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Noroviruses at Retail: Current Challenges and Future Opportunities

Lee-Ann Jaykus, Professor
Dept. of Food, Bioprocessing and Nutrition

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

NoroCORE
Food Virology
Collaborative for Outreach, Research & Education

Outline

- The Challenges
  - Methodology
  - Control
  - Capacity
- The Opportunities

The Challenges--Methodology

- Human noroviruses (HuNoV) cannot be cultivated *in vitro* and there is no relevant animal model
- Adequacy of cultivable surrogates
- Strain diversity
- No licensed or officially approved detection methods (clinical, food, or environmental samples)
- The infectivity dilemma

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The Relevance of Surrogates

Surrogates for the Study of Norovirus Stability and Inactivation in the Environment: A Comparison of Murine Norovirus and Feline Calicivirus

JENNIFER L. CANNON,[1] EFSTATHIA PAPAFRAGKOU,[2] GEUNWOO W. PARK,[1] JASON OSBORNE,[3] LEE-ANN JAYKUS,[2] AND JAN VINJÉ[1]*

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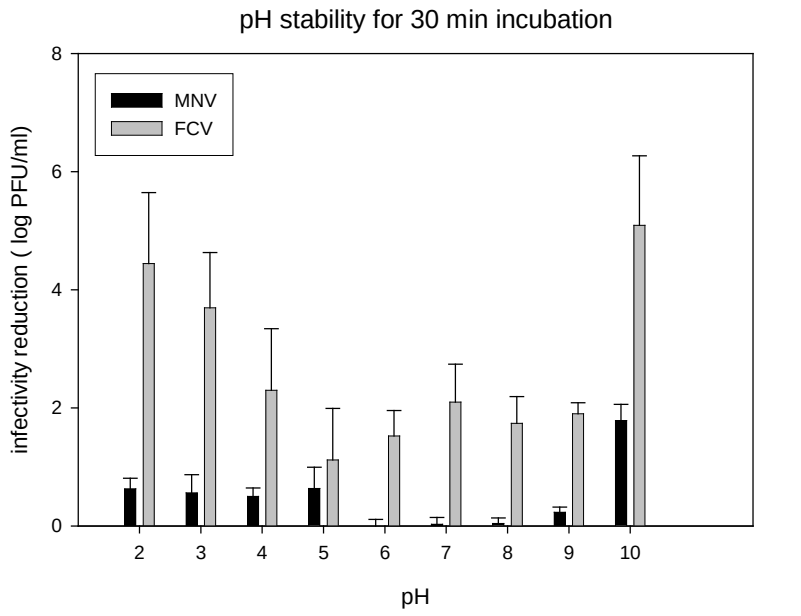
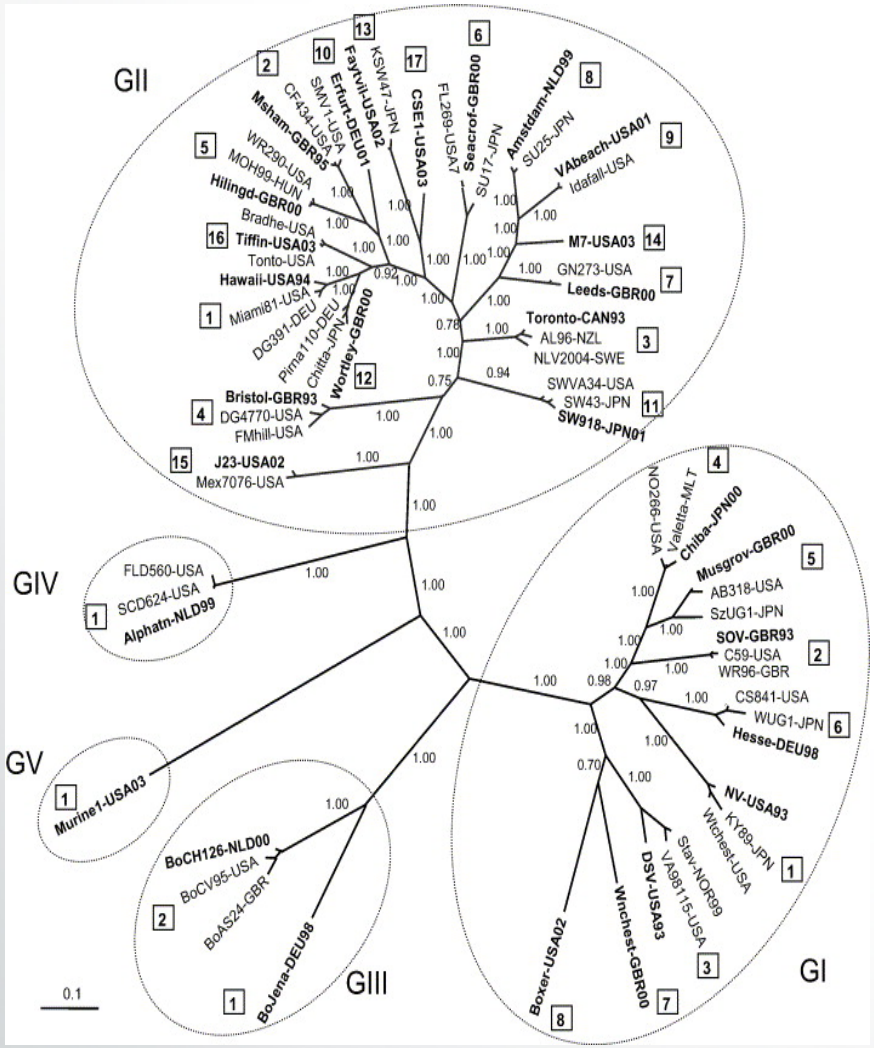
Comparative Efficacy of Seven Hand Sanitizers against Murine Norovirus, Feline Calicivirus, and GII.4 Norovirus†

GEUN WOO PARK,[1]* LESLIE BARCLAY,[1] DAVID MACINGA,[2] DUANE CHARBONNEAU,[3] CHARLES A. PETTIGREW,[3] AND JAN VINJÉ[1]

			Reduction in titer[a]			
			MNV		FCV	
Active ingredient	Concn (%)	Contact time (min)	qRT-PCR (log RNA copy/ml)	Plaque assay (log PFU/ml)	qRT-PCR (log RNA copy/ml)	Plaque assay (log PFU/ml)
Ethanol	50	1	0.1 ± 0.2	0.3 ± 0.4	0.3 ± 0.6	0.3 ± 0.1
		5	0.2 ± 0.1	0.4 ± 0.3	0.0 ± 0.3	2.2 ± 0.6
	70	1	3.2 ± 0.5	>3.6	0.3 ± 0.5	0.5 ± 0.6
		5	3.9 ± 0.5	>3.6	0.0 ± 0.2	2.6 ± 0.3
	90	1	3.1 ± 0.6	3.2 ± 0.7	0.1 ± 0.4	0.1 ± 0.2



Strain Diversity



- Strain Diversity
  - Antigenic
  - Genetic
  - Propensity toward mutation and recombination
- Take home message:
  - Lots of strains
  - Frequent emergence of new strains
  - Few broadly reactive reagents

From Zheng, 2005

Clinical Detection Challenges

Diagnostic Accuracy and Analytical Sensitivity of IDEIA Norovirus Assay for Routine Screening of Human Norovirus[▽]

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Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; Oregon State Public Health Laboratory, Hillsboro, Oregon 97124[2]; Commonwealth of Virginia, Division of Consolidated Laboratory Services, Richmond, Virginia 23219[3]; Virology Department, Leeds Teaching Hospitals NHS Trust, Leeds LS1 3EX, United Kingdom[4]

Received 29 March 2010/Returned for modification 14 May 2010/Accepted 10 June 2010

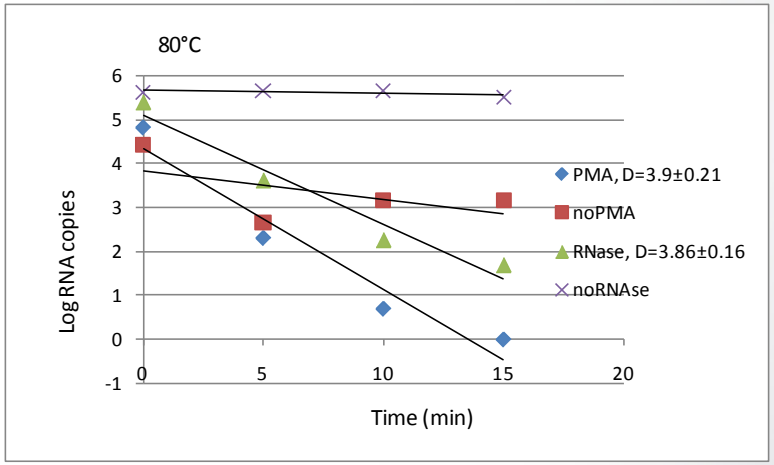
Noroviruses (NoVs) are recognized as the leading cause of epidemic and sporadic acute gastroenteritis. Early detection of NoV is crucial to control the spread of the disease. In this study, we evaluated the diagnostic accuracy, analytical sensitivity, and analytical reactivity of the IDEIA Norovirus assay (an enzyme immunoassay [EIA]) in a prospective and retrospective study design. A total of 557 prospectively collected fecal samples and a panel of 97 archived fecal samples, including 21 different GI and GII genotypes, were tested by conventional reverse transcription-PCR (RT-PCR)/bidirectional sequencing, real-time RT-PCR, and electron microscopy. The sensitivity and specificity of the EIA were 57.6% and 91.9%, respectively. The sensitivity for detecting NoV in fecal samples from outbreaks improved from 44.1% when three samples were tested to 76.9% when five samples per outbreak were tested. The EIA was able to detect strains from 7 GI and 11 GII genotypes. The analytical sensitivity of the EIA was 3.1×10^6 and 1.6×10^7 virus particles g^{-1} of fecal sample for NoV GI and GII strains, respectively. Most GII samples positive by EIA had a threshold cycle (C_T) of <26.5, and 50% of the GII samples negative by EIA had a C_T of >25.6, suggesting that, although strains from genotypes GI.8, GII.10, and GII.16 were not detected, the low sensitivity of the EIA is primarily caused by low virus concentration. In conclusion, the current EIA may be of use as a rapid screening test during a norovirus outbreak investigation when multiple fecal samples are available; however, sporadic samples should be tested by molecular methods.

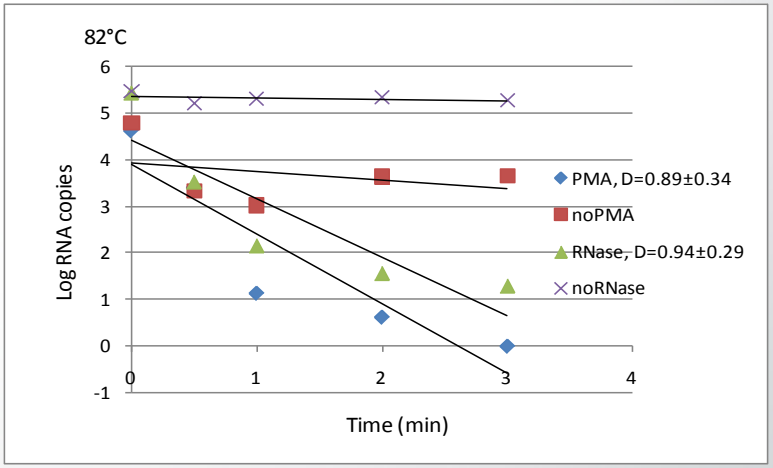
Detection Challenges--Food and Environmental Samples

- No approved commercial methods available in the U.S.
- Detection in foods
  - Low levels of contamination
  - Unable to cultivate
  - Genetically/antigenically diverse
  - Large sample sizes relative to small amplification volumes
  - Diverse, complex matrices
  - Interference/inhibition
  - **Virus concentration and purification**

The "Infectivity Dilemma"

- What constitutes a "positive" for infectious virus?
  - Naked RNA vs. infectious virus
  - Particle:infectious particle ratio
  - Viral aggregation?
  - Gradual vs. instantaneous?
- Can we use RT-qPCR to measure virus infectivity?
  - Measuring capsid integrity
  - Measuring virion integrity
  - Likely to be process-specific
- The goal: positive RT-qPCR signal = Infectious particle





The Challenges--Control

- Characteristics of virus infection
- Environmental persistence and transferability
- Resistance to commonly used disinfectants
- Resistance to common food preservation and processing practices
- Vomiting
- Managing behavior of food handlers

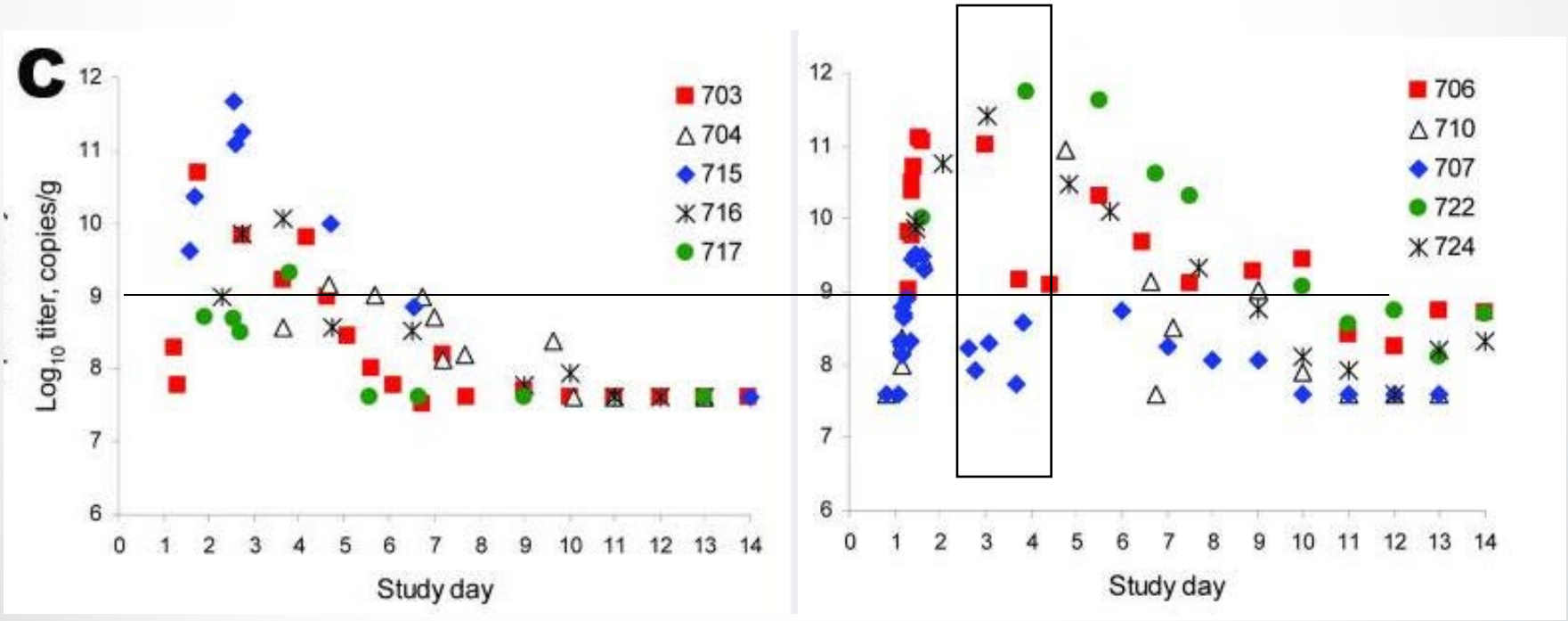
Transmissibility of HuNoV: Contributing Factors

- Low infectious dose (50% of susceptible individuals at ~5 x 10^3 quantifiable genome copies)
  - How many infectious particles?
  - How much fecal material?
- High level of shedding (10^6-10^{10} particles/g feces)
- Prolonged post-symptomatic shedding
- Asymptomatic shedding
- Role of vomitus

Human Challenge Studies

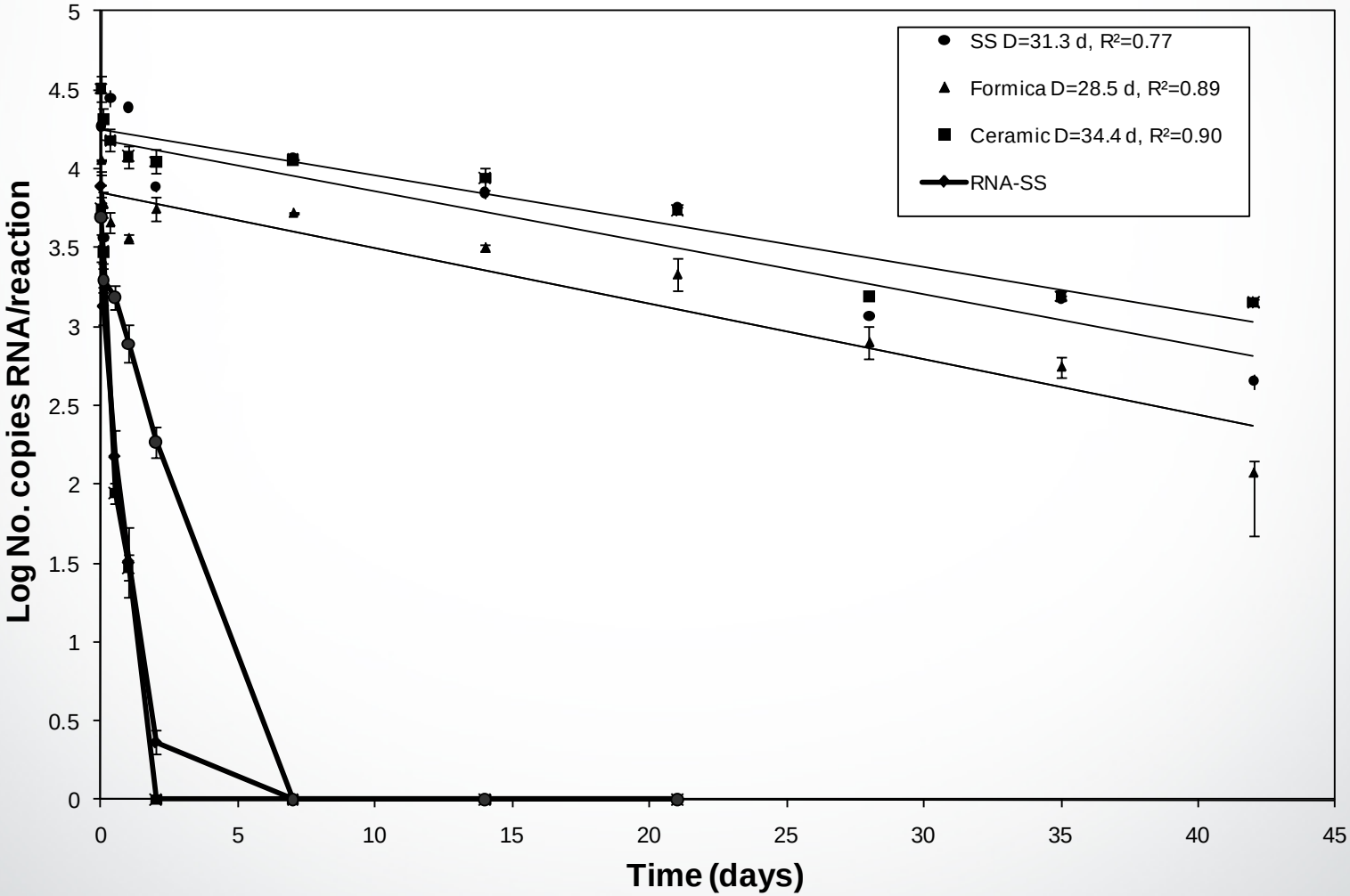
Asymptomatic

Vomiting/diarhea



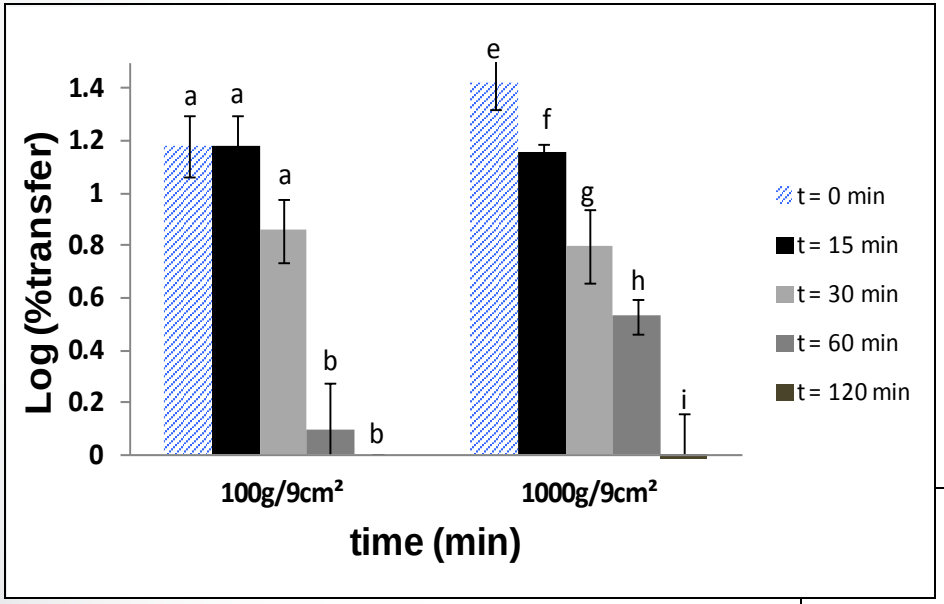
Atmar et al., 2008.

Persistence of HuNoV on Surfaces

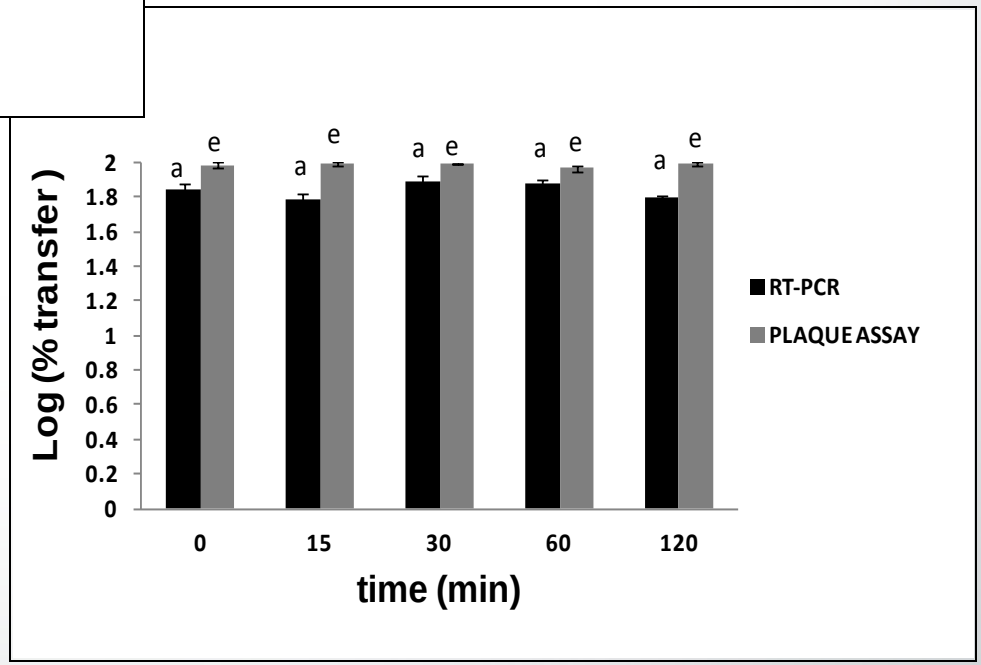


From: Escudero-Abarca et al., 2012

Transferability



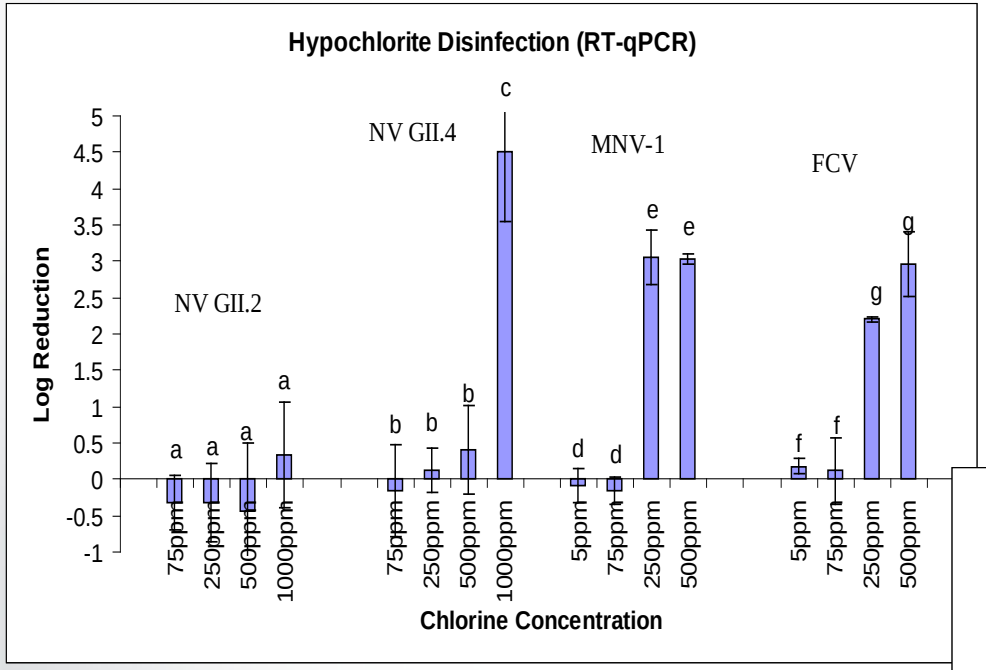
GII HuNoV and Lettuce



MNV-1 and Deli Turkey Meat

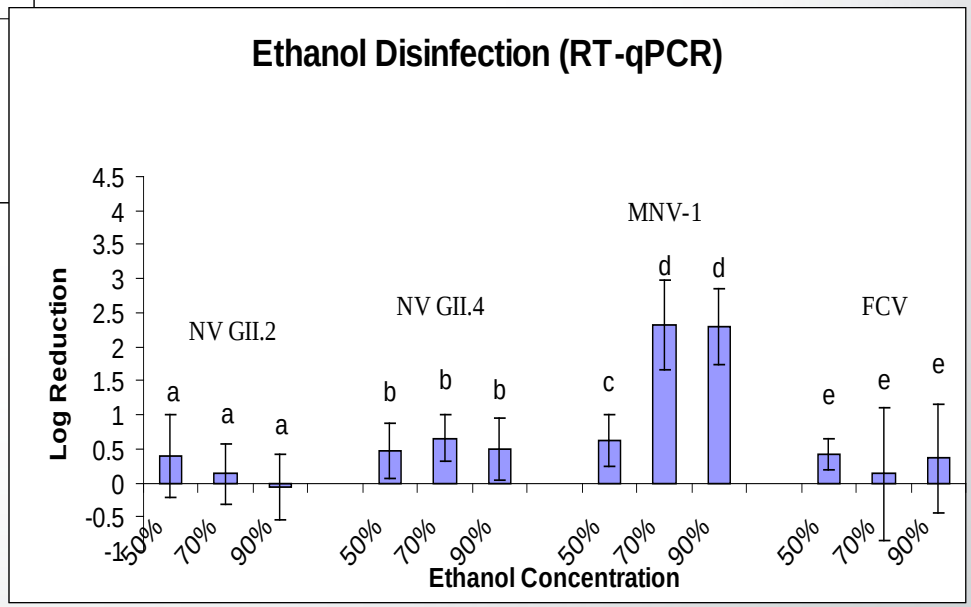
From: Escudero-Abarca et al., 2012

Disinfection Efficacy



Comparative Efficacy of Seven Hand Sanitizers against Murine Norovirus, Feline Calicivirus, and GII.4 Norovirus†

GEUN WOO PARK,[1]* LESLIE BARCLAY,[1] DAVID MACINGA,[2] DUANE CHARBONNEAU,[3] CHARLES A. PETTIGREW,[3] AND JAN VINJÉ[1]



Improved Inactivation of Nonenveloped Enteric Viruses and Their Surrogates by a Novel Alcohol-Based Hand Sanitizer[▽]

David R. Macinga,[1]* Syed A. Sattar,[2] Lee-Ann Jaykus,[3] and James W. Arbogast[1]

Efficacy of Ethanol-Based Hand Sanitizers

Effectiveness of Liquid Soap and Hand Sanitizer against Norwalk Virus on Contaminated Hands[▽]

Pengbo Liu,[1] Yvonne Yuen,[1]§ Hui-Mien Hsiao,[1] Lee-Ann Jaykus,[2] and Christine Moe[1]*

Center for Global Safe Water, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322,[1] and Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695[2]

TABLE 1. *In vivo* efficacies of hand wash agents against NV evaluated by standard and modified ASTM methods[a]

Exposure	Standard ASTM method			Modified ASTM method		
	n	Avg log reduction (SD)		*n*	Avg log reduction (SD)	
		No RNase	RNase		No RNase	RNase
Dry control	20	0.20 (0.28)[A]	0.16 (0.06)[X]	20	0.22 (0.25)[A]	0.20 (0.24)[X]
Hand sanitizer	20	0.14 (0.31)[A]	0.27 (0.12)[X]	10	0.22 (0.22)[A]	0.34 (0.22)[X]
Liquid soap[b]	20	0.94 (0.46)[B]	0.67 (0.47)[Y]	10	1.20 (0.64)[B]	1.10 (0.49)[Y]
Water rinse[c]	20	0.75 (0.63)[B]	0.58 (0.37)[Y]	20	1.58 (0.48)[B]	1.38 (0.49)[Y]

[a] Different superscript capital letter designations in a column indicate statistically significant differences ($P < 0.05$) between mean $\log_{10}$ reductions by disinfection/removal treatments for the finger pad eluates with no RNase treatment (A and B) or with RNase treatment (X and Y) prior to RT-qPCR.

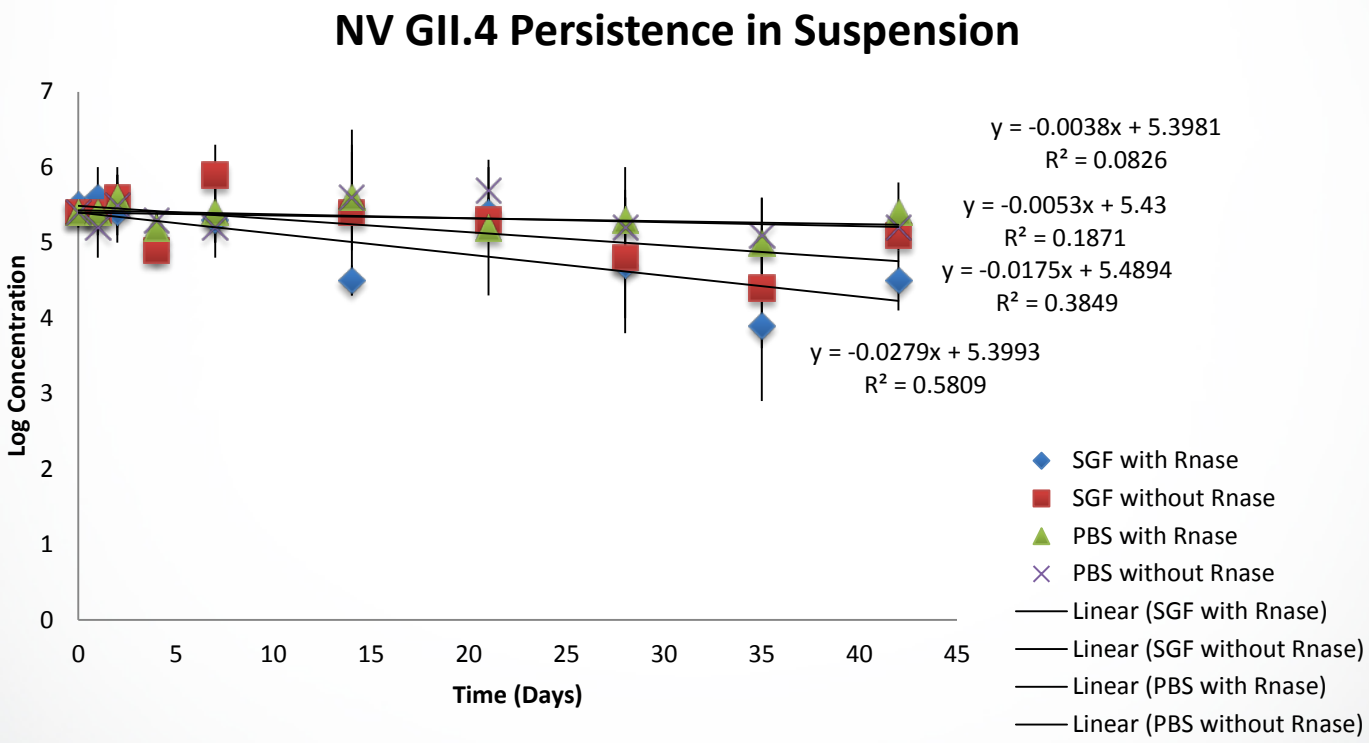
[b] There was a marginal statistical difference ($P = 0.048$) between the results from the standard and modified ASTM methods for samples that received the RNase treatment.

[c] In comparing the results of the standard and modified ASTM methods for each disinfection/removal treatment, statistically significant differences ($P < 0.05$) were observed for treatments either with no RNase treatment or with RNase treatment prior to RT-qPCR.

Conventional Preservation and Processing Controls

- Traditional microbiological indicators of fecal contamination
- Intrinsic parameters
  - a_w
  - pH
- Extrinsic parameters
  - Temperature (heat and cold)
  - Preservatives
- Standard food processes

Persistence of GII.4 in SGF Suspension



HuNoV GII.4 persistence in suspension of SGF or PBS, over the course of 42 days, with or without RNase pretreatment prior to RT-qPCR

Emerging Processing Technologies

Randomized, Double-Blinded Clinical Trial for Human Norovirus Inactivation in Oysters by High Hydrostatic Pressure Processing[▽]†

Juan S. Leon,[1]‡ David H. Kingsley,[2]‡ Julia S. Montes,[1]‡ Gary P. Richards,[2] G. Marshall Lyon,[3] Gwen M. Abdulhafid,[3] Scot R. Seitz,[3] Marina L. Fernandez,[1] Peter F. Teunis,[1] George J. Flick,[4] and Christine L. Moe[1]*

Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322[1]; United States Department of Agriculture, Agricultural Research Service, Delaware State University, Dover, Delaware 19901[2]; Emory University, Atlanta, Georgia 30322[3]; and Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061[4]

TABLE 2. Distribution of study subject infection status among oyster treatment groups

Phase	Treatment conditions	No. of subjects infected/total (%) postchallenge with:		*P* value[b]
		HPP-treated oysters	Untreated oysters[a]	
1	400 MPa, 25°C, 5 min	3/5 (60)	7/15 (47)	1.0000
2	600 MPa, 6°C, 5 min	0/10 (0)	7/15 (47)	0.0202
3	400 MPa, 6°C, 5 min	3/14 (21)	7/15 (47)	0.2451

[a] The control group represented the combined number of controls over phase 1 through phase 3 (n = 15) because each control received untreated HuNoV-seeded raw oysters with the same amount of HuNoV inoculum.

[b] Fisher's exact two-sided test compared each treatment group to all of the controls (i.e., the total number of subjects challenged with non-HPP treated oysters).

The Role of Vomiting?

Recurring Norovirus Transmission on an Airplane

Craig N. Thomley,[1] Nicola A. Emslie,[2] Tim W. Sprott,[2] Gail E. Greening,[3] and Jackie P. Rapana[1]

[1]Auckland Regional Public Health Service, Auckland District Health Board, Auckland, New Zealand; [2]Air New Zealand Medical Unit, Air New Zealand, Auckland, New Zealand; and [3]Environmental Health Food Group, Institute of Environmental Science and Research, Porirua, New Zealand

(See the Editorial Commentary by Lopman, on pages 521-22.)

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doi:10.1017/S0950268810000981

A norovirus outbreak associated with environmental contamination at a hotel

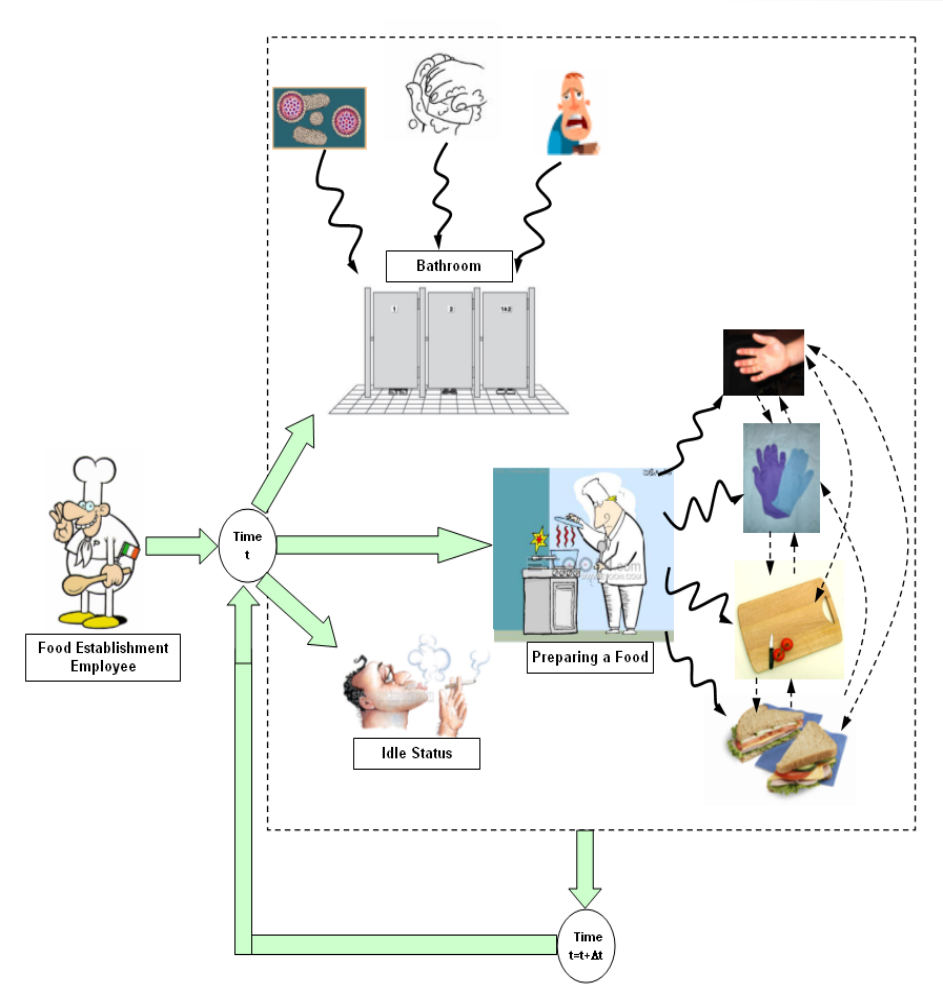
H. KIMURA[1]*, K. NAGANO[2], N. KIMURA[3], M. SHIMIZU[2], Y. UENO[4], K. MORIKANE[5] AND N. OKABE[6]

Disease Transmission and Passenger Behaviors during a High Morbidity Norovirus Outbreak on a Cruise Ship, January 2009

Mary E. Wikswo,[1] Jennifer Cortes,[1] Aron J. Hall,[1] George Vaughan,[2] Christopher Howard,[2] Nicole Gregoricus,[1] and Elaine H. Cramer[2]

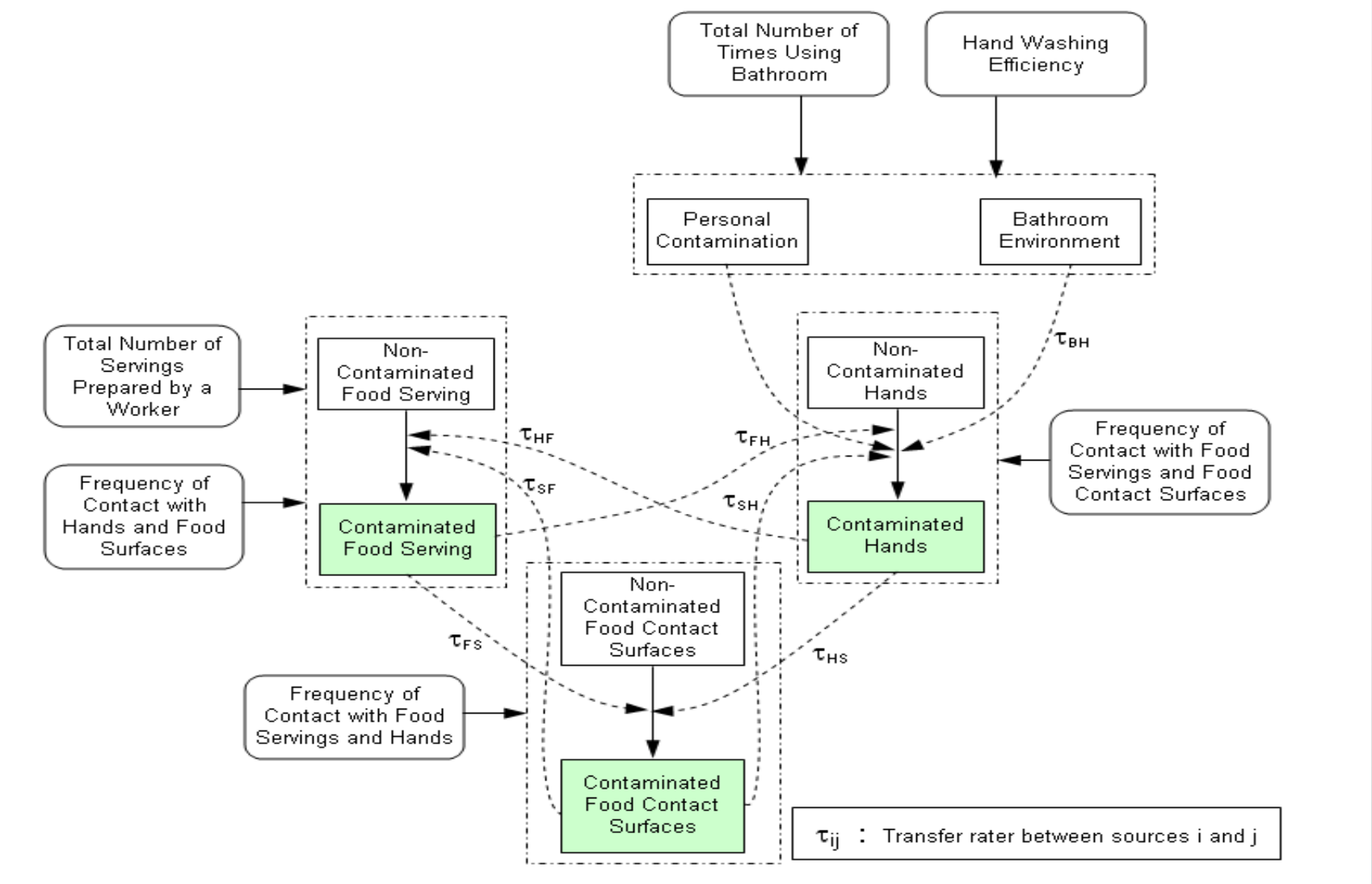
[1]National Center for Immunization, and Respiratory Diseases, and [2]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia

Food Handling



From: Mokhtari and Jaykus, 2008

Schematic Diagram of the Model



Input	Description	Distribution
LR	Log inactivation in the number of NoV particles on hands per minute (log/min)	Triangle(0.01,0.03,0.05)
m_{FH}	Mass of feces per hand (g/hand)	$10^{\text{Triangle}(-8,-3,-1)}$
n_{FS}	Number of times that food touches surface in a minute (per min)	Uniform(1,3)
n_{HF}	Number of times that hands touch food in a minute (per min)	Uniform(1,10)
n_{HS}	Number of times that hands touch surface in a minute (per min)	Uniform(1,5)
nv_{CE}	NoV contamination in the bathroom environment (log/cm^2)	Uniform(0,1)
nv_{CF}	Viral concentration in feces (log/g)	Triangle(2,6,8)
p_{CB}	Probability of hands become contaminated in bathroom	Triangle(0,0.25,0.7)
p_G	Probability of using gloves	Uniform(0,0.5)
p_{CG}	Probability of changing gloves	Uniform(0,0.1)
p_{WH}	Probability of washing hands in bathroom	Triangle(0.3,0.8,1.0)
r_{HW}	Hand washing removal efficiency (%)	Uniform(30,90)
r_S	Surface washing removal efficiency (%)	Uniform(30,90)
tr_{EH}	Transfer rate from environment to hands (bathroom)	Beta(0.56126,2.1455)
tr_{HS}	Transfer rate from hands to surface in the production line	Beta(1.1645,7.7930)
tr_{SH}	Transfer rate from surface to hands in the production line	Beta(1.1351,15.0810)
tr_{HF}	Transfer rate from hands to food in the production line	Beta(1.1651,2.6298)
tr_{FH}	Transfer rate from food to hands in the production line	Beta(2.2567,15.502)
tr_{SF}	Transfer rate from surface to food in the production line	Triangle(0.02,0.07,0.17)
tr_{FS}	Transfer rate from food to surface in the production line	Triangle(0.02,0.07,0.17)

What-If Scenario Analysis: Hand Washing Compliance vs. Efficiency

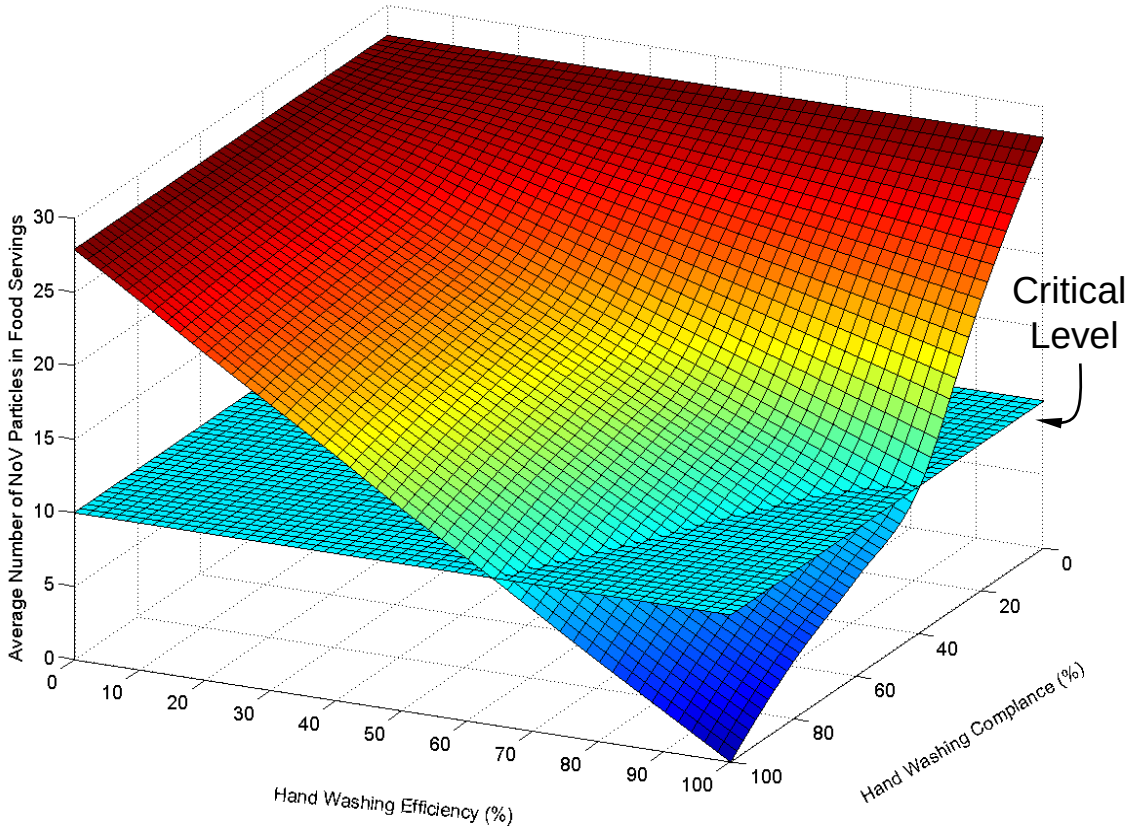


Table: Number of recommended and actual handwashing (HW) episodes per hour, and estimated handwashing time needed to comply with frequency recommendations in selected institutional food service, restaurants and retail food store facilities

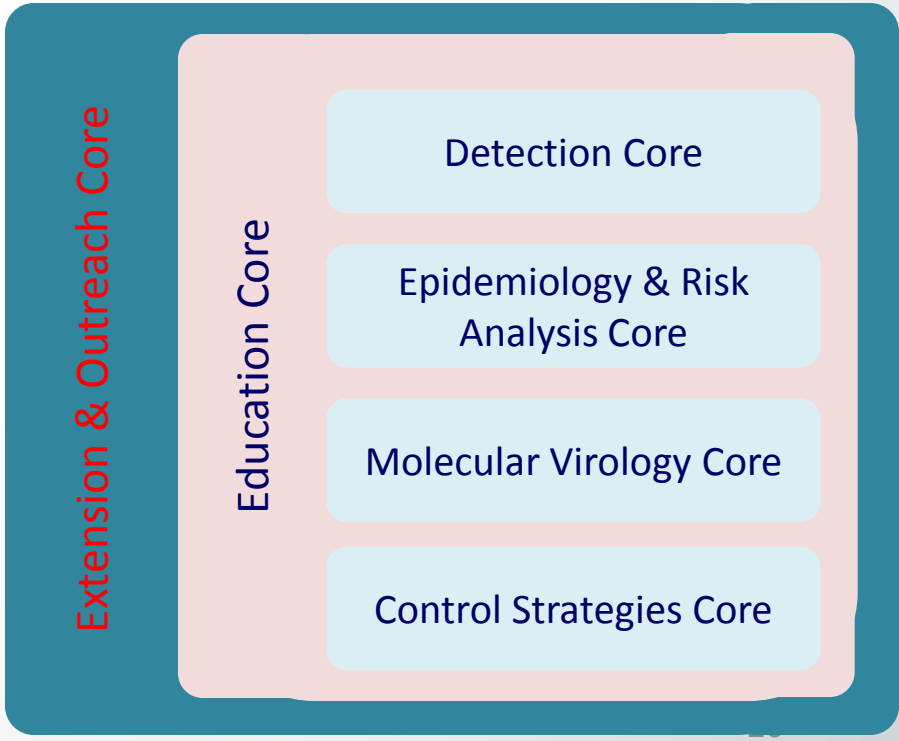
Setting	Number of recommended handwashing episodes[1]	Number of actual handwashing episodes performed[1]	Estimated time to perform recommended number of HW episodes[2]	Source
Institutional Foodservice				
Assisted-living facilities	7	1	7	Strohbehn et al., 2008
Childcare centers	9	2	9	Strohbehn et al., 2008
Schools	11	1	11	Strohbehn et al., 2008
Commercial Foodservice				
Restaurants	9	2	9	Green et al., 2006
Restaurants	29	0	29	Strohbehn et al., 2008
Catering businesses[3]	17	5	17	Clayton and Griffith, 2004
Deli departments, chain stores	27[a]	4[a]	27	Lubran et al., 2010
Deli departments, independent stores	17[a]	1[a]	17	Lubran et al., 2010

The Challenges--Capacity

- Reagent availability
- Standardized protocols
- Trained professionals
- Need for trans-disciplinary collaboration
- Cost
- Public and industry awareness

The USDA-NIFA Food Virology Collaborative

- Long Term Goal: To reduce the burden of food borne disease associated with viruses, particularly noroviruses
- Approach: Multi-disciplinary team working in an integrated manner to develop improved tools, skills, and capacity to understand and control food borne virus risks
- Objectives (Cores):
  - Molecular virology
  - Detection
  - Epidemiology and Risk Analysis
  - Prevention and Control
  - Extension and Outreach
  - Education and Capacity Building



Partners in the Food Virology Collaborative

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Stakeholder Engagement

capps. Center for Advanced Processing and Packaging Studies

CFS UGA CENTER FOR FOOD SAFETY

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH

MATRIX MICROSCIENCE

ECOLAB

FMI FOOD MARKETING INSTITUTE

NATIONAL FISHERIES INSTITUTE

CHARM SCIENCES INC

GOJO

DUPONT

BIOMÉRIEUX

SILLIKER Food Safety & Quality Solutions

United Fresh PRODUCE ASSOCIATION

NATIONAL RESTAURANT ASSOCIATION

IFT

International Association for Food Protection

Seafood Network Information Center
Sea Grant Extension Program

USDA United States Department of Agriculture Food Safety and Inspection Service

Food Safety Begins on the Farm

IFPTI INTERNATIONAL FOOD PROTECTION TRAINING INSTITUTE

AFDO

FDA

NORTH CAROLINA FRESH PRODUCE SAFETY TASK FORCE
www.ncfreshproducesafety.org

NOAA FISHERIES SERVICE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

Research Activities

- Molecular Virology: Develop improved methods to facilitate the study of foodborne viruses
- Detection: Develop and validate sensitive, rapid, and practical methods to detect and genotype HuNoV in relevant sample matrices
- Epidemiology and Risk Analysis: Collect and analyze population data on the burden of virus-associated foodborne disease, including epidemiological attribution and characterization of risk and costs
- Prevention and Control: Improve understanding the occurrence and behavior of HuNoV in the food safety continuum so as to inform development of scientifically justifiable control measures.

Extension, Outreach, and Education Activities

- Extension and Outreach: Translate and disseminate new knowledge about foodborne viruses into practices that reach target audiences in relevant work environments and across a wide array of stakeholder groups
- Capacity Building: Build scientific and human capacity to support increased and sustained efforts in food virology by fostering information and exchange, expanding professional capacity through formal student education and training initiatives

Conclusions

- We're working on it…..
- Thorough and frequent handwashing!
- Do not assume that simple surface disinfection with standard chlorine concentrations will eliminate viruses on fomites or other surfaces
- Take care to manage vomiting events in food processing and retail establishments
- Pay attention to restrooms
- Give us your feedback, experiences
- Stay tuned, more to come….

Acknowledgements

- Jaykus Lab/Office Students and Staff
  - Senior researchers: Dr. Helen Rawsthorne, Dr. Rebecca Goulter, Dr. Blanca Escudero-Abarca
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NoroCORE

Food Virology

Collaborative for Outreach, Research & Education