

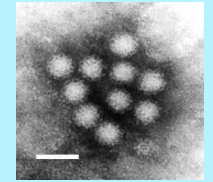
Studies of Norovirus Infectivity, Persistence and Reduction

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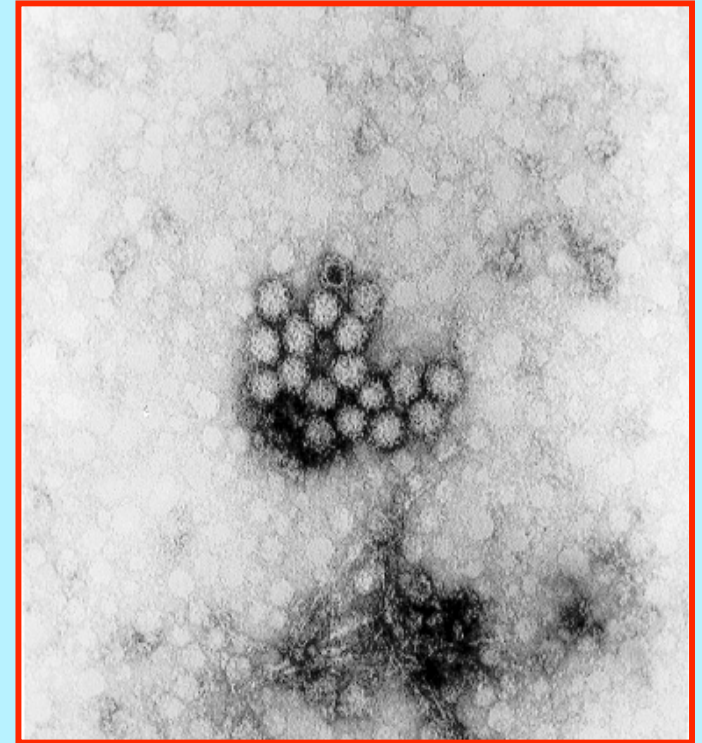
Overview



- Introduction to noroviruses
- Norovirus outbreaks
- Studies of norovirus infectivity
- Studies of norovirus persistence
 - Water
 - Environmental Surfaces
 - Human Hands
- Efficacy of various of handwash agents against Noroviruses
- Summary of what we know so far

Noroviruses

- Group of “small round structured viruses” 27-33 nanometer diameter
- RNA viruses
- Classified as *Caliciviridae*
 - *Two human genera: noroviruses and sapoviruses*
 - *Two genogroups of human norovirus:*
 - *Genogroup I and Genogroup II*
 - *Hundreds of different strains*



Norwalk virus
S. Miller

Norovirus Phylogenetic Tree

A. Genogroup I

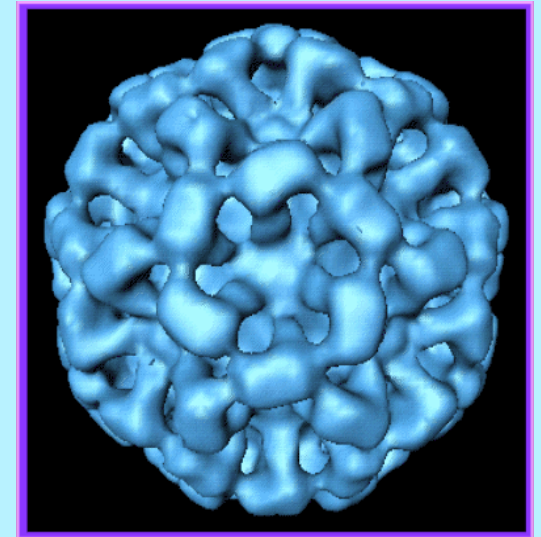


B. Genogroup II



Norovirus Background

- Most common cause of epidemic acute gastroenteritis
 - Est. 23 million cases in US annually
- Paradigm since 1972
 - Can not be grown outside the host
 - Culture in 3-D rotating cell system
Straub et al., 2007
 - “No animal model”
 - Infection of germfree piglets
Cheetham et al., 2006
 - Two animal caliciviruses used as surrogates for human virus because they can be grown in cells outside the host
 - Feline calicivirus - respiratory virus
 - Murine norovirus - infects multiple organs



B.V. Prasad

Use of Animal Caliciviruses as Surrogates for Human Norovirus

- Feline calicivirus (FCV) and murine norovirus (MNV) can be grown in cell culture - measure infectivity
- Used as model for human norovirus
- Uncertain how closely the characteristics of these viruses match the characteristics of human noroviruses
- Differences between FCV and MNV in pH sensitivity, temperature sensitivity

Clinical Presentation of Norovirus Infection

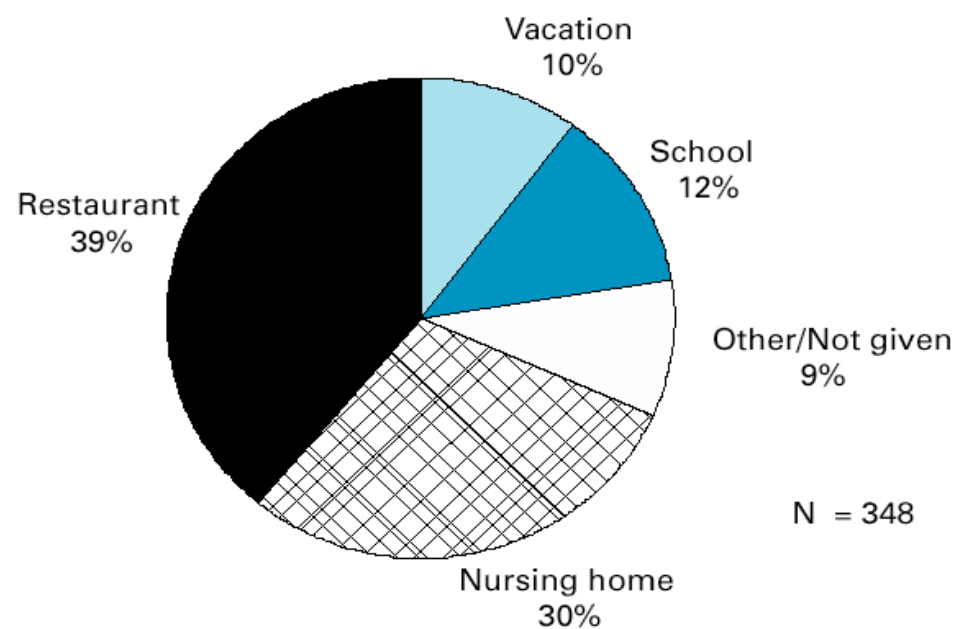
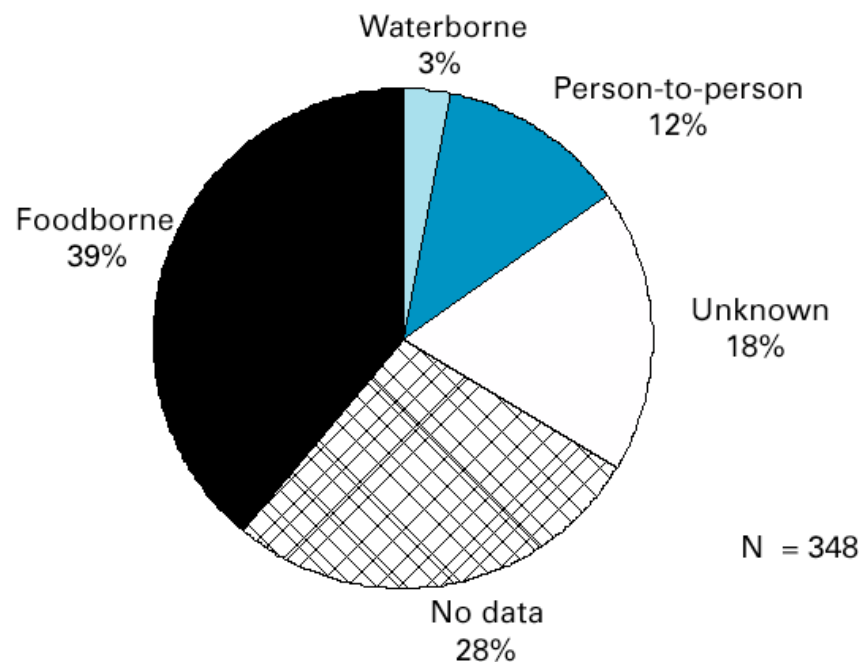
- Acute gastroenteritis with predominant vomiting, nausea, abdominal cramps, diarrhea (“Stomach Flu”)
- Incubation period: 24 - 72 hours
- Duration: 12 - 72 hours
- High attack rates: ave 45%

Norovirus Outbreaks



- o Carnival cruise ship "Liberty"
- o Port Everglades, Florida, November 2006
- o 700 ill passengers and crew
- o 16 day trans-Atlantic cruise

Transmission Mode and Settings of 348 Norovirus Outbreaks: USA, 1996-2000



Foods implicated in outbreaks of viral gastroenteritis or hepatitis

- Salads
- Fruit salad and cut fruits
- Produce (green onions)
- Raspberries, strawberries (fresh and frozen)
- Shellfish
- Deli meats
- Sandwiches
- Cake icing



How do foods become
contaminated with
Noroviruses?

Opportunities for Fecal Contamination of Food during Growth and Harvest

- Soil
- Irrigation water
- Hands
- Equipment



Susan Lance-Parker, GA Dept of Health
D Bacon, 1996. <http://dbacon.igc.org/Mexico/border00.htm>

<http://www.roncastle.com/images/new-orleans-pictures/oysters.jpg>

Viral Outbreaks linked to Produce

Year	Virus	Vehicle	Source	Cause
Multiple	NoV	Salad	Multiple	Infected foodhandler
1997 1998 2005	NoV	Raspberries (multi-country)	Eastern Europe	Contaminated agricultural waters
1996	HAV	Strawberries (multi-state)	Mexico	Poor sanitation and hygiene => contam pickers' hands
2003	HAV	Scallions (multi-state)	Mexico	Contamination during harvest?

Opportunities for Food Contamination during Processing and Preparation



- Processing water/ice
- Hands
- Equipment/surfaces



Norwalk-Like Virus Crosses the Scrimmage Line:



Transmission During a Football Game

KM Becker, CL Moe, KL Southwick and JN MacCormack. (2000) Transmission of Norwalk Virus During a Football Game. *N Engl J Med* 343(17):1223-1227.



Why are noroviruses such successful pathogens?

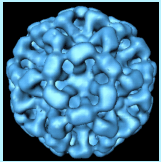
- Highly infectious?
- Widespread host susceptibility?
- No protective immunity?
- Environmental persistence?
- Resistance to disinfection?
- Prolonged viral shedding?
- High numbers of virus shed in stool?

Studies of Norovirus Infectivity in Human Volunteers

Norwalk Virus

- Genogroup I norovirus - “prototype Norovirus”
- Associated with 1968 outbreak in a school in Norwalk, Ohio
- First seen by electron microscopy by Kapikian in 1972
- Several previous human challenge studies
- Sequenced by Jiang and Estes in 1990
- Molecular detection by RT-PCR developed 1992

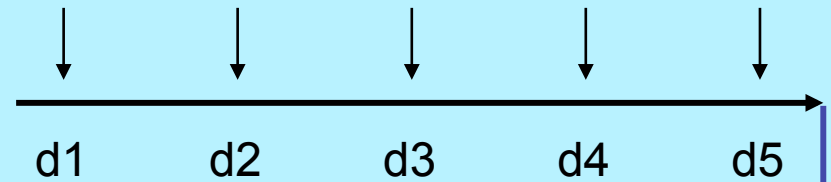
Safety-
tested
NoV
Inoculum



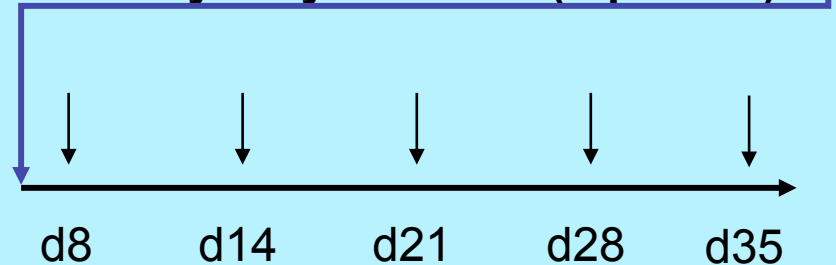
Healthy
Adult
Volunteer



Collect serum, saliva, stool, emesis,
symptoms



5 Day Stay at GCRC (Inpatient)



5 Outpatient Follow-up visits

Determine Infection Status:

Test stool samples by RT-PCR to determine viral shedding

Test sera samples by ELISA to determine NV seroconversion

Key Findings of Human Challenge Studies

- Noroviruses are extremely infectious - most infectious agent ever described (Teunis et al., 2008)
- Symptoms typically last 2-5 days
- 20-30% of infected subjects did not have symptoms
- Virus can be shed in feces for up to 35 days in a normal infected person

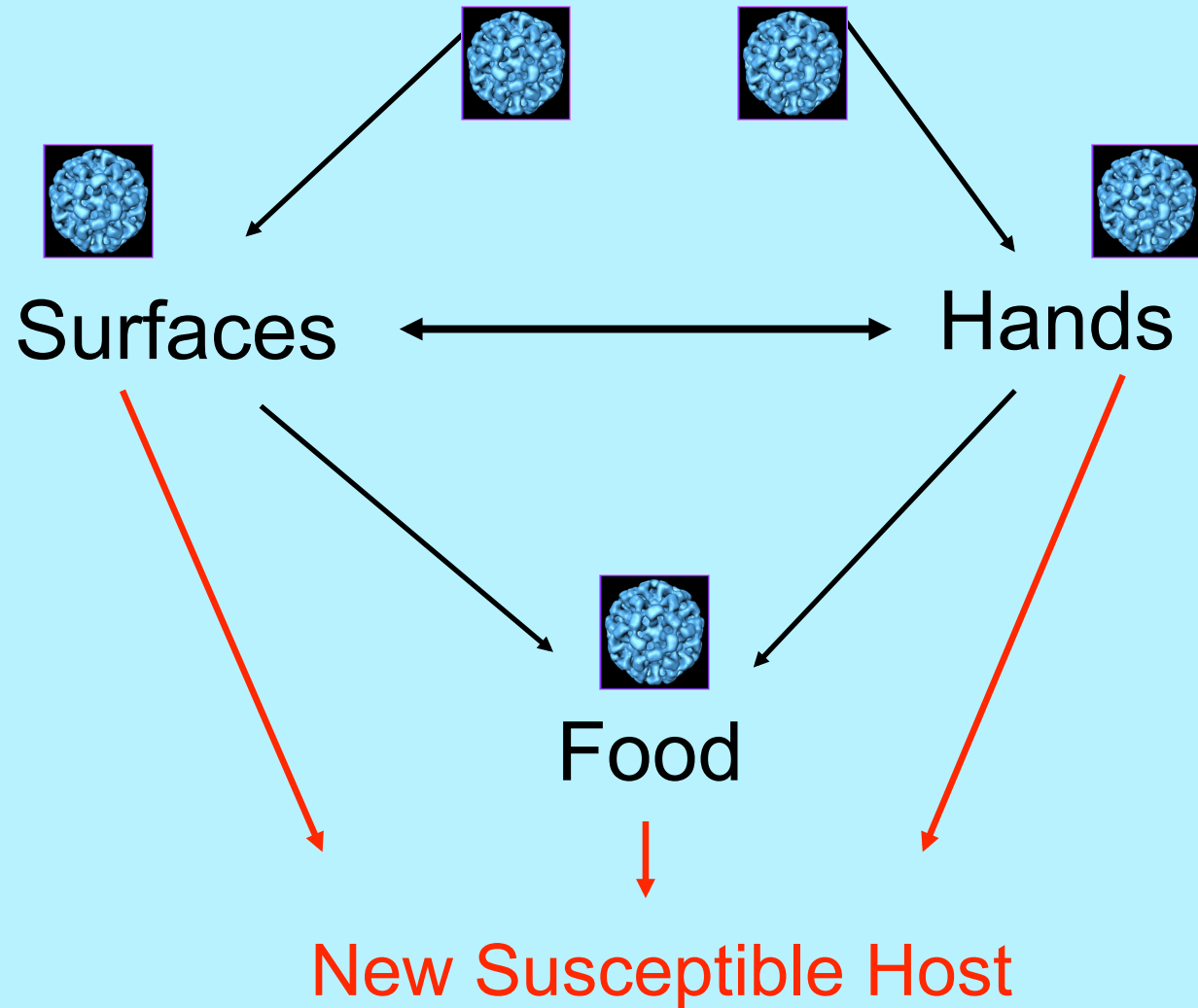
Norwalk Virus vs. Snow Mountain Virus Infectivity

- Both NV and SMV are HIGHLY infectious
- NV was more infectious at low doses than SMV
- NV – Flatter curve. More variation in dose response
- SMV – Steeper curve. More subjects were susceptible to infection
- SMV infection more likely to cause illness than NV
- SMV symptoms lasted longer than NV symptoms

Studies of Norovirus Persistence

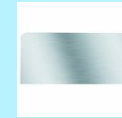
- Objectives:
 - Examine Norwalk Virus (NV) and Snow Mountain Virus (SMV) persistence in water, on environmental surfaces and hands
 - Examine Norovirus removal by various handwash agents

Viral shedding in feces



Norovirus Persistence in Environment

- Food Processing Surfaces



- Temperature



- pH

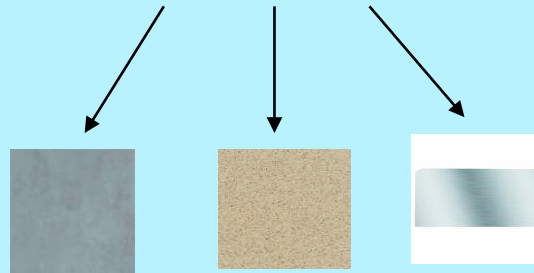


- Human Hands



Norovirus Persistence on Environmental Surfaces

20% Norovirus-positive stool suspension



Ceramic

Formica

Stainless Steel

(Room temperature 24°C, 40% humidity)

Serial Time Points (~4 weeks for NV, ~ 6 weeks for SMV)

Elute virus from surface

Concentrate virus

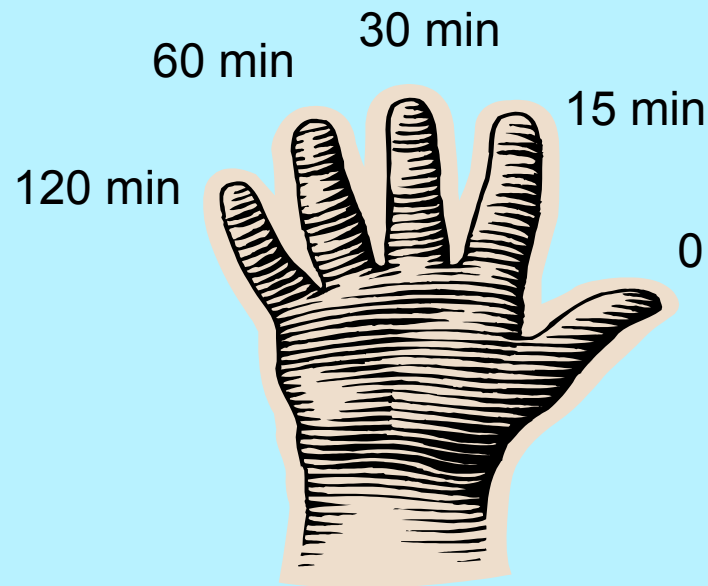
Store at -80°C until RT-PCR assay

Summary of Persistence Studies

- Norwalk virus remained infectious in water stored at room temperature in the dark for at least 61 days
- Norwalk virus in a stool suspension gradually declines over time on common kitchen surfaces (1.5 to 3 logs (99.9%) over 28 days).
- Both Norwalk virus and Snow Mountain virus RNA were still detected on surfaces after 4 weeks and 6 weeks, respectively
- Snow Mountain virus seems more stable than Norwalk virus
- Noroviruses persist longer on surfaces at cooler temperatures
- Naked NV RNA degrades after 7 days on stainless steel and formica surfaces

Norovirus Persistence on Human Hands

Norwalk Virus and Snow Mountain Virus Persistence on Human Hands



Virus: 20% NV- or SMV-positive
stool suspension

Subjects: 6 volunteers, both hands

Summary of Norovirus Persistence on Human Hands and in Water

- Noroviruses are stable on human hands. Almost no change in NoV RNA levels after more than two hours on human hands

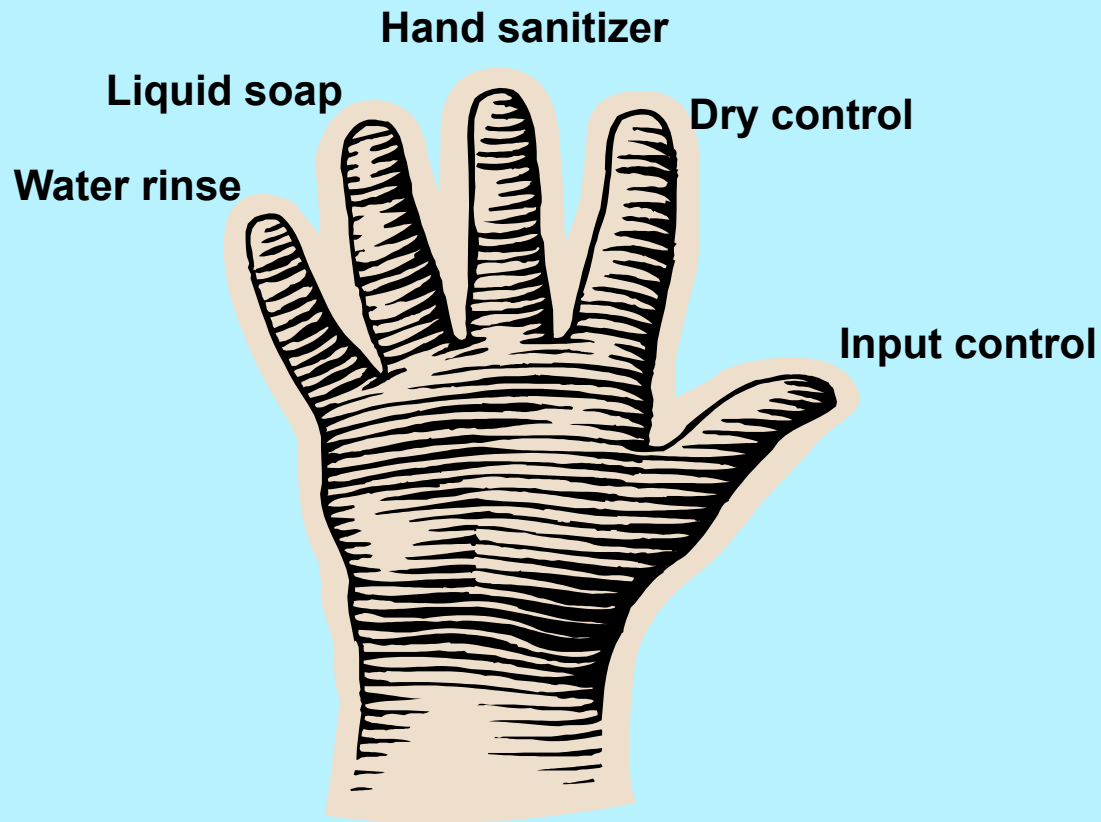
Efficacy of Various Handwash Agents against Noroviruses

- Handwash Study 1: efficacy of standard benchmark PURELL
- Handwash Study 2: efficacy of VF447
- Handwash Study 3: efficacy of VF481
- Handwash Study 4: efficacy of handwash regimens

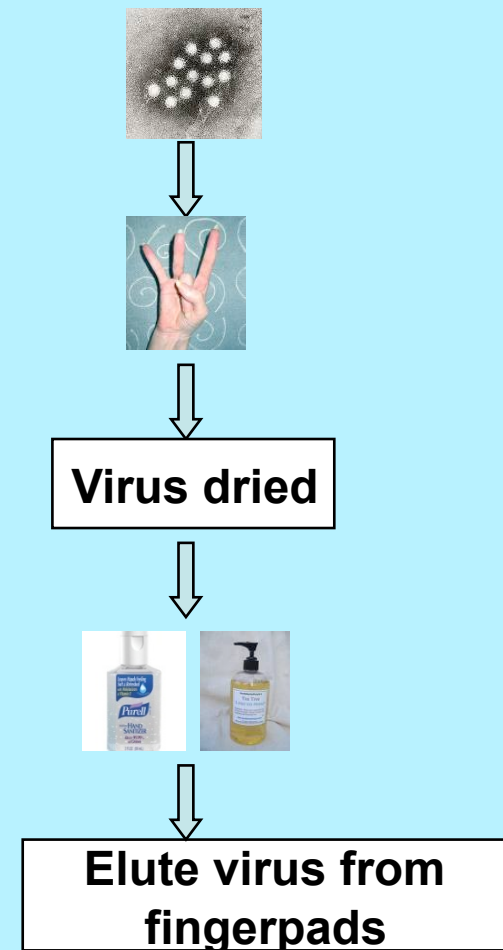
Handwash Study 1: Efficacy of Benchmark PURELL and Liquid Soap against Norwalk Virus

- **Virus ----** 20% NV-positive stool sample
- **Subject ----** 5 volunteers
- **ASTM Standard Method**
- **Replicate Trials ----**
 - Both left and right hands,
 - Day 1 and Day 2
- **Test products**
 - Hand sanitizer (from grocery store): 60% ethyl alcohol
 - Liquid soap (lab): antibacterial, Triclosan
 - Water rinse: tap water

American Standard Test Method for in vivo evaluation of the activity of handwash agents using the fingerpad (ASTM E 1838-02)



Study procedures



Fingerpad Method

1. Wash hands, dry, mark fingerpads



2. Add virus suspension to fingerpad



3. Allow virus to dry on fingerpads



Fingerpad Method

4. Expose fingerpad to handwash agent



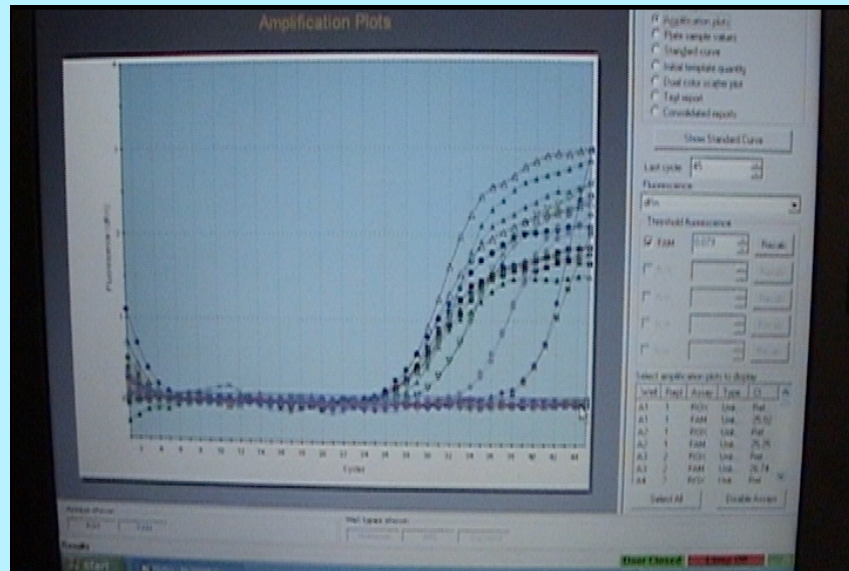
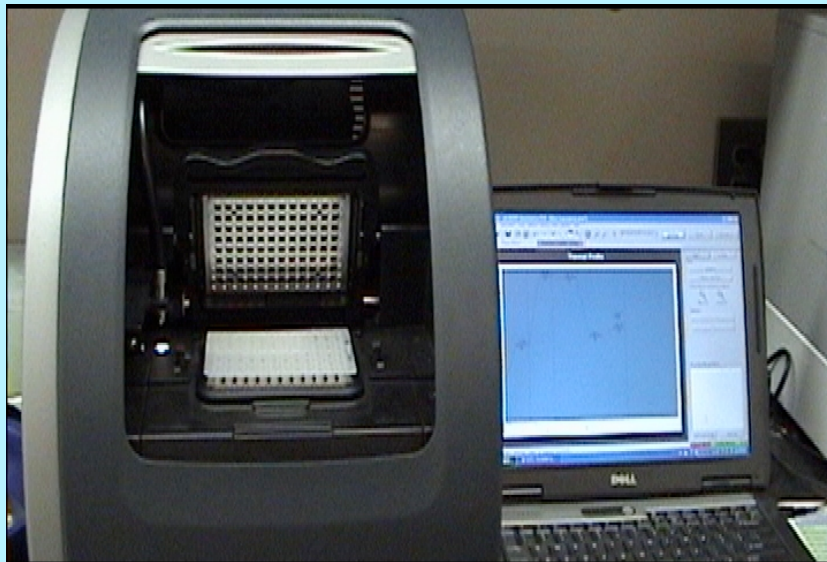
5. Handwash agent removes and/or inactivates virus



6. Expose finger to buffer to remove any remaining virus

Fingerpad Method

7. Test buffer by RT-PCR to measure amount of remaining virus.
8. Compare to input level of virus on control finger to calculate virus reduction



Summary of Handwash Study 1

Results

- Benchmark PURELL (62% ethanol) is less effective than liquid soap or water at removing Norwalk virus from hands
- Implications for health care settings and food preparation establishments
 - Settings where norovirus outbreaks commonly occur
 - Settings where alcohol-based sanitizers are commonly used

Handwash Study 2: Efficacy of VF447 (GOJO) against Norovirus

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2008, p. 5047–5052 Vol. 74, No. 16
0099-2240/08/\$08.00_0 doi:10.1128/AEM.00487-08
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Improved Inactivation of Nonenveloped Enteric Viruses and Their Surrogates by a Novel Alcohol-Based Hand Sanitizer

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Received 28 February 2008/Accepted 19 June 2008

VF 447

- 70% ethanol with additional ingredients that serve as alcohol potentiators
- Data from animal calicivirus surrogates indicate that synergistic action provides better virus removal/inactivation

Handwash Study 3: Efficacy of VF481 (GOJO) against Norovirus

VF 481

- 70% ethanol with additional ingredients that serve as alcohol potentiator

Hand wash Study 4: Removal of Norovirus by Handwash and VF481 Regimens

Summary of Handwash Results

- Significant differences in hand sanitizer performance. Not all hand sanitizers created equal.
- Benchmark PURELL was not effective against norovirus
- PURELL VF447 was effective at reducing Norwalk Virus on human hands but appeared not effective against SMV
- PURELL VF481 was very effective for both NV and SMV
- The regimen of MICRELL Antibacterial Foam Handwash followed by PURELL VF481 was significantly better than MICRELL or VF481 alone for removing NV on human hands. The combined regimens could achieve approximately >6 log reduction from virus-contaminated hands
- Since we tested norovirus RNA titers in all studies, the actual efficacies of the hand sanitizers were probably underestimated.

What do we know about Noroviruses?

- Data from human challenge studies confirm that **noroviruses are highly infectious**
- Prolonged virus shedding and infections without symptoms make it easier to transmit virus
 - **Need simple diagnostic tests to identify who is infected and who is still shedding virus**
- Majority of reported norovirus outbreaks are associated with food service settings or events
- Norovirus RNA can persist on common kitchen surfaces for at least 3-6 weeks
 - **Need EFFECTIVE cleaning and disinfection agents and protocols [Sodium hypochlorite (bleach)]**

What do we know about Noroviruses?

- RT-PCR data indicates that Norwalk virus RNA is stable in groundwater for at least 70 days.
- Human challenge study indicates that Norwalk virus remains infectious in water for at least 61 days.
 - Need to examine persistence of infectivity in water at longer time points.
- Development of norovirus vaccines is in very early research stages. Food service workers are an important target group for norovirus vaccines.

What do we know about Noroviruses?

- Norovirus RNA is very stable on human hands for at least two hours
- Some new products used alone (VF481) or in combination appear to be effective at reducing noroviruses on hands
- Need to continue to develop more effective products for reducing noroviruses on hands to reduce risks of foodborne and person-to-person transmission

Acknowledgements

- Moe Research Team
 - Dr. Pengbo Liu
 - Dr. Juan Leon
 - Marina Fernandez
 - Esther Wong
 - Hui-Mien Hsiao
 - Melissa Dowd
 - Kizee Etienne
 - Dr. Yu-Wen Chien
 - Yvonne Yuen
 - Laura Kovalchick
 - Allison King
 - Hannah Cluck
- Dr. Marshall Lyon
- Gwen Abdulhafid
- Emory GCRC
- Dr. Lee-Ann Jaykus (NC State Univ)
- Dr. Kellogg Schwab (Johns Hopkins Univ)
- Dr. Kim Green (NIH)
- Dr. Michele Hardy (Montana State Univ)
- USEPA Coop Agreement
- FDA
- USDA
- GOJO Industries
 - Dr. Dave Macinga
 - Dr. James Arbogast
 - Carrie Zapka

Collaborators

- Inocula
 - John Herrmann
 - Neil Blacklow
 - John Treanor
 - Mark Sobsey
- Recombinant viral proteins
 - Mary Estes
 - Ralph Baric
 - Boyd Yount
 - Robert Johnston
 - Nancy Davis
- Electron Microscopy
 - Sarah Miller
- Dose-Response Modeling
 - Peter Teunis
 - Paul Stewart
 - Burton Levine, Michelle Harb, Sharon Murray, Lauren Lindblad
- UNC GCRC
 - Susan Pusek
 - William Heizer, Cathy Kapoor, James Trogden, Bruce Gilliam, Meera Kashkari, David Wohl, Walter Straus
 - GCRC Nursing Staff
- RT-PCR and serology
 - Frances Tseng
 - Erin-Joi Collins McNeal
 - Lisa Lindesmith
 - Deanne Rhodes, QingHong Yang, Cornelia Kaydos, Thao Vo, Amica Simmons