Particles (VLPs)

Three GI virus-like particles (VLPs) [GI.1, GI.6, and GI.7], and six GII VLPs [GII.2 (Snow Mountain, SMV), GII.4 (Houston, HOV; Grimsby, GRV), GII.6, GII.7, GII.12 and GII.17] (courtesy of Dr. Robert Atmar, Baylor College of Medicine, Houston, TX), were used in this study.

2.8 Plate-Based ApoH Capture Assay

VLPs described above were diluted in PBS to a final concentration of 3mg/ml. ApoH-coated microplates (ApoH Technologies, Villaneuve St Georges, France) were incubated with 100µl of VLP suspension per well (four wells per replicate) at 37°C for 90 min as per manufacturer instructions. Negative controls (four wells of PBS containing no VLPs) were included in all replicates. Suspensions were removed from the wells, and then blocked using Superblock (Thermo Fisher) for 2 h at 4°C. Wells were thrice washed using 200µl PBS-T. One hundred microliters of primary antibody (Ab3901 for GI, AbNS14 for GII; courtesy of Dr. Robert Atmar, Baylor College of Medicine) was added to each well at a concentration of 3mg/ml. Samples were incubated at 37°C for 60 min, followed by washing three times with 200µl PBS-T. One hundred microliters of

secondary antibody (Ab6789; Abcam, Cambridge, UK) was then added to each well and incubated at 37°C for 60 min. Wells were washed again thrice with PBS-T and 100µl of TMB 2-Component Microwell Peroxidase Substrate System (Fisher Scientific, Waltham, MA) was added with incubation at room temperature for 15 min. Absorbance was measured at 450nm using a TECAN Infinite M200 Pro plate reader (TECAN Systems, Inc. San Jose, CA). Values were considered significant if the ratio between the test sample and the negative control was greater than 2.0 (Tian et al, 2006).

2.9 Statistical Analysis

Three independent replicates were performed for each experiment. Data for all studies were expressed as mean $\pm$ standard deviation of Log₁₀ concentration (tube-based capture assays) or sample/negative control ratio (plate-based assay). All statistical comparisons between samples were done in JMP (SAS Institute, Cary, NC) using the Tukey-Kramer honest significant difference test and considered significant if $p < 0.05$.

3.0 Results

3.1 ApoH Bead-Based Capture of Whole Virus Particles

A comparison of the RT-qPCRU corresponding to whole virus particles before and after capture using ApoH-coated beads is presented in Figure 1. In virtually all instances, there was no statistically significant difference between input virus concentration and that recovered using the ApoH beads ($p > 0.05$), indicating a high degree of capture efficiency. On a percentage basis, >23% of the input virus was captured by the beads; hence, virus loss during capture was always $< 1 \text{ Log}_{10}$ RT-qPCRU. The limit of detection for TV

capture-RT-qPCR occurred at the 10^{-6} dilution, or approximately 100 input RT-qPCRU (Figure 1a); for NV, 10^{-4} , or approximately 10 input RT-qPCRU (Figure 1b); and for GII.4, 10^{-4} , or approximately 10 input RT-qPCRU (Figure 1c).

Figure 1. Capture of virus particles using the ApoH-CaptoVIR kit. Serial dilutions of virus stocks were subjected to ApoH capture, magnetic separation, RNA extraction of the virus-bound beads, and detection/enumeration by RT-qPCR. Capture efficiency (%) was calculated as the ratio of captured virus to input virus as measured in RT-qPCR amplifiable units (RT-qPCRU). There were no statistically significant differences ($p < 0.05$) between input and captured virus at a given input virus concentration.

3.2 ApoH Capture Assay for VLPs

Because of an interest in evaluating the reactivity of ApoH with a variety of human norovirus strains, 11 VLP types were used in a plate-based ApoH capture assay. Seven VLP types could be captured by the plate assay (positive/negative ratio >2.0; Figure 1). VLPs GI.6, GI.7, GII.3 and GII.6 showed mean test sample/negative control ratios of <2.0, meaning poor capture efficiency (Tian et al, 2006). The highest degree of capture was observed for the GII.4 HOV VLP strain, although significant capture and detection was also observed for the GI.1, GII.2 SMV, GII.4 GRV, GII.7, GII.12 and GII.17.

Figure 2. Human norovirus VLP binding to ApoH-coated microplates as determined using a capture assay approach. Binding (capture) was considered significant when positive/negative ratios were greater than 2.0 (Tian et al, 2006). Different letters (a, b, c, d) indicate statistically significant differences between binding efficiency when comparing VLP genotypes ($p < 0.05$).

3.3 ApoH Capture of Bacteria

The capture efficiency, as evaluated by qPCR and cultural enumeration, for the four bacteria tested using the ApoH-CaptoVIR kit are shown in Figure 3a. Using the qPCR method and *E. coli* O157:H7, there was a significant difference ($p < 0.05$) between the input concentration and that captured. Specifically, capture efficiency for *E. coli* O157:H7 ranged from 2.1-5.0% for all bacteria concentrations tested, indicating close to a 2 Log₁₀ loss during the capture step. For the Gram-positive bacteria and *S. enterica*, the capture efficiencies were much better, ranging from 29.2%- 92.0% (for all initial bacteria concentrations), or < 1 Log₁₀ loss during the capture step. On the other hand, there were no statistically significant differences ($p > 0.05$) when comparing capture efficiencies for all four bacteria based on results using the cultural method (Figure 3b). When comparing the performance of the ApoH-CaptoBAC kit to the ApoH-CaptoVIR kit, a significantly reduced efficiency of capture using both the qPCR and cultural methods was again observed for *E. coli* O157:H7 (data not shown). The ApoH-CaptoVIR kit was chosen for subsequent experiments due to the desire to evaluate one single kit for its efficacy in capture of both bacteria and viruses.

Figure 3. Capture of select bacteria using the ApoH-CaptoVIR kit. Serial dilutions of fresh bacterial stocks were subjected to ApoH capture, magnetic separation, DNA extraction of the bacteria-bound beads, and detection/enumeration by RT-qPCR (A); or else by cultural enumeration of the discarded supernatant from the capture step (B). Capture efficiency (%) was calculated as the ratio of captured bacteria to bacteria virus as

258 measured in CFU equivalents (A) or as measured by loss to supernatant (B). *Statistically
259 significant difference ($p < 0.05$) between input and captured bacteria at a given input
260 bacteria concentration.

3.4 ApoH Capture from Mixed Bacteria-Virus Samples

To evaluate the potential for competition between bacteria and viruses when in the same solution, ApoH capture of GII.4 Sydney human norovirus in the presence of different bacterial pathogens (Figure 4a) and bacterial capture in the presence of virus (Figure 4b), was evaluated. Specifically, input bacteria concentrations for bacteria-virus co-capture experiments remained constant at approximately 10^6 cfu/mL, while virus input concentration (10^4 , 10^3 and 10^2 RT-qPCRU) was varied. The virus capture data under these circumstances was consistent with that obtained using virus-only suspensions, with an approximate 1 Log₁₀ loss in viral genome copy number after capture compared to input, across all bacteria at all concentrations. The bacterial capture performance was also consistent with the previous studies, with a <1 Log₁₀ loss across three out of four bacteria, regardless of viral load. As was the case for bacteria only, the *E. coli* O157:H7 maintained the previously observed 1-2 Log₁₀ loss during ApoH capture when suspended in solutions also containing norovirus. There was no effect on either human norovirus or pathogenic bacteria capture efficiency when non-target bacteria and viruses (10^8 cfu/ml and 10^{12} pfu/ml, respectively) were added to mixed samples prior to ApoH capture. This suggests that ligand saturation did not occur due to the presence of excess background microflora.

Figure 4. ApoH co-capture of whole human norovirus particles and bacterial pathogens.

The bacterial pathogens remained at a constant concentration, 10⁶ cfu/ml, while the amount of virus varied, as shown (10⁴, 10³, 10² RT-qPCRU). (A) Virus capture in the presence of the indicated bacterial pathogens, as determined by RT-qPCR. (B) Bacteria

capture in the presence of the indicated virus concentration. Bacterial capture efficiency was based on the qPCR method. *Statistically significant differences between input and captured (A) virus or (B) bacteria.

Discussion

The need to shorten and simplify methods to detect bacterial and viral pathogens in food and environmental samples is well established. The most commonly used approach to achieve this is concentration and purification of the target from the sample matrix just prior to detection. Pathogen concentration methods range from general (e.g. high speed centrifugation, filtration) to specific (immunomagnetic separation), with a emphasis on separating the organisms from sample matrices, removing inhibitors and reducing sample size while maintaining viability/infectivity. Physical and chemical approaches are useful but usually lack specificity. Biological approaches have greater specificity, often using antibodies or phages to target individual bacterial strains, with high capture efficiencies (Dwivedi & Jaykus, 2011). For example, cell wall-binding domains of bacteriophage endolysins for specific bacteria were conjugated to paramagnetic beads and shown to capture select bacteria with efficiencies of over 90% (Kretzer et al, 2007). Similar approaches using magnetic beads conjugated to virus cellular receptors and nucleic acid aptamers, to name a few, have been used for capture and detection of human norovirus (Tian et al., 2008; Escudero-Abarca et al., 2014). Unfortunately, concentration strategies such as these are limited in scope, as specialized approaches focus on capturing and detecting a single bacterial or viral pathogen per test. For a more broadly reactive application, a system needs to be developed where strain variation, pathogen type

(bacterial, viral, protozoal) or emerging, as yet unidentified pathogens, may be captured and enumerated.

ApoH-bacteria interactions have been documented in the literature, but the protein has not been used before as a capture ligand. For example, anti-ApoH antibodies have been found to be associated with several bacterial infections, and multiple pathogens directly interact with the ApoH protein, including *S. aureus* and *S. pyogenes* (Zhang et al, 1999). Furthermore, binding to LPS causes ApoH to undergo a conformational change leading to binding and clearance of bacteria during infection (Agar et al, 2011). This binding is mediated largely by ionic and hydrophobic interactions (Hammel et al, 2001), instead of the protein-specific interactions observed for some Gram-positive bacteria. This difference in interaction mechanism may explain the slightly higher capture efficiency observed for Gram-positive (67.7-92.0%) versus Gram-negative (2.1-66.4%) bacteria (Figure 3).

The capture efficiency for bacteria observed in this study was comparable to similar affinity-based separations, where, using different matrices, recovery has ranged from 33-215% in broth to 13-50% in ground beef and milk (Stevens & Jaykus, 2008). Unfortunately, the recovery of *E. coli* O157:H7 using ApoH (2.1-5.0%) was lower than density gradient centrifugation (20-45%) or crude filtration (10-95%) (Stevens & Jaykus, 2008), suggesting that the ApoH method sometimes underperforms relative to other concentration approaches. A complementary plate-based capture assay showed that ApoH bound to a variety of human norovirus VLPs. Taken together, the results

demonstrate that ApoH has the potential to be a broadly reactive ligand for separating and concentrating foodborne pathogens, with an affinity for a variety of human norovirus strains, in addition to select Gram-negative and Gram-positive of foodborne significance.

A single strategy to concentrate both enteric viruses and bacteria has not been previously explored, making ApoH a unique capture ligand. A major advantage of joint capture is the ability to use a single reagent for concentration and purification of multiple pathogen targets. Of course, the specificity of ApoH-bacteria interactions is unknown, and it is possible that the molecule shows broad binding affinity to a variety of microorganisms, both pathogenic and non-pathogenic. Even if that were the case, ApoH would have utility in food microbiology, although perhaps in a slightly different application . The fact that the ApoH magnetic beads were able to capture bacteria amongst a background of viruses, and vice versa, without significant loss of efficiency, bodes well for its use in more complex sample matrices. However, the studies described here did not take into account the potential impact of the sample matrix itself, which can be substantial. Unfortunately, due to the proprietary nature of the ApoH product, we were unable to obtain unconjugated beads for use as a negative control. In addition, a comprehensive binding specificity study using related and unrelated bacteria and viruses was not undertaken in our study. It therefore is unclear if the ligand-pathogen interaction is specific or if a complex enough sample matrix could competitively saturate the beads. Because the impact of the matrix is a black box, future studies should focus on using ApoH to concentrate and separate both viral and bacterial pathogens from relevant food and environmental samples.

354

355 In summary, in this proof-of-concept study, ApoH was used for the concentration of key
356 foodborne bacteria and viruses prior to detection by nucleic acid amplification. For all
357 three viruses studied at all concentrations, >10% capture efficiency (<1 Log₁₀ loss in RT-
358 qPCR) was observed, even when viruses were co-captured in the presence of bacteria.
359 The same can be said for the bacterial pathogens tested, with the exception of *E. coli*
360 O157:H7 (approximately 1% capture efficiency, or 2 Log₁₀ loss). The efficiency of the
361 capture steps did not vary as a consequence of input target concentration or in the
362 presence of an abundance of background microflora. The ApoH ligand performed well
363 for several human norovirus strains, as well as three different pathogenic bacteria,
364 making it relatively broadly reactive and amenable to concentration of multiple,
365 structurally different organisms. Further, the capture step appeared to work well in the
366 presence of background microflora. The next logical step is to evaluate the performance
367 of the methods in complex sample matrices, studies that are underway in our laboratory.

368

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564 **Table 1.** Primers and probes used for RT-qPCR detection of viruses and qPCR detection of bacteria.

565 Pathogen	Primers and Probes	Reference
GI.1 Norwalk	COG1F 5'-CGYTGGATGCGNTTYCATGA-3' COG1R 5'-CTTAGACGCCATCATCATTYAC-3' RING1a 5'-6-FAM-AGATYGCGATCYCCTGTCCA-BHQ-3' RING1b 5'-6-FAM-AGATCGCGGTCTCCTGTCCA-BHQ-3'	Kageyama et al (2003)
GII.4 New Orleans	JJV2F 5'-CAAGAGTCAATGTTTAGGTGGATGAG-3' COG2R 5'-TCGACGCCATCTTCATTCACA-3' RING2P 5'-6-FAM-TGGGAGGGCGATCGCAATCT-BHQ-3'	Jothikumar et al (2005); Kageyama et al (2003)
Tulane Virus	TV2F 5'-GAGATTGGTGTCAAACACTCTTTG-3' TV2R 5'-ATCCAGTGGCACACACAATT-3' TVP 5'-6-FAM-AGTTGATTGACCTGCTGTGTCA-BHQ-3'	Sestak et al (2012)
<i>E. coli</i>	ECN1254F 5'-GCAAGGTGCACGGGAATATT-3' ECN1328R 5'-CAGGTGATCGGACGCGT-3' ECL1277P 5'-6-FAM-CGCCACTGGCGGAAGCAACG-BHQ-3'	(Takahashi et al, 2009)
<i>L. monocytogenes</i>	hlyQF 5'-CATGGCACCACCAGCATCT-3' hlyQR 5'-ATCCGCGTGTTTCTTTTCGA-3' hlyQP 5'-6-FAM-CGCCTGCAAGTCCTAAGACGCCA-BHQ-3'	(Rodríguez-Lázaro et al, 2004)
<i>S. enterica</i>	ttr-6 5'-CTCACCAGGAGATTACAACATGG-3' ttr-4 5'-AGCTCAGACCAAAAGTGACCATC-3' ttr-5 5'-6-FAM-CACCGACGGCGAGACCGACTTT-BHQ-3'	(Malorny et al, 2004)
<i>S. aureus</i>	nucF372 5'-TGTAGTTTCAAGTCTAAGTAGCTCAGCAA-3' nucR465 5'-TGCACTATATACTGTTGGATCTTCAGAA-3' nuc 5'-6-FAM-TGCATCACAAACAGATAACGGCGTAA-BHQ-3'	(Alarcón et al, 2006)