USDA-NIFA Food Virology Collaborative

Objective 2 – Virus Detection
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* Mary K. Estes
* Timothy G. Palzkill
* Robert L. Atmar
Norovirus VLP Availability

Genogroup II
- Porcine
- GII.4 Variants:
  - Grimsby/1996
  - Houston/2002
  - Farmington Hills/2002-like
  - New Orleans/2009
  - Sydney/2012
- GII.20
- GII.19
- GII.18
- GII.16
- GII.15
- GII.13
- GII.11
- GII.10
- GII.9
- GII.8
- GII.7
- GII.6
- GII.5
- GII.4
- GII.3
- GII.2
- GII.1

Genogroup I
- Bovine
- GI.4
- GI.3
- GI.2
- GI.1
- GI.9
- GI.8
- GI.7
- GI.6
- GI.5
- GI.4
- GI.3
- GI.2
- GI.1

Genogroup IV
- Feline/Canine
- GIV.1
- GIV.2

Genogroup V
- Murine
- GV.1

Genogroup VI
- Canine
- GVI.1
- GVI.2

Available VLPs

In production
### Summary of MAb Epitope Mapping by Competition ELISA

#### A

<table>
<thead>
<tr>
<th>Biotin MAb</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>NV23</td>
<td>NV37</td>
<td>F8</td>
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<tr>
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<td>NV7</td>
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#### B

![Venn Diagram]

- **Group 1**: NV23, NV37, NV3, NV57, NV7, NS22, NS941
- **Group 2**: F8, F120

Crawford et al. Manuscript in revision
## Comparison of MAb Reactivity by Assay Format (Capture vs. Direct EIA)

<table>
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<th>MAbs</th>
<th>GI.1</th>
<th>GI.2</th>
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<th>GI.7</th>
<th>GI.8</th>
<th>GI.1</th>
<th>GII.2</th>
<th>SMV</th>
<th>GI.3</th>
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<th>GRV</th>
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### Kₐ’s of Different NoV-Specific MAbs by SPR (nM)

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<th>GI.2</th>
<th>GI.4</th>
<th>GI.6</th>
<th>GI.7</th>
<th>GI.8</th>
<th>GII.1</th>
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<th>SMV</th>
<th>GII.3</th>
<th>GII.4</th>
<th>Hov</th>
<th>Sydn.</th>
<th>GIL.6</th>
<th>GIL.7</th>
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<th>GIL.12</th>
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<th>GIV.1</th>
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## Detection of GI Strains Using NV23 for Capture

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<table>
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<tr>
<th>Genotype</th>
<th>NoV VLP LLoD (ng/well)</th>
<th># Ag (+) Fecal Samples/Total (%)</th>
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<tbody>
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<td>GI.1</td>
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<td>5/5 (100%)</td>
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<td>GI.4</td>
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<td>(0)</td>
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<td>GI.6</td>
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<td>0/2 (0)</td>
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<tr>
<td>GI.7</td>
<td>25</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>GI.8</td>
<td>25</td>
<td>1/3 (33%)</td>
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### Detection of GII & GIV Strains Using NV23 for Capture

<table>
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<tr>
<th>Genotype</th>
<th>Strain/variant</th>
<th>LLoD (ng/well)</th>
<th>Fecal #(+)/Total (%)</th>
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<td>SMV/1976</td>
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<td>GII.3</td>
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<td>6/7 (86%)</td>
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<td>GII.4</td>
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<td>HOV/2002</td>
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<td>13/14 (93%)</td>
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<td>Sydney/2012</td>
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<td>GII.6</td>
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<td>4/4 (100%)</td>
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<td>10</td>
<td>4/7 (57%)</td>
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<tr>
<td>GIV.1</td>
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</table>
* VLPs from many genotypes available
* Several NoV-specific MAbs available
* NV23 works reasonably well as a capture antibody, although LLOD varies by genotype
Yang Lab

North Carolina Central University
VLP binding:
Overnight vs. 1 h

Blocking:
Overnight vs. 1 h

Temperature:
4°C vs. 25°C
3-hour Fluorescent Immunoassay

Average Fluorescence vs. VLP Concentration (µg/mL)

- 2.5 µg/mL Alexa Fluor® 488 Goat Anti-Mouse Ab
  - Equation: $y = 30161x + 63654$
  - $R^2 = 0.88$

- 5.0 µg/mL Alexa Fluor® 488 Goat Anti-Mouse Ab
  - Equation: $y = 26454x + 38473$
  - $R^2 = 0.99$

- 0.002 mg/mL mAb 3901 Anti-GI.1 VLP Ab
- 0.008 mg/mL mAb 3901 Anti-GI.1 VLP Ab

Excitation Emission (485 nm) (520 nm)

Alexa Fluor® 488 Anti-Mouse Ab

Anti-VLP Ab

VLPs
"Signal-down" capture ELISA

**Without VLPs**

- **TMB**
- **Products** (abs. 450nm)
- HRP-conjugated anti-mouse Ab
- Anti-GI.1 VLP Ab

**With VLPs**

- **TMB**
- **Products** (abs. 450 nm)

Graph:
- **y = -0.0903x + 0.4063**
  - $R^2 = 0.99$
- **y = -0.0448x + 0.2298**
  - $R^2 = 0.94$
# Table 1: Comparison of Correlation Values, Ranges, and Limits of the evaluated immunoassays

<table>
<thead>
<tr>
<th>Method</th>
<th>$R^2$ Value</th>
<th>Detection Range ($\mu$g/mL)</th>
<th>Detection Limit$^a$ ($\mu$g/mL)</th>
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<tbody>
<tr>
<td>Three-Day (3-d) ELISA</td>
<td>0.97-0.99</td>
<td>0-0.555</td>
<td>0.012 (L1)$^b$; 0.106 (L2)$^c$</td>
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<tr>
<td>Three-Hour (3-h) ELISA</td>
<td>0.98-0.99</td>
<td>0-0.925</td>
<td>0.033 (L1); 0.098 (L2)</td>
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<tr>
<td>Three-Hour (3-h) Fluorescent Immunoassay</td>
<td>0.88-0.99</td>
<td>0-3.000</td>
<td>- (L1); 0.097 (L2)</td>
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<td>“Signal-Down” Capture ELISA</td>
<td>0.94-0.99</td>
<td>0-2.040</td>
<td>0.00044 (L1); 1.477 (L2)</td>
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$^a$ Detection limit was calculated using $LOD=X_{Blank}+2\delta$ with 95% confidence level.

$^b$L1 denotes the lower primary (3-d, 3-h ELISA) or secondary (3-h immunoassay) antibody employed in the assay

$^c$L2 denotes the higher primary (3-d, 3-h ELISA) or secondary (3-immunoassay) antibody employed in the assay

Proof of concept Impedance biosensor for GI.1 VLP detection

Electron transfer resistance

Interdigitated microelectrodes
Selection, Characterization and Application of Nucleic Acid Aptamers for the Capture and Detection of Human Norovirus Strains

Blanca I. Escudero-Abarca*, Soo Hwan Suh†a, Matthew D. Moore, Hari P. Dwivedi†b, Lee-Ann Jaykus

Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina, United States of America

Figure 5. Binding of aptamer 25 to HuNoV stool specimens derived from outbreaks. Partially purified 10–20% stool suspensions were diluted and tested using ELASA. Negative controls consisted of PBS alone and HuNoV-negative human stool suspensions (NVF); the positive control was GI.4 (Houston) VLP. Results are expressed as ratios between absorbance readings for test sample versus negative control (T/N). Experiments were done in triplicate. Statistically significant differences between the ratios obtained from the positive stool specimens and the NVF are designated with an asterisk (p<0.05).
doi:10.1371/journal.pone.0106805.g005

Figure 6. Performance of the AMC-RT-qPCR method (using aptamer SMV 25) as applied to artificially contaminated lettuce samples. Lettuce samples were inoculated with varying concentrations of a 20% suspension of HuNoV GI.4 fecal stock. They were pre-treated for virus concentration and purification using a combined elution-PEG precipitation method prior to AMC-qPCR. The negative controls consisted of the AMC using blocked beads in the absence of the aptamer. Experiments were done in triplicate. Statistically significant differences in recovery efficiencies are designated with an asterisk (p<0.05).
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Problem

* HuNoV are difficult to detect in food and environmental samples
  * Lack of cell culture necessitates use of RT-qPCR
  * Processing complex samples
    * Low recovery
    * Variable recovery efficiency
Purpose and Objectives

Evaluate Differences in Behavior of Candidate Process Controls (MNV, Tulane virus [TV], MS2, Turnip Crinkle Virus (TCV), Mengovirus) for Extraction of HuNoV from Complex Samples

* Aim 1: Determine which virus(es) have a similar RNA extraction efficiency to HuNoV GI and GII
* Aim 2: Determine which virus(es) have a similar PEG precipitation efficiency to HuNoV GI and GII
* Aim 3: Determine which virus(es) have a similar elution efficiency to HuNoV GI and GII from foods


* See POSTER for details

* Greatest variability associated with RNA extraction steps

* Loss during elution and PEG precipitation lower and less variable
Aptamers for Capsid Integrity/Functionality

* Infectivity dilemma—way to discriminate infectious particles in vitro?
  * Genome integrity and capsid integrity/functionality methods
    * Enzymatic treatment, receptor binding assays, long RT-PCR
* Purpose: To determine if ssDNA aptamers can be used to approximate capsid integrity
* Further Characterize capsid degradation as a function of receptor/ligand binding
* Analyzed using ELASA, HBGA binding plate assay (HELPA), TEM
  * GII.2 Snow Mountain (SMV), GII.4 Houston (HOV), GII.4 Sydney 2012
* To compare ISO/TS 15216 methods for recovery of HAV from fresh and dried produce and to evaluate murine norovirus (MNV-1) as a process control for virus recovery method

* Produce types tested:
  * Fresh and dried pomegranate, strawberry, raspberry
  * Fresh blueberry
  * Dried cranberry, chives, tomatoes
  * Trail Mix with nuts, chocolate and raisins

See POSTER
RNA Extraction Comparison

* To assess the impact of sample matrix and RNA extraction method on RT-qPCR inhibition and virus recovery from food and soil samples

* Sample Matrices tested using an ISO/TS 15216-based method; 30 min adsorption step included for virus cocktail
  * Lettuce – Iceberg Lettuce
  * Deli Meat – Black forest ham
  * Mixed Berries – Berry medley (black, rasp and blue berries)
  * Pasta Salad – Macaroni salad
  * Soil* – top soil (known to contain RT-PCR inhibitors)
* RNA extraction Methods
  * Lysis Buffer - guanidine thiocyanate lysis buffer made in-house, with omega spin columns
  * UNEX Buffer – similar protocol to ‘Lysis’, but different buffer.
  * Trizol-Chloroform extraction method
  * Qiagen viral RNA kit.

* With and without a chloroform extraction step

* Internal amplification controls (IAC) added to investigate PCR inhibition
Ct value distribution for IACs with different sample matrices and RNA extraction methods.
Recovery of MNV-1, GI.1 and GII.4 viral RNA from different sample matrices using four RNA extraction methods A) without or B) with a preceding chloroform extraction step.
Results Summary

* Efficiency of RNA extraction methods in reducing RT-qPCR inhibition depended on
  * sample matrix
  * chloroform extraction after elution-concentration
* The chloroform extraction step reduced PCR inhibitors in the sample and improved viral RNA recovery in nearly all cases
* The soil sample matrix showed the highest level of PCR inhibition followed by pasta the salad matrix; the least amount of PCR inhibition observed was from the lettuce sample matrices
Results Summary

* For viral RNA recovery and PCR inhibition,
  * The GuSCN Lysis buffer and the Esphere Unex buffer were generally comparable
  * Efficiency of Qiagen QiAmp kit and Trizol buffer method varied with sample matrix

* PCR inhibition observed in this study was mainly in the GII assay rather than GI assay,
  * variation in the RT-PCR mastermix used may be responsible for the observed differences