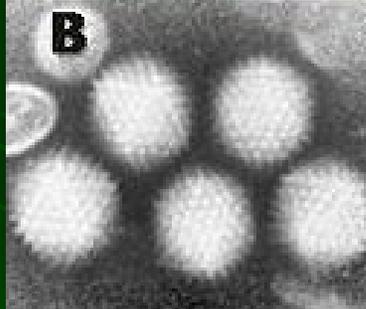
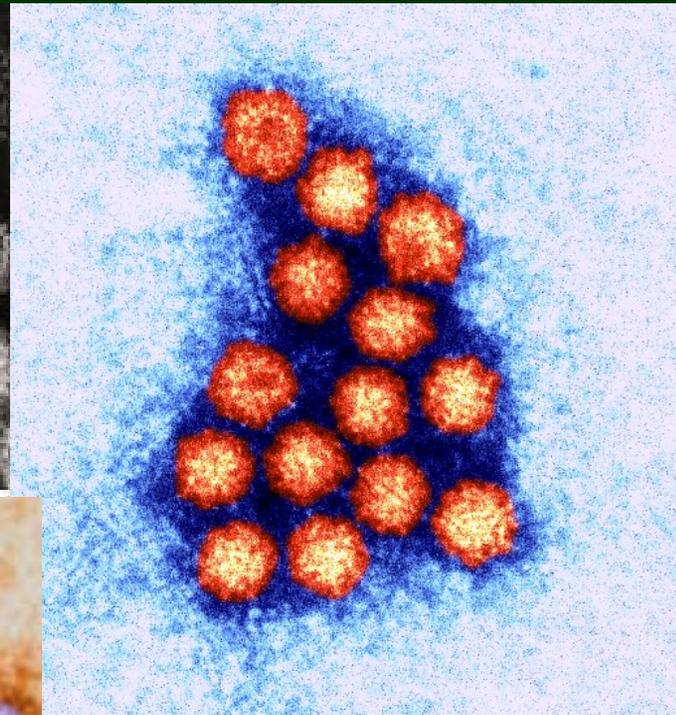
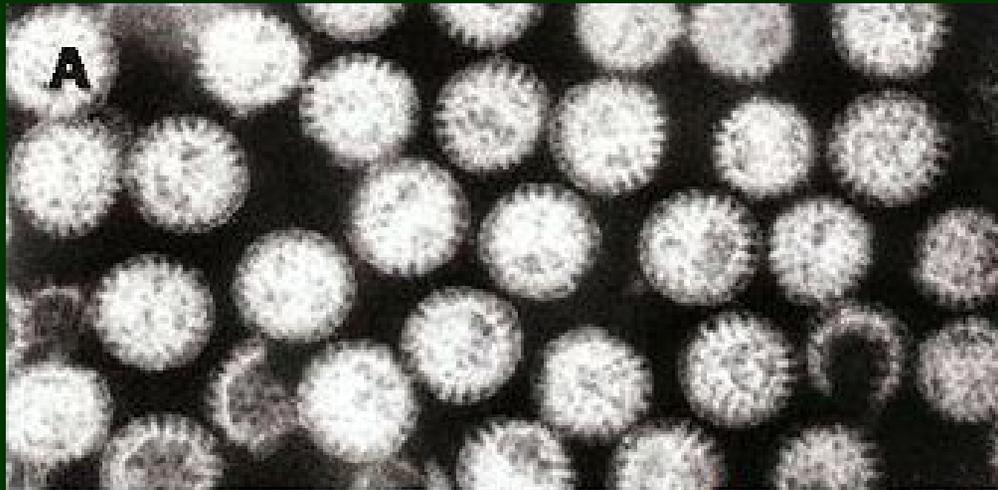


Detection of Norovirus GI/GII from food



Wellcome Images



Norovirus is a problem for food service industry

INTERNATIONAL FOOD SAFETY NETWORK INFOSHEET APRIL 23, 2008

Over 100 reported ill with norovirus after conference at Hilton hotel

More than 100 people reported illness after attending a conference at the Hilton hotel in New York City. The illness was identified as norovirus, a highly contagious virus that causes "stomach flu" or gastroenteritis. Symptoms include nausea, vomiting, and diarrhea. The outbreak was linked to a buffet breakfast served at the hotel on the day of the conference.

What you need to know about norovirus:

- Some norovirus infections occur without symptoms
- Infected people can shed large amounts of norovirus in their poop
- Viral shedding (passing the virus on in poop) can sometimes occur for 3 weeks after symptoms have resolved
- The majority of reported norovirus outbreaks are associated with food service settings or events
- Norovirus can persist on common kitchen surfaces for at least 3-6 weeks
- Some hand sanitizers are not effective at reducing norovirus on hands

INTERNATIONAL FOOD SAFETY NETWORK INFOSHEET APRIL 23, 2008

NOROVIRUS OUTBREAK HITS OHIO CHIPOTLE

Chipotle MEXICAN GRILL

OVER 400 CUSTOMERS HAVE BECOME ILL WITH VOMIT, DIARRHEA AND NAUSEA. THREE WERE HOSPITALIZED

WHAT YOU NEED TO KNOW ABOUT NOROVIRUS

- Some norovirus infections occur without symptoms
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Health officials say norovirus is likely to blame for making hundreds of people sick after they recently ate at a Chipotle restaurant in Kent, Ohio. According to Kent health officials, 432 people have reported norovirus symptoms as of Monday, April 21.

Officials said no cases have occurred since the restaurant reopened on April 19. It had closed on April 18 after people reported on April 19. It had closed on April 18 after people reported symptoms.

The restaurant was cleaned and sanitized and new employees were brought in from other restaurants not of a outbreak.

Chipotle has announced that the restaurant chain plans to reinforce health care costs for customers who became ill as a result of a visit to the restaurant.

FOR MORE INFORMATION CONTACT THE CHIEF OF PUBLIC HEALTH SERVICES AT 330.426.7000

ALL NEW!

WEEKLY WORLD NEWS

THE WORLD'S ONLY RELIABLE NEWSPAPER

COMPUTER VIRUS SPREADS TO HUMANS!

Sickness bug forces over 50 hospital wards to be closed

By Tom Kelly and Daniel Bates

Over 50 hospital wards in Kent, Ohio, have been closed due to a norovirus outbreak. The virus, which causes "stomach flu" or gastroenteritis, is highly contagious and can be spread through food, water, and contact with surfaces. The outbreak has forced the closure of wards at several hospitals, including the Kent State University Medical Center. Health officials are urging the public to practice good hygiene, such as washing hands frequently with soap and water, to help prevent the spread of the virus.

SICKNESS BUG TOLL TO HIT 200,000 A WEEK

A STOMACH bug sweeping the nation could hit 200,000 people a week, doctors warned yesterday.

The norovirus, which causes vomiting and diarrhea, is spreading rapidly across the country. Health officials estimate that the virus is responsible for over 200,000 cases each week. The outbreak is particularly concerning because the virus is so contagious and can be spread through food, water, and contact with surfaces. Doctors are warning that the toll of the virus could continue to rise if more people do not take steps to prevent infection.

ATTACK OF THE NOROVIRUS*

a group of highly contagious viruses that cause "stomach flu" or gastroenteritis (GAS-tro-en-ter-I-tis)

Repel it by frequently:

- washing your hands with soap + water
- OR using an alcohol-based hand sanitizer (especially after toilet visits and before eating and/or preparing food)
- AND avoiding sharing food, utensils and drinking containers.

Most individuals recover from norovirus in 1-2 days on their own by treating symptoms and staying hydrated.

Symptoms can begin suddenly, and may include:

- Nausea
- Vomiting
- Diarrhea
- Stomach cramping
- A low-grade fever
- Chills
- Muscle aches
- General sense of tiredness

www.health.arizona.edu

THE UNIVERSITY OF ARIZONA

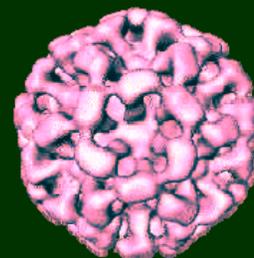
CAMPUS HEALTH SERVICE



DO NOT USE
Restrooms for
VOMITING! Vomit goes
OVER RAIL ONLY!

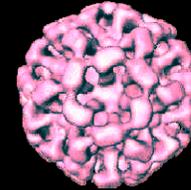


What is Norovirus?



- Norovirus is an RNA virus which causes approximately 90% of epidemic non-bacterial outbreaks of gastroenteritis around the world.
- NV is responsible for more foodborn outbreaks than any another other foodborn pathogen.
- Symptoms causing acute gastroenteritis develops between 24 and 48 hours after exposure, and lasts for 24–60 hours.
- Nausea, vomiting, diarrhea, and abdominal pain; and in some cases, loss of taste. General lethargy, weakness, muscle aches, headache, and low-grade fever may occur.

Top 10 of food borne pathogens (us)



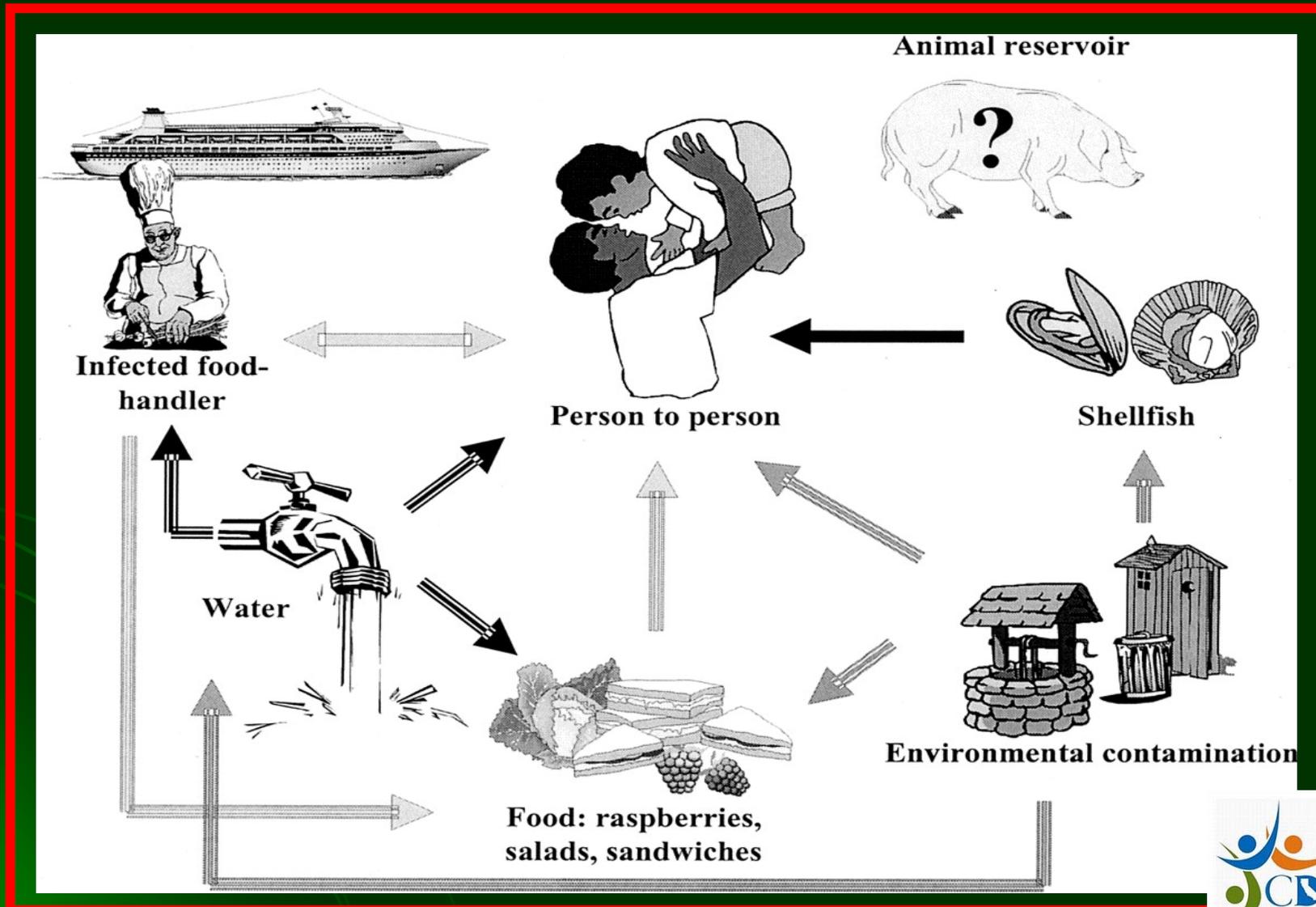
1. Norwalk like viruses	9,200,000
2. Campylobacter	1,963,000
3. Salmonella (non-typhoid)	1,342,000
4. Clostridium perfringens	249,000
5. Giardia lamblia	200,000
6. Staphylococcus	185,000
7. Toxoplasma gondii	112,000
8. Shiga Toxin E. coli	92,000
9. Shigella	90,000
10. Enterotoxigenic E. coli	56,000



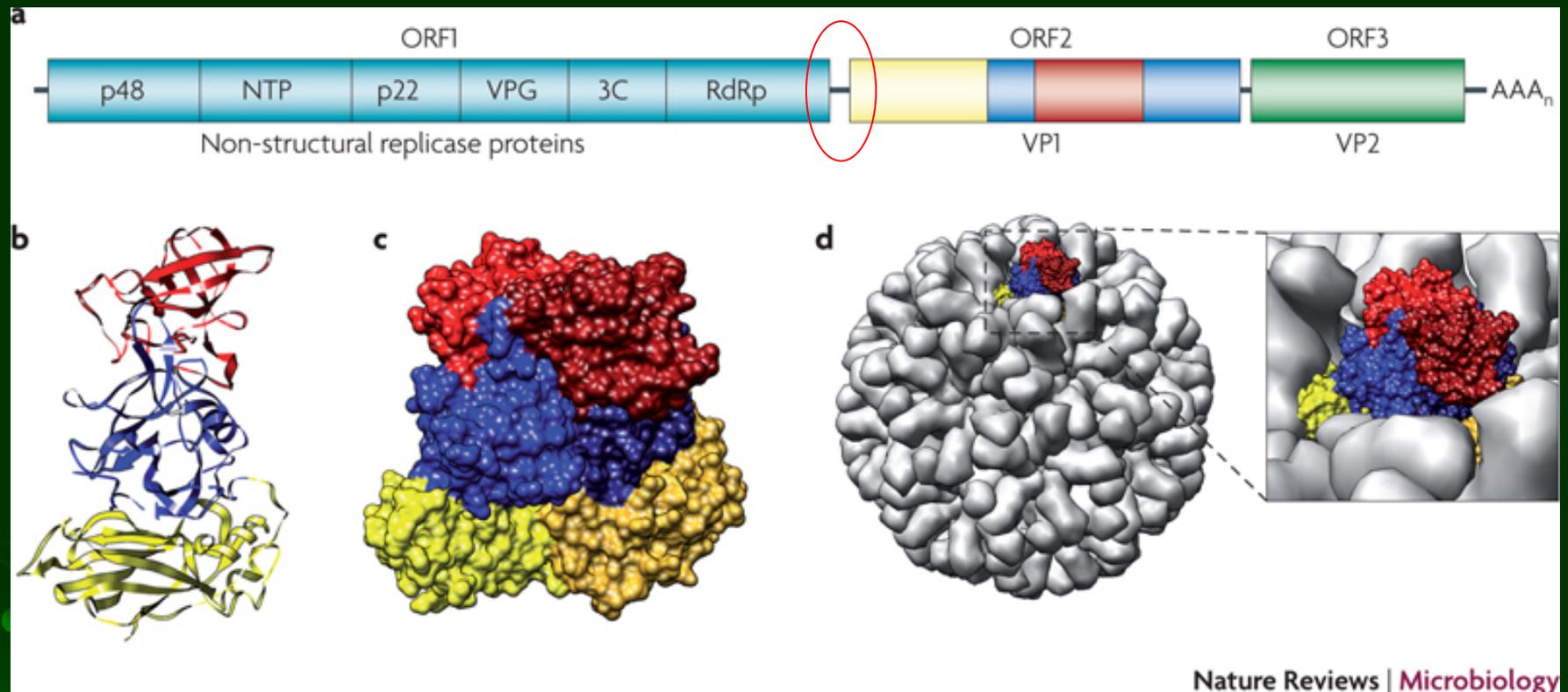
ANIMAL SCIENCES GROUP
WAGENINGEN UR



Means of transmission



Virus structure



- Positive sense RNA genome ~7.5 Kb
- 5 Genogroups
- Virus is between 27-38nm in size

Why is it difficult to detect Norovirus in foods?

- No pre-enrichment for Norovirus...you get what is there.
- Low viral titers and low infectious dose.
- Viral recovery is complicated by the food matrices.
- Inhibitors may be present.

Methods

- PEG extraction
- Ultracentrifugation
- Ultrafiltration
- Flocculation
- Immunocapture methods

Our goal is to develop a simple non-toxic method of viral capture and detection, from foods, that could be automated.

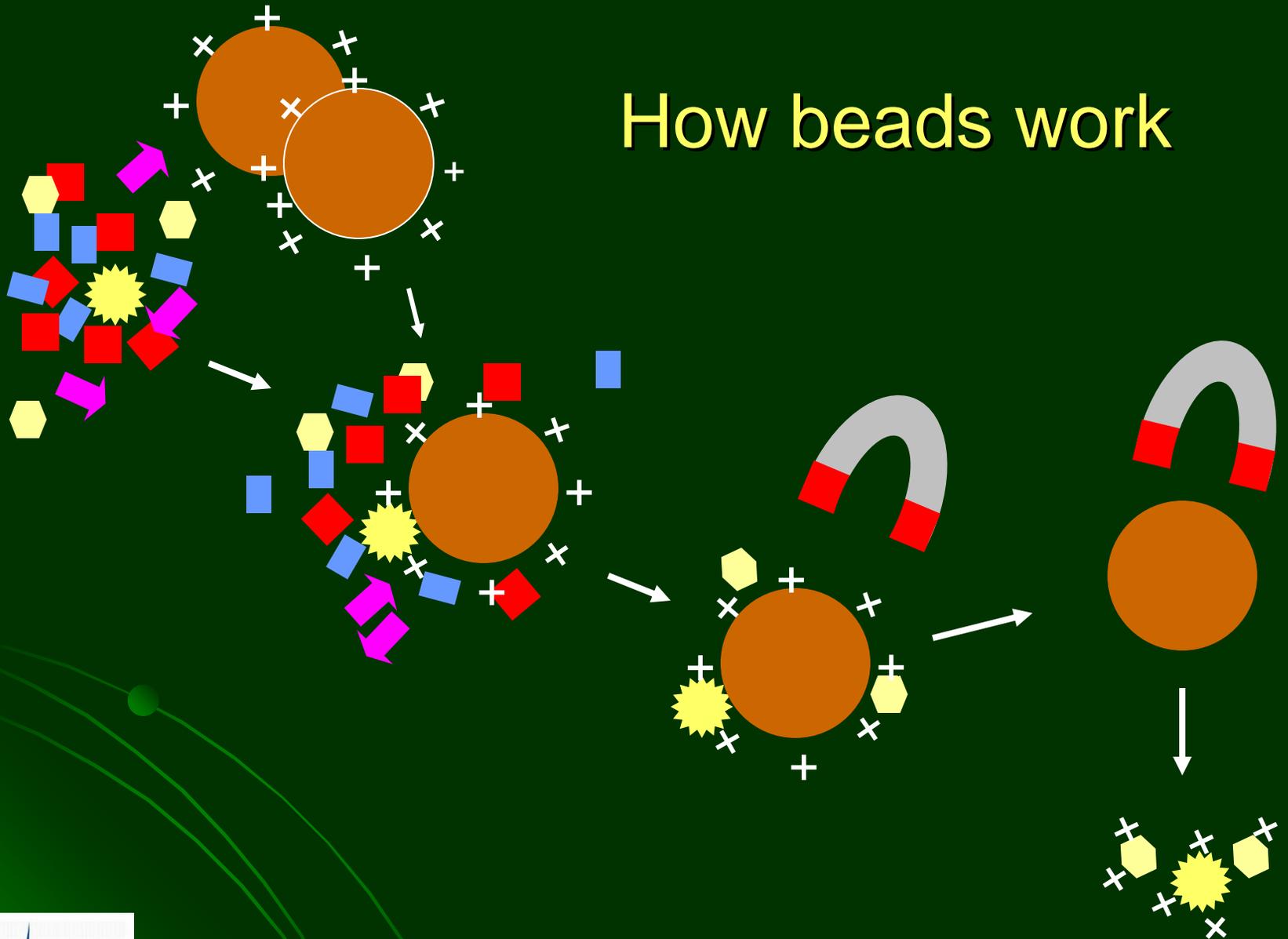




Why beads?

- Beads concentrate target and select against matrix and inhibitors.
- PMB can be collected by centrifugation or magnet.
- Can easily modify them for different applications.
- They can be cost effective.

How beads work



Beads are a tested technology

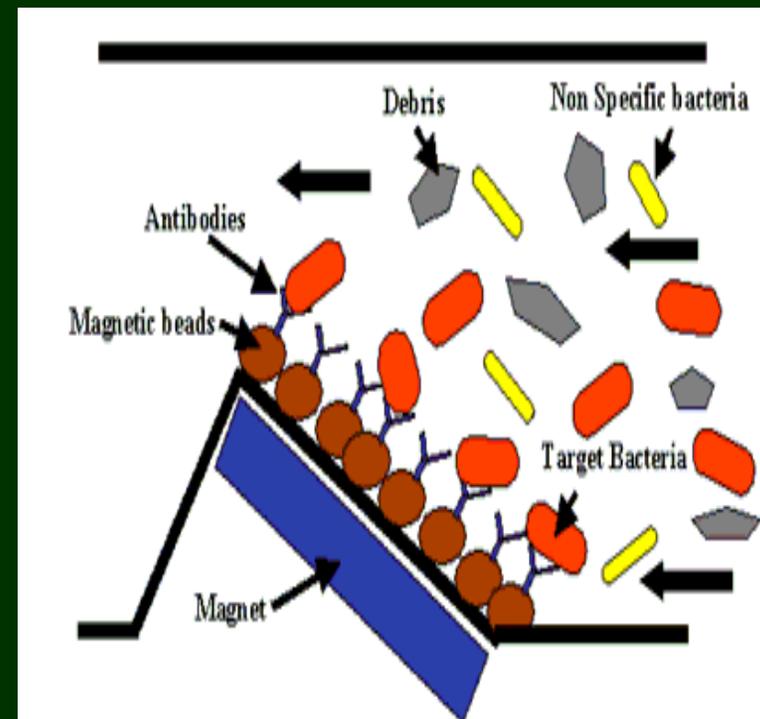
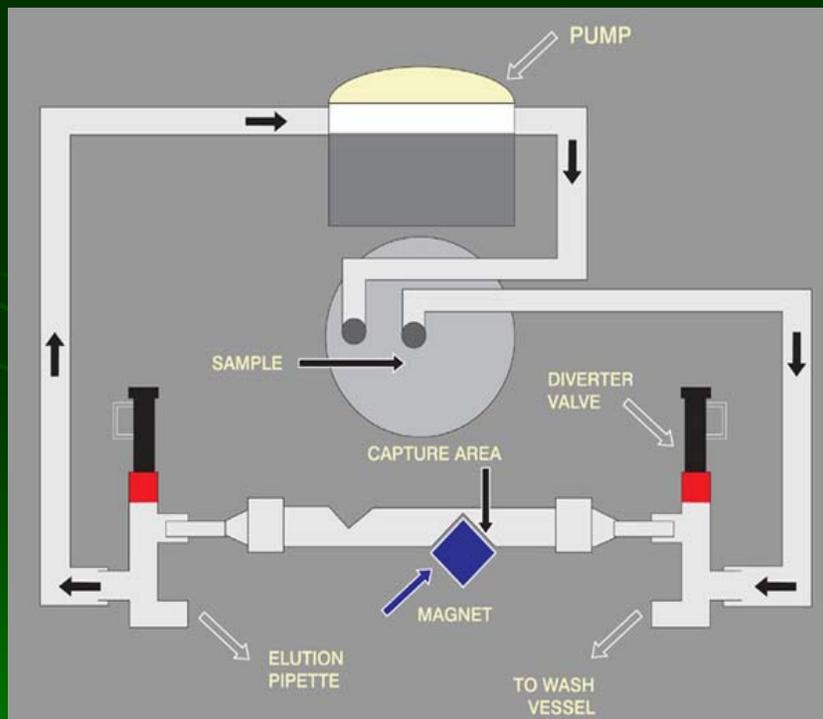
- Numerous commercially available Immuno capture beads do exist for bacteria (Dynal, Pathatrix, Pierce).
- These beads are antibody based and are target specific.
- Charged beads are not target specific and utilize charge to capture a number of different viral targets.
- Charged beads are attractive for viral capture due to the small size of viruses.

Bead performance: strategy

- Test beads in a clean system
- Compare beads with commercially available CAT (cationic) beads
- Test beads in various food matrices
- Evaluate sensitivity
- Make assay amendable for automation

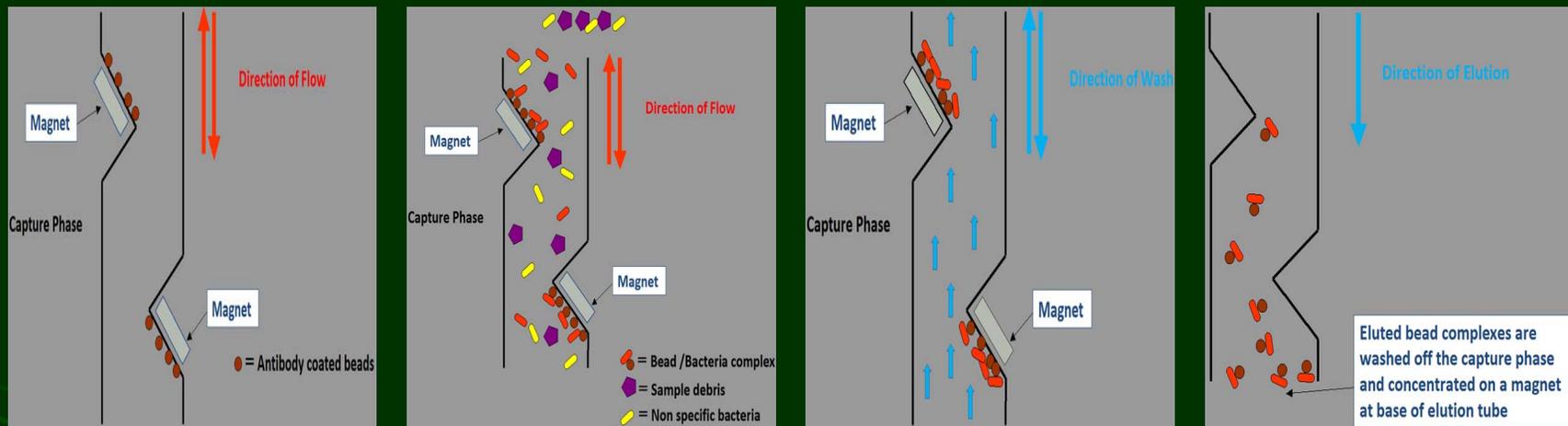
Objective

- Our objective was to couple the use of charged magnetic beads for the capture of norovirus with the Pathatrix RIMS system.



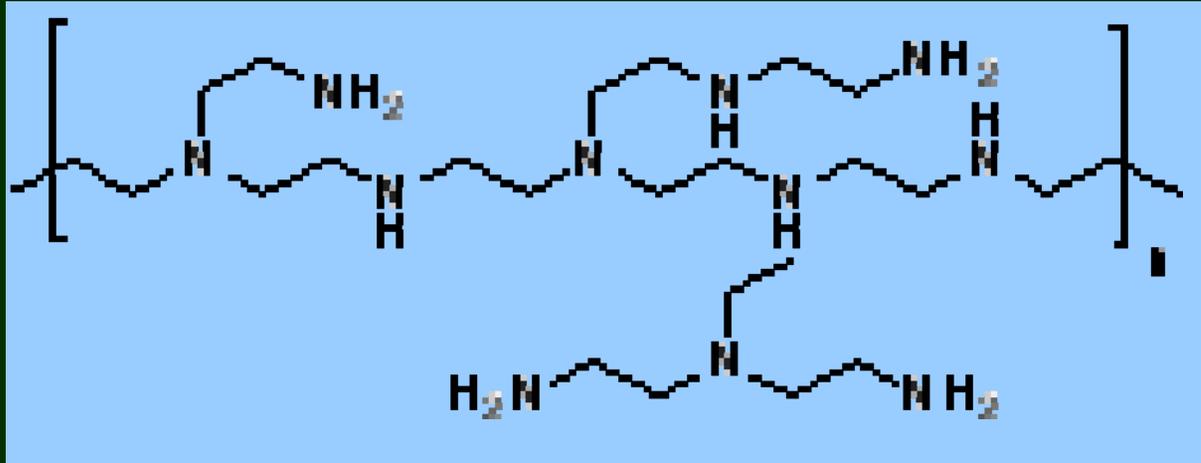
Modified objective

- Pathatrix unit can have a high degree of bead loss and are not ideal.
- Matrix has since modified there units to re-circulate a 10% sample repeated over a dual magnet system in a total volume of 50ml.



- Based on this, we tested 50ul of charged beads to a 10% food matrices solution in a total volume of 50ml. Beads are simply recovered by centrifugation or magnetic capture.
- Beads are then removed, washed, processed and RNA is extracted from the sample for RT-PCR.

PEI beads



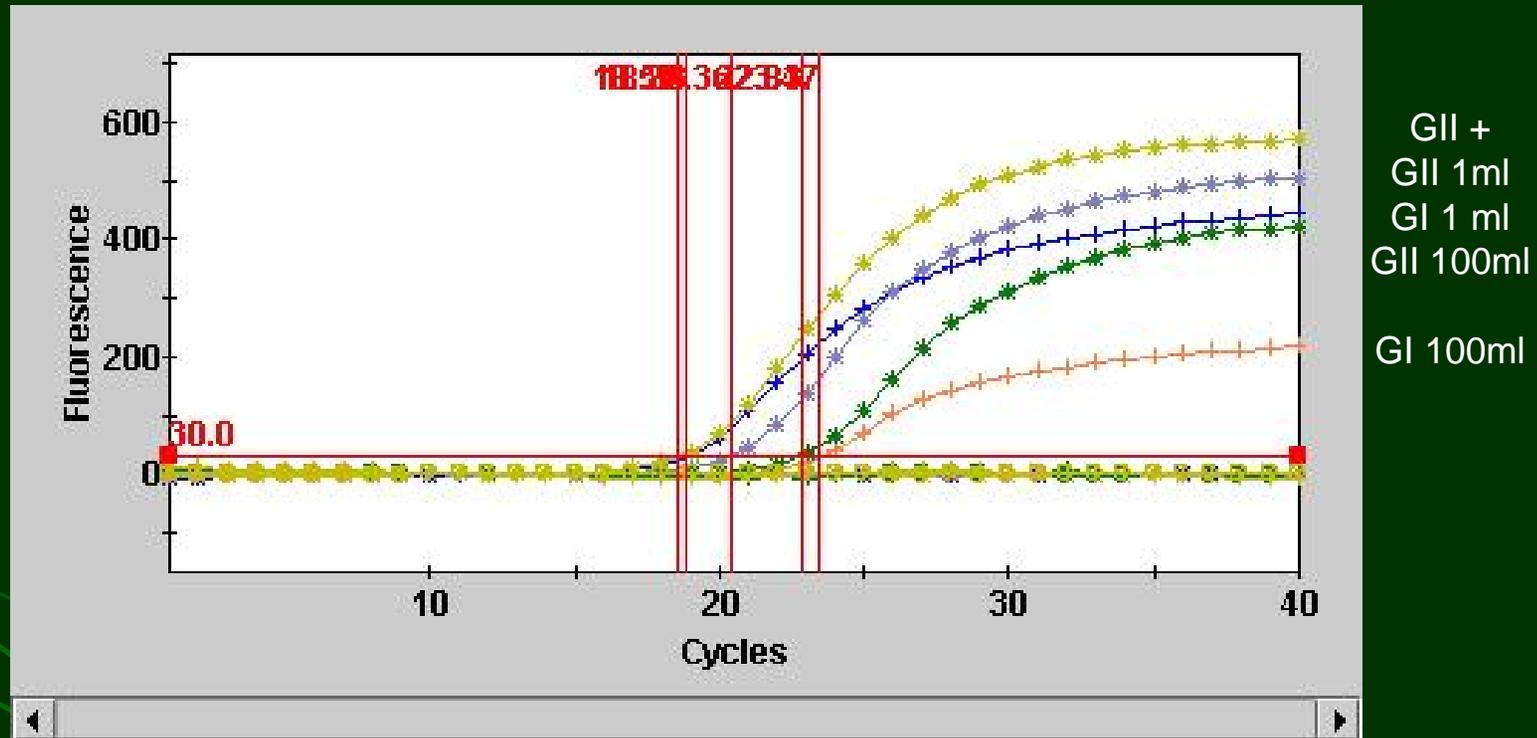
- PEI (polyethylenimine) conjugated magnetic beads have a strong cationic charge under sub-acidic conditions.
- Beads have been reported to bind a number of different viruses including HAV, HCV, and Herpes simplex virus



PEI beads

- The virus adsorption mechanism of the PEI beads is unclear.
- It is speculated the branching structure and positively charged field of the PEI molecule may strongly interact with the negatively charged surface lipids or proteins on the virus.
- May have an advantage over immuno-based capture in that it is not limited to a single virus type and be used to detect multiple types of viruses that may be present in a single sample.

Test of bead binding with GI and GII Norovirus in different volumes



Tested beads capture ability of GI and GII in different volumes 1ml, and 100ml
Ct values ranged from 18.8- 25.8

GII spike $\sim 1 \times 10^5$ and GI spike $\sim 3.1 \times 10^4$

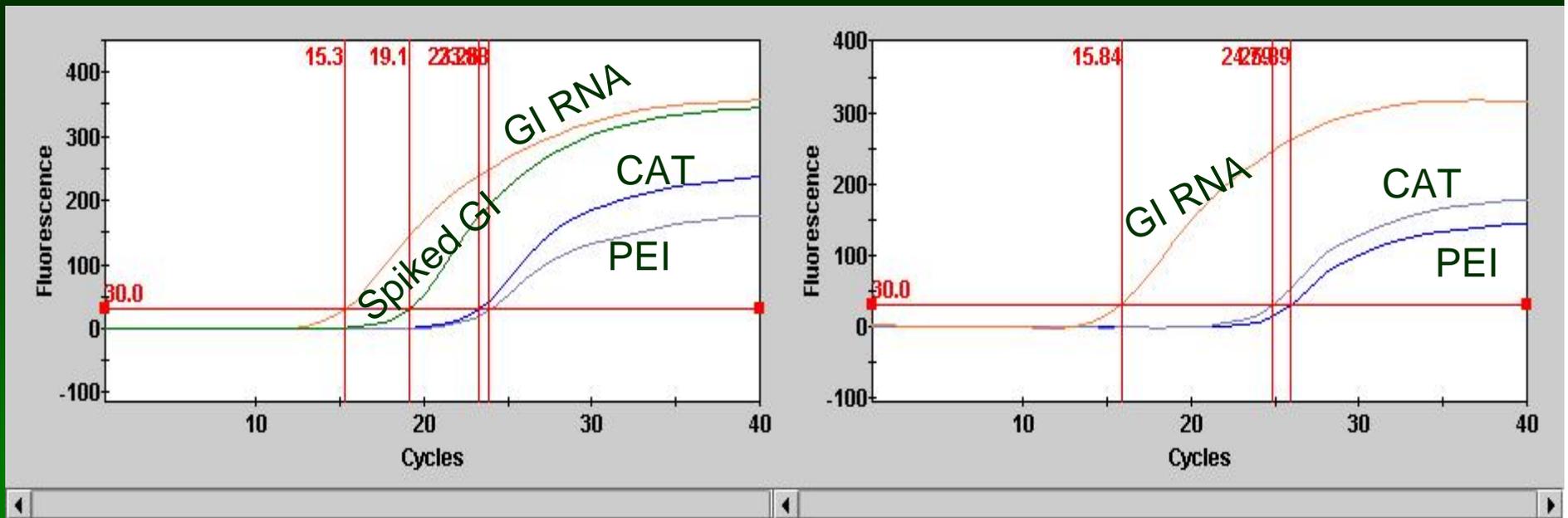
Experiments determined that 50ul of bead suspension is optimal

Comparison of PEI and the Pathatrix CAT bead

- In this study we have been comparing Pathatrix CAT beads vs the PEI beads. CAT beads were used as our reference performance standard.
- CAT beads are cationically charged like PEI. The charged agent is proprietary. CAT beads are currently marketed for viral capture.

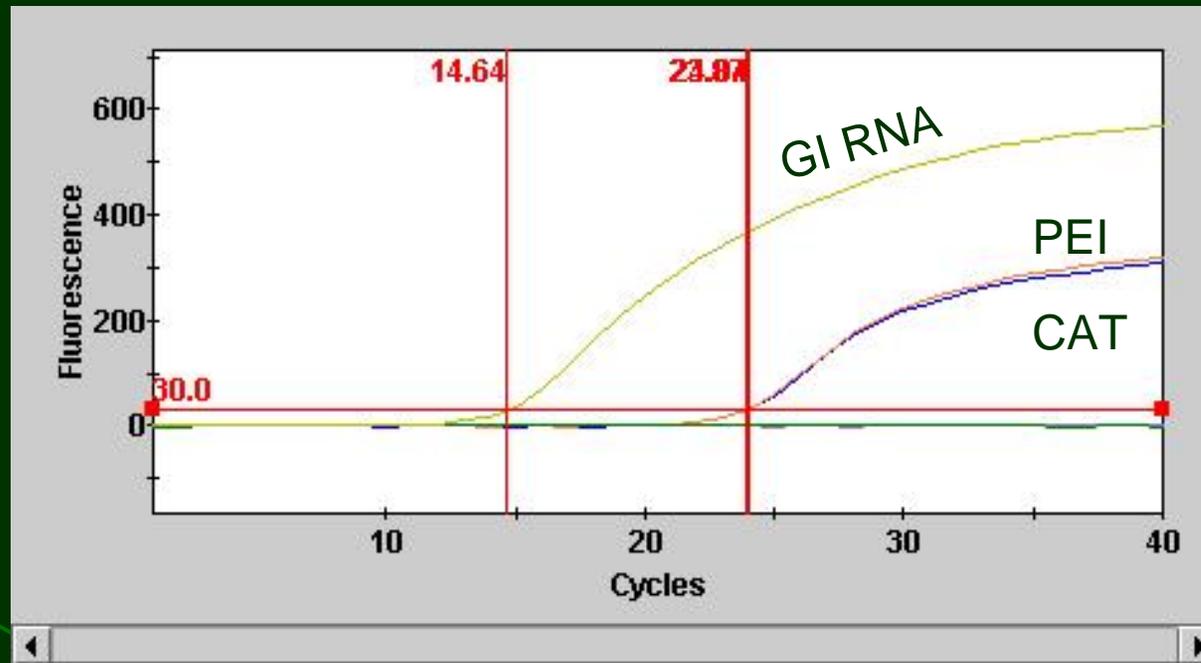
Cat vs PEI bead in 100ml

Cat vs PEI bead in 250ml



Performance is very similar in most situations and food matrices tested (oysters, mussels, potato salad and coleslaw)

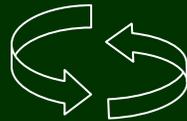
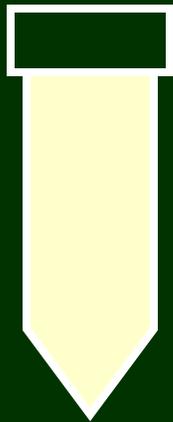
PEI vs CAT bead binding of GI in oyster cecum



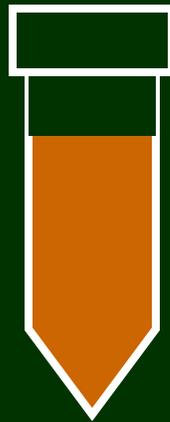
- PEI beads need to have a short PK treatment to cleave captured virus off the beads. Pathatrix CAT beads do not.
- PEI beads are preferred since we can make and modify beads in-house and they are very cost effective.

Basic protocol most foods

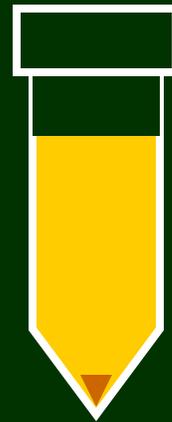
Homogenized sample



Add beads incubate



Collect beads



Wash beads



Free virus



Pk treatment

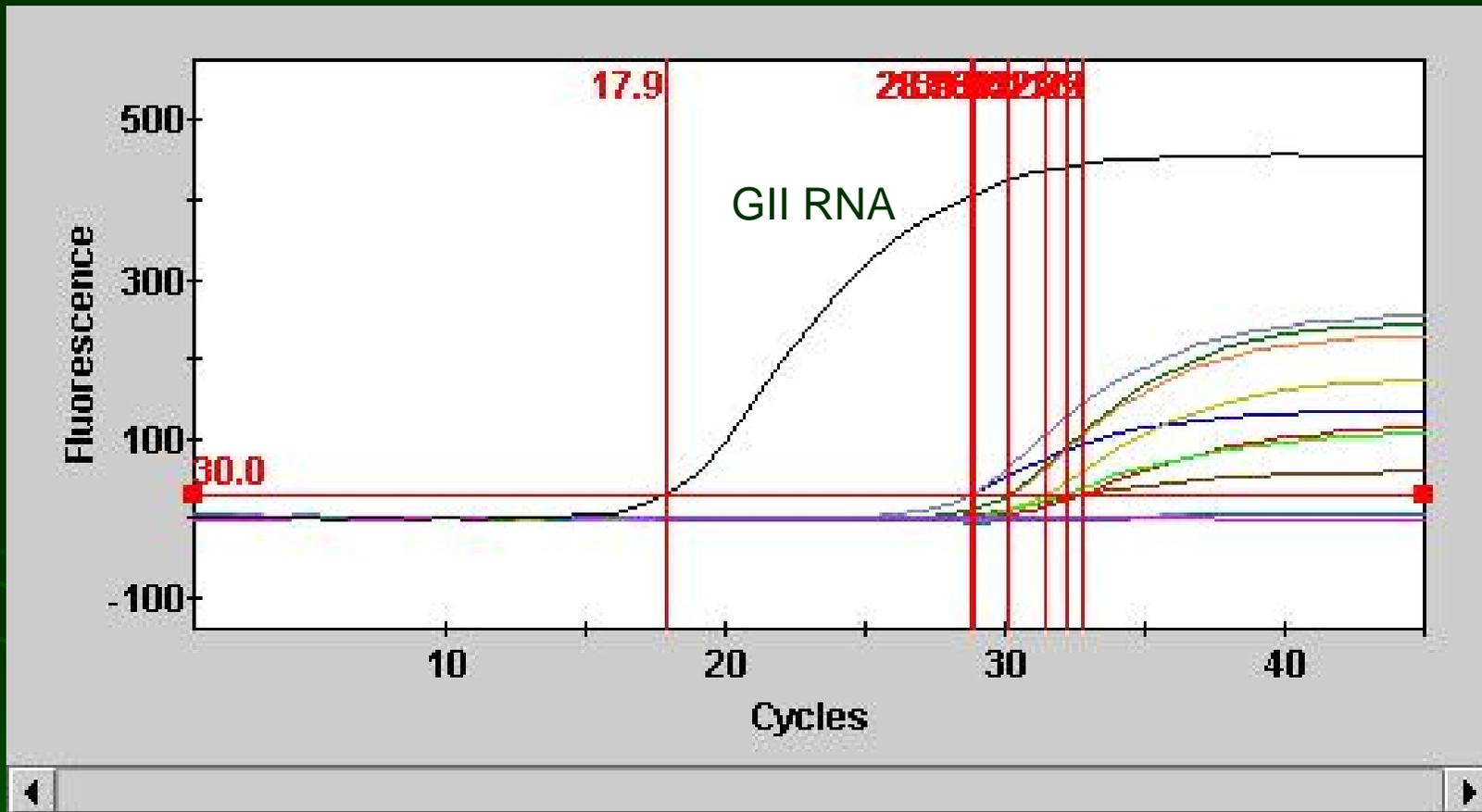
***RNA Extraction
and PCR***



got milk?

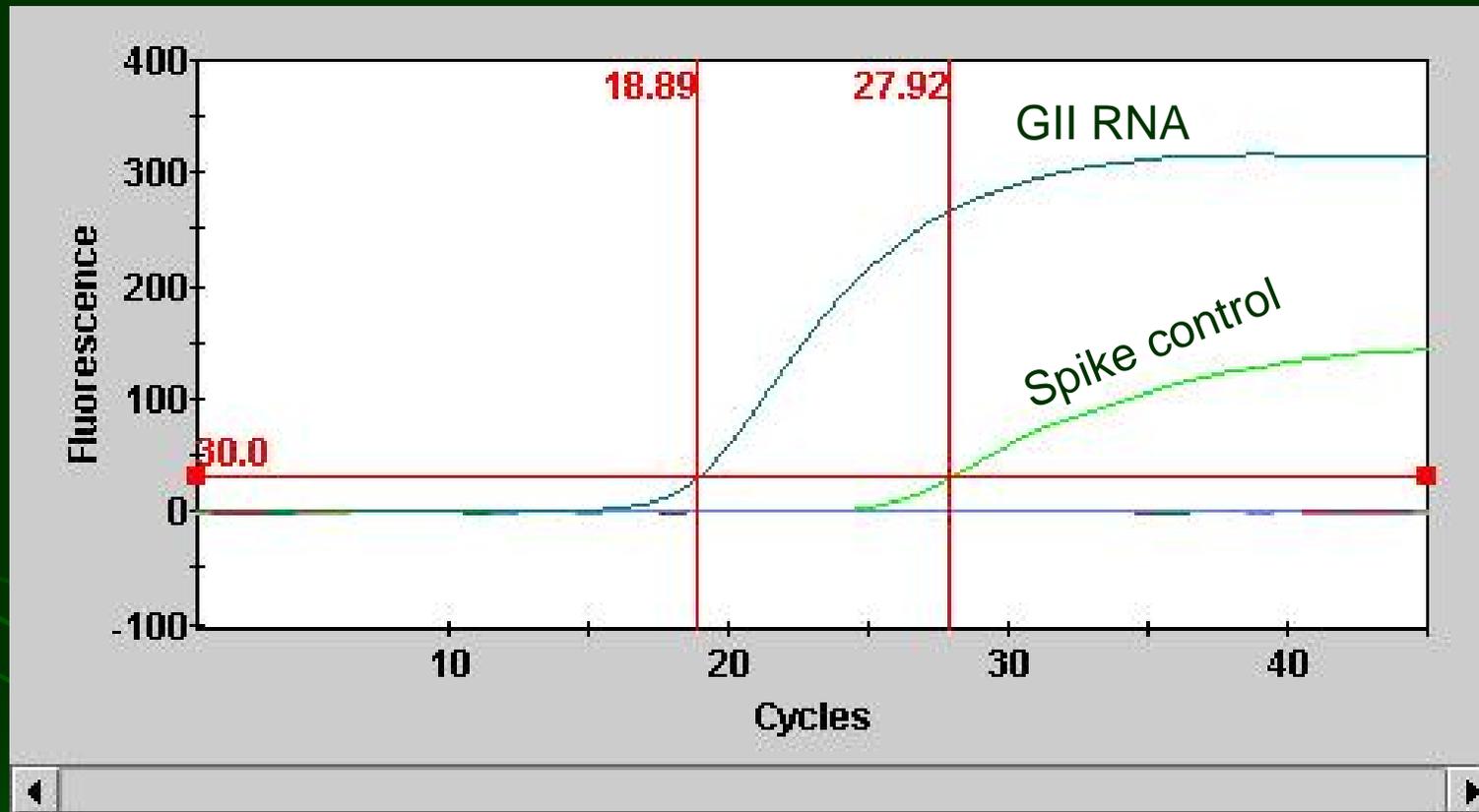


Dilution series of Noro GII in Cecum



In oyster dilution series from 5×10^4 to 1×10^3 viral particles seeded. Could detect norovirus at the 5×10^3 level or 100 viral particles/ml.

Test Yaquina Bay Oyster Outbreak, 2010 Newport, OR



6 dozen oysters were tested. No positive oysters were detected by our assay or by the FDA in Dauphin Island, AL



Matrices tested

Matrices	Detected?	Ct range
mBPW	Yes	21.1-24.8
Water	Yes	22.3-.8
Milk 100%	No	-
Milk 10%	Yes	23.7
Similac (infant formula)	Yes	29.7
Whole oyster	Yes	26.4-.6
Oyster cecum	Yes	23.8-30.5
Mussels	Yes	26.9
Coleslaw	Yes	26.0-.3
Potato salad	Yes	26.2-3
Leaf lettuce	Yes	25.8
Strawberry	Yes	24.4-27.5
Raspberry	Yes	22.3-25.7



Berries were challenging



- Standard homogenization methods do not work well.
- Maceration, blending, freezing/thaw, enzymatic treatments of the berries was also tested.
- Virus seems to get trapped in the fruit pulp and lost.
- So...don't make fruit pulp! Wash the virus off the fruit surface. This strategy worked well!

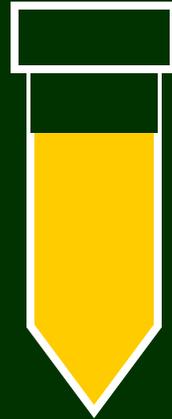
Modified protocol for berries

Wash berries

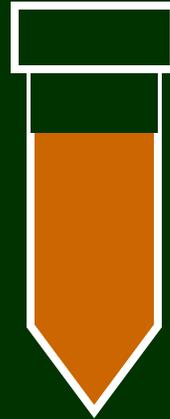
10g/50ml



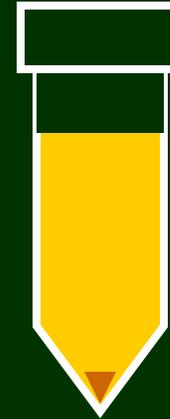
Remove wash
buffer



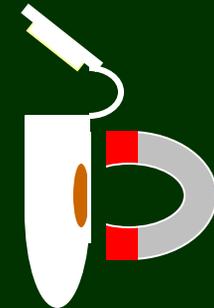
Add beads
incubate



Collect
beads



Wash
beads

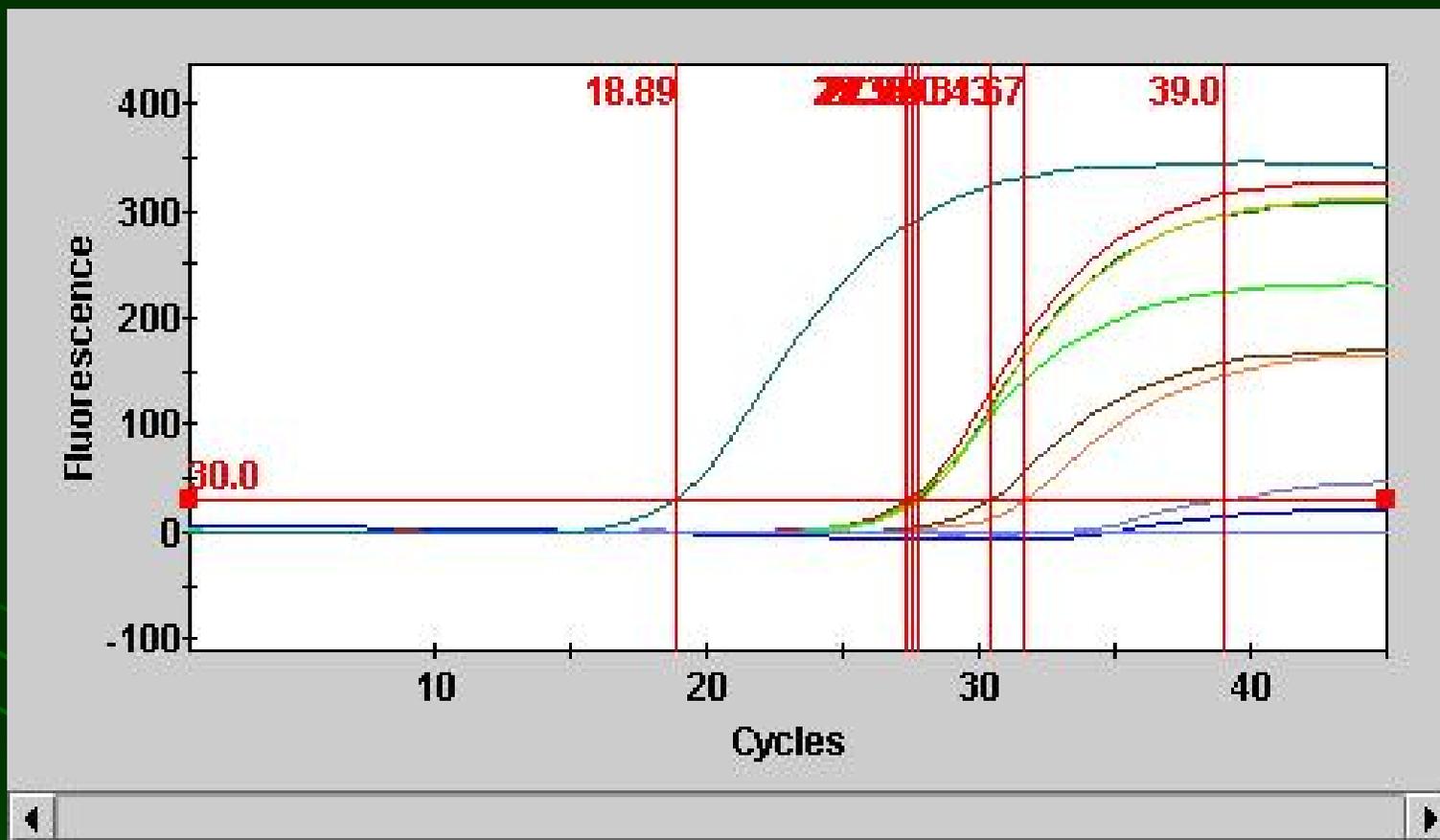


***RNA Extraction
and PCR***

150g/250ml
mBPWp with 0.05%
Tergitol (NP-7)

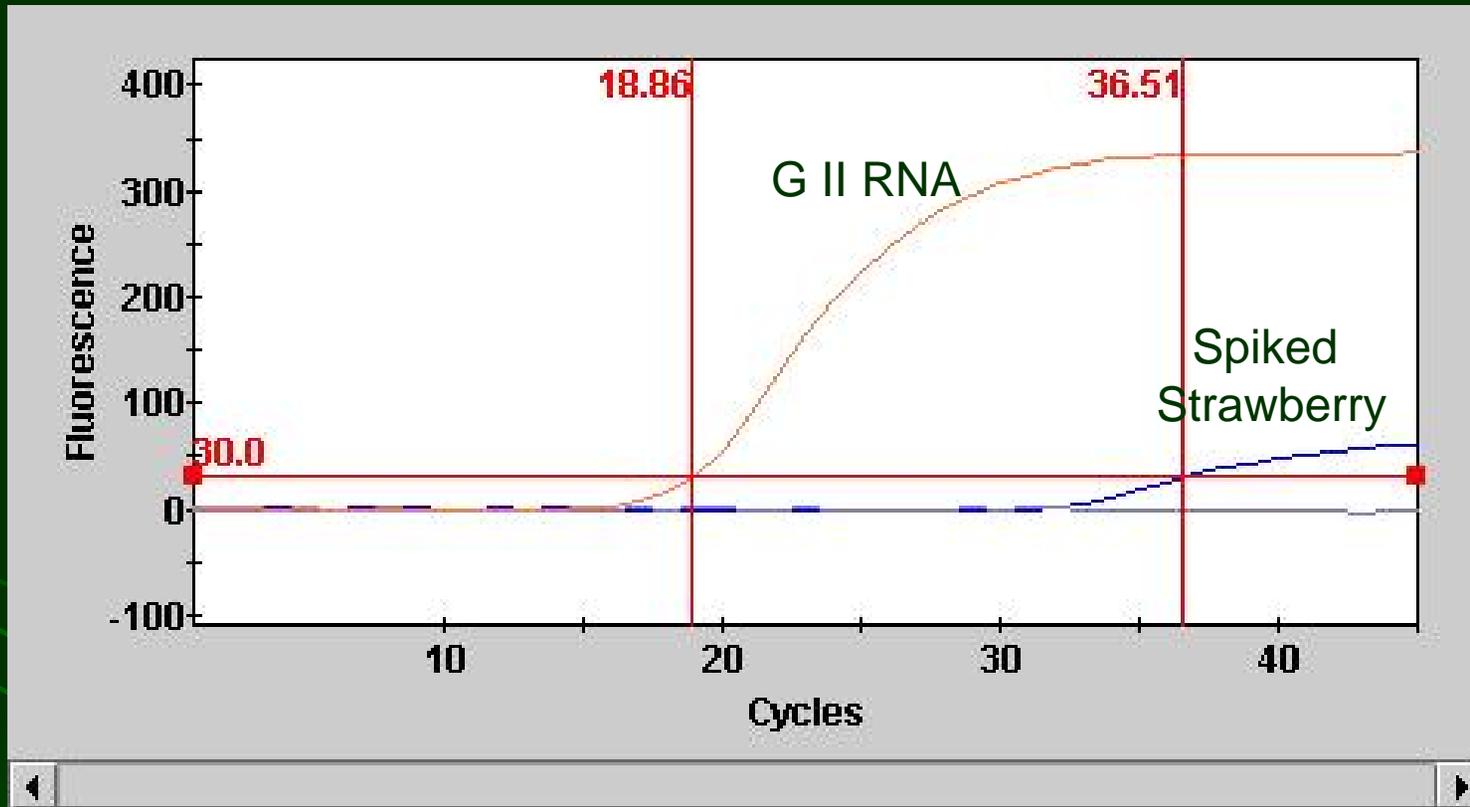


Spike dilution series in strawberry



Detection of Norovirus GII seeded at 5×10^4 , 1×10^4 , 5×10^3 , 1×10^3 or 1000, 200, 100 and 20 viral particles per ml

Test in strawberry: Will it work in a large real world scenario?



Tested the detection by seeding one berry with 2×10^4 viral particles.
150g for non-seeded strawberry in 250ml of wash buffer. (80 particles/ml)

Summary

- Pleasantly surprised with the performance of the beads.
- Worked well in all 12 matrices tested.
- Effective method of concentrating virus and removing inhibitors
- Protocol is simple/no specialized equipment
- Easy to make and are very stable
- Cost effective
- Could be automated or coupled with existing beads based systems
- Can easily be used to test for other viruses.

So where do we go from here?

- So far I have tested the breadth of the assay against a variety of foods that are likely to be contaminated with Norovirus.
- Need to establish sensitivities of the assay in different matrices.
- Need to test naturally contaminated samples when possible.
- Begin investigating how well the developed assays work with Hepatitis A.
- Couple with new Pathatrix system. Could save a lot of time.

Acknowledgments

- FERN research grant FSIS-C-03-2007/03
- California Dept. of Public Health, Food and Drug Lab, Microbiology

- **References**

PEI beads: Satoh K et al, J. Vir Methods 114(2003) 11-19.

Uchida E, et al J. Vir Methods 143(2007) 95-103

Norovirus PCR: Jothikumar N, et al App Env Micro, vol 71, 2005
1870-75

- **Contact:** michael.zwick@cdph.ca.gov

