# Salivary antibody responses as an indicator of waterborne infections: pilot community study before and after installation of UV treatment

# Co-investigators and main contributors

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# Study objectives

- Test and validate novel infection surveillance technique that uses salivary antibody as a biomarker of infection
- Apply this technique to assess health benefits of EPA water quality regulations in a selected community
- Identify sites for future studies utilizing this methodology

# Study Design

- Study sites:
  - Lawrence, MA (population 70,000):
    - Water from a microbiologically-challenged river
    - In March 2007, the city replaced an old plant (built in 1938) with a new plant ($ClO_2$, UV) meeting LT2 requirements
  - Lowell - control community using the same river
- Participants: Local families with at least one 1 to 11 y.o. child
- Study cohorts: “before” (June 2006 – Jan. 2007) and “after” (planned in June 2008 – Jan. 2009)
  - Planned 100 families for a full year but the start was delayed. Recruited ~400 families to compensate for shorter follow-up
  - Monthly exposure and illness questionnaires
  - Monthly saliva samples
- Supplemental water monitoring project (Crypto, Giardia, viruses, aerobic endospores, other bacterial indicators)

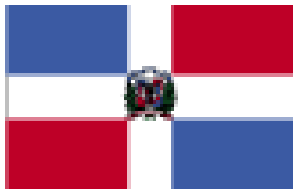
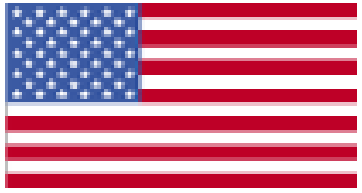
# Data analysis

- Immunoconversion (a steep increase in antibody response to a specific pathogen) as an indicator of infection
- Compare the incidence of immunoconversions before and after new water treatment
  - Compare temporal changes in Lawrence with temporal changes in Lowell
  - Asymptomatic vs. symptomatic infections (immunoconversion following diarrhea/vomiting)
  - Effect of non-boiled tap water consumption
- Compare the results of risk assessment with epidemiological results

# “Before new treatment” cohort

# Demographics of the study population in Lawrence

- 85% Hispanic (mainly Dominican Republic and Puerto Rico)
- Income
  - 79% had a household income below $25k
  - 94% had a household income below $50k
- Education
  - 36% of adults did not complete high school
  - 32% had only a GED or high school diploma


# Summary of saliva sampling by month

| Month | Samples from Lawrence | Samples from Lowell | Total number of samples |
|---|---|---|---|
| **June** | **8** | **36** | **44** |
| **July** | **39** | **33** | **72** |
| **August** | **206** | **47** | **253** |
| **September** | **698** | **80** | **778** |
| **October** | **1088** | **235** | **1323** |
| **November** | **948** | **204** | **1152** |
| **December** | **1282** | **256** | **1538** |
| **The entire "before" period** | **4269** | **891** | **5160** |

Total number of families: 391

Total number of individuals: 1398

# 24 hour liquid consumption

## (average numbers of 8 oz glasses)

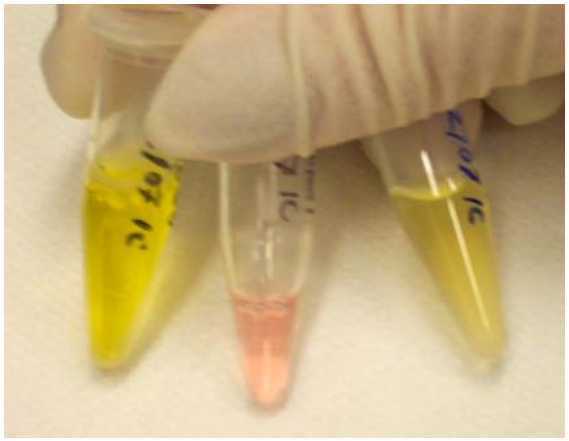
| City | Soda | Milk | Bottled water | Boiled water | Filtered non-boiled tap water | Non-filtered non-boiled tap water | All non-boiled tap water | All drinks |
|---|---|---|---|---|---|---|---|---|
| Lawrence | 2.3 | 1.9 | 1.7 | 1.0 | 1.1 | 0.7 | 1.8 | 8.8 |
| Lowell | 1.8 | 1.6 | 1.3 | 0.6 | 0.9 | 1.3 | 2.2 | 7.5 |

- **Relatively low consumption of untreated tap water in Lawrence**
- **Extensive use of home water filters in Lawrence but not in Lowell:**
  - **Lawrence – 46 % of participants**
  - **Lowell – 22 % of participants**

# Objective 1

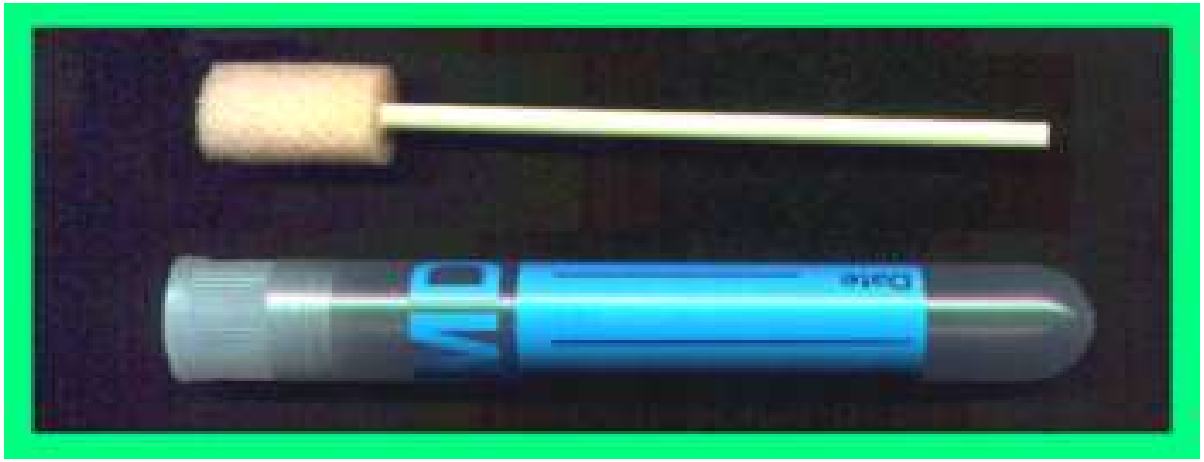
Test and validate novel infection surveillance technique that uses salivary antibody as a biomarker of infection

# Salivary antibody – advantages and challenges



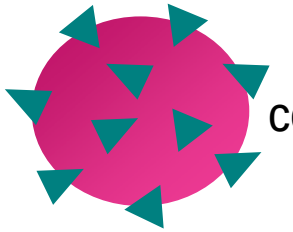
- Advantages:
  - Sampling well tolerated by children
  - Multiple samples are possible
- Challenges:
  - Substantial variability in antibody concentrations
  - Precipitation of antibody-protein complexes
  - Non-specific reactivity
  - Lower concentrations of antibodies than in serum

# Saliva sampling

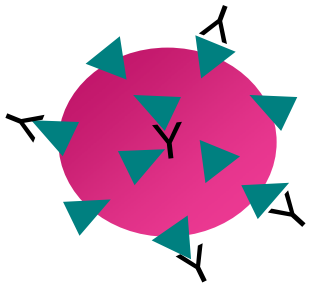


- Oracol™ oral fluid samplers
- Centrifugation to separate saliva from the sponge and debris from saliva
- Storage at -80° C until analysis
- Analysis at EPA using Luminex™ multiplex microbead immunoassay

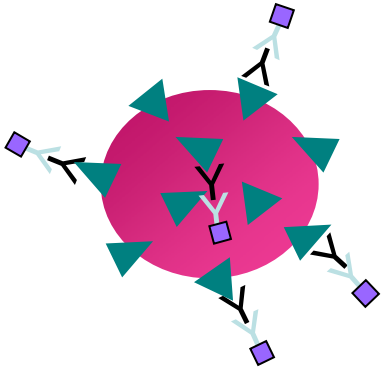
# Luminex xMAP microsphere suspension microplate immunoassay



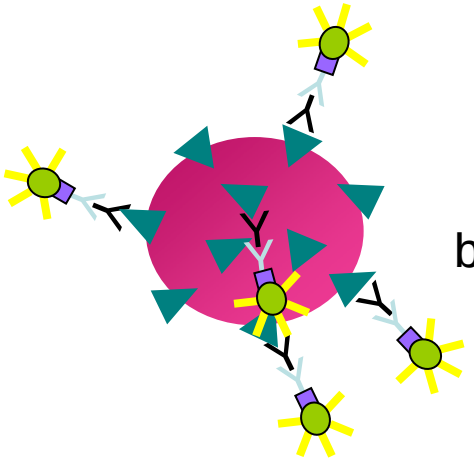
1. Microscopic bead is coupled with one specific protein



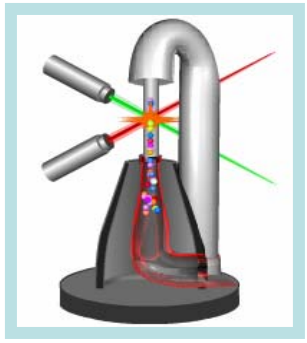
2. Saliva is incubated with beads; salivary antibodies react with protein



3. Samples are incubated with biotinylated anti-human detection antibody

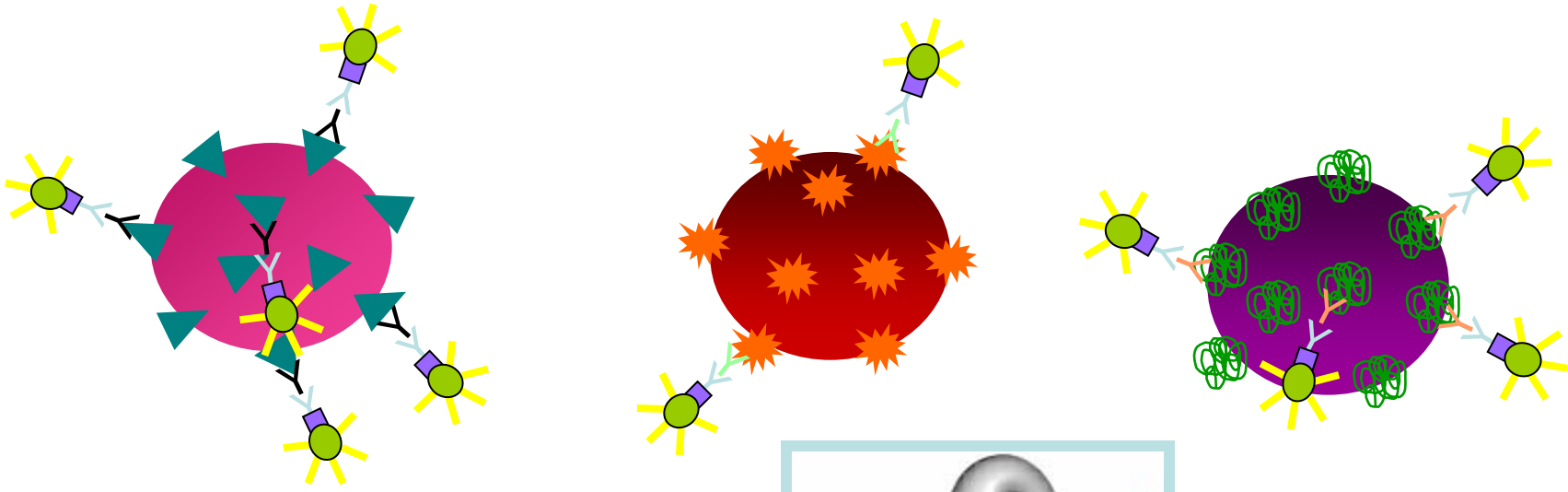


4. SAPE is added to wells to bind biotinylated detection antibodies

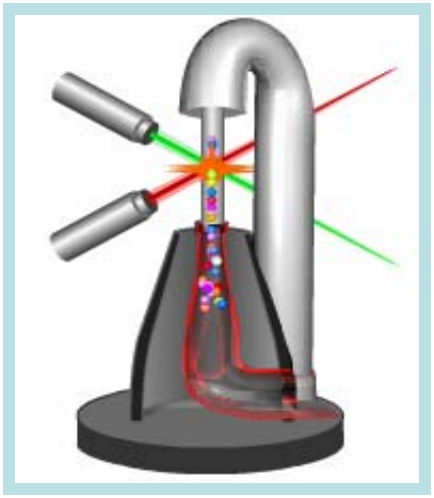


5. Microplates are analyzed using Luminex instrument

# Multiplex assay



- Color-coded sets of beads coupled to different proteins
- Dual laser flow cytometer determines the type of bead and measures signal intensity



# Selected potentially waterborne pathogens

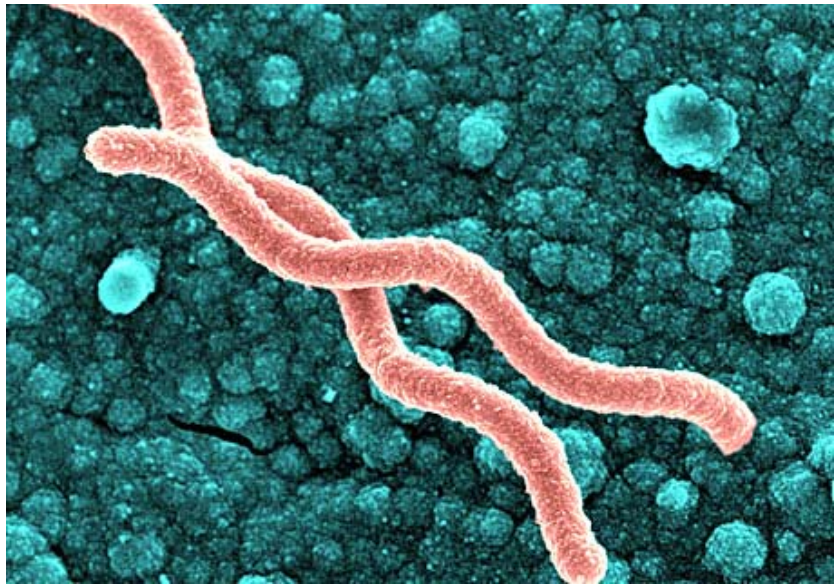
- *Cryptosporidium*
- Noroviruses
- Rotaviruses
- *Helicobacter pylori*
- *Toxoplasma gondii*

# Assay development

- Samples used:
  - Serum and saliva samples from EPA volunteers
  - Selected saliva samples from participants of main study
- Selection and acquisition of proteins
- Expression and purification of recombinant proteins
- Optimization of protein-bead coupling
  - Coupling confirmation tests using antigen-specific antibodies
- Selection of saliva dilution ratio and dilution buffer
- Internal controls (GST- and BSA-coupled beads)
- Total antibody and total protein concentrations
- Effects of sample volume, storage, freezing, etc.
- Validation of salivary tests for chronic infections

# *Helicobacter pylori*

- Active chronic infection of the stomach
- Causes gastritis, ulcers, cancer
- >30% of US adults infected
- CCL2 pathogen
- Proteins:
  - Flagellin
  - VAC protein
  - CAG protein
  - Small subunit urease
  - Soluble antigen extract (strain )

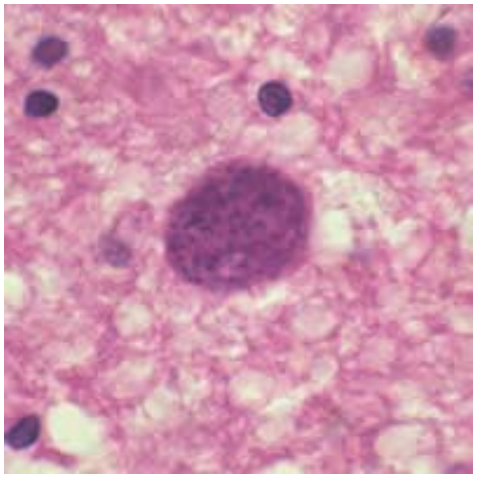


# *Toxoplasma gondii*

- Protozoan parasite of felines, forms environmental cysts
- Forms latent tissue cysts in muscles and brain of intermediate hosts including humans
- ~25% of US adults are infected
- Can infect human fetus and cause severe neurological damage
- Reported waterborne outbreaks

**Proteins:**

- Soluble proteins from tachyzoites
- P30 protein
  - Recombinant
  - Purified from HeLa human cells
  - Purified from mice
- GRA7 protein
- MIC3 protein

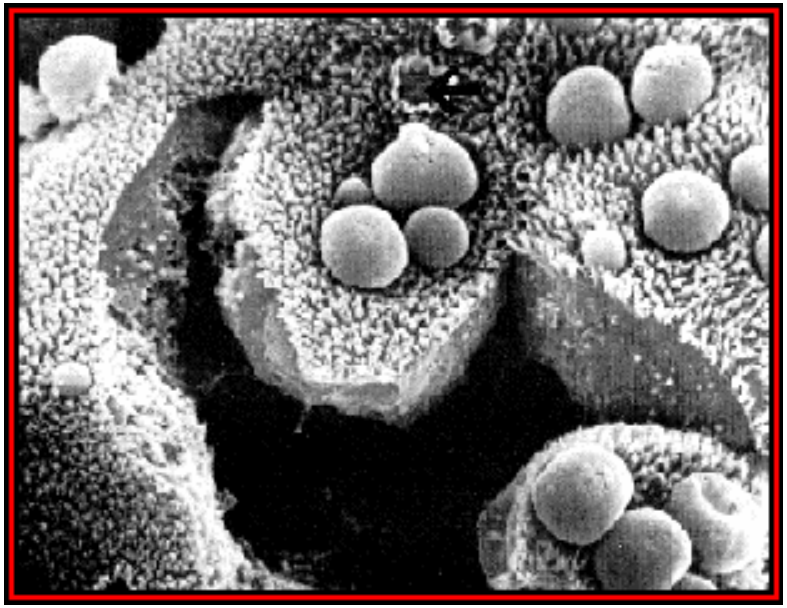


*T. gondii* cyst in brain tissue

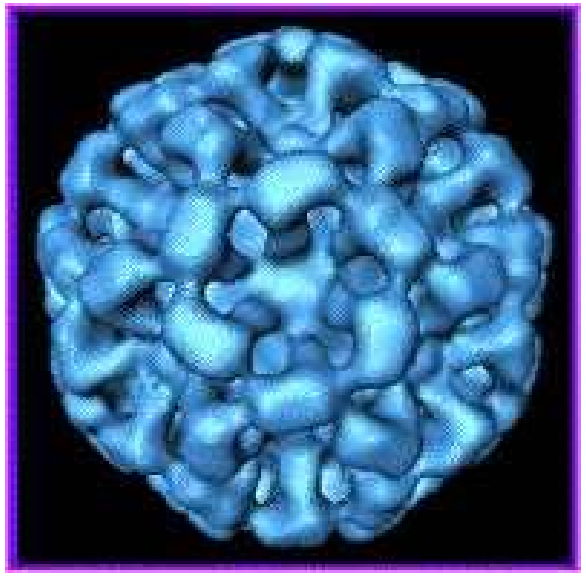
*http://www.dpd.cdc.gov*

# *Cryptosporidium*

- Oocysts resistant to chlorine
- Reported incidence 1 per 100,000 PY
- Major waterborne outbreaks
- LT2 is based on RA for endemic cryptosporidiosis
- Antigen extract from *C. parvum* oocysts
- Recombinant 27 kDa *C. parvum* protein
  - Transformed *E. coli* was provided by Jeffrey Priest (CDC)
  - Expressed and purified glutathione *S*-transferase (GST)-tagged protein
  - Use GST-coupled beads as internal control



# Noroviruses



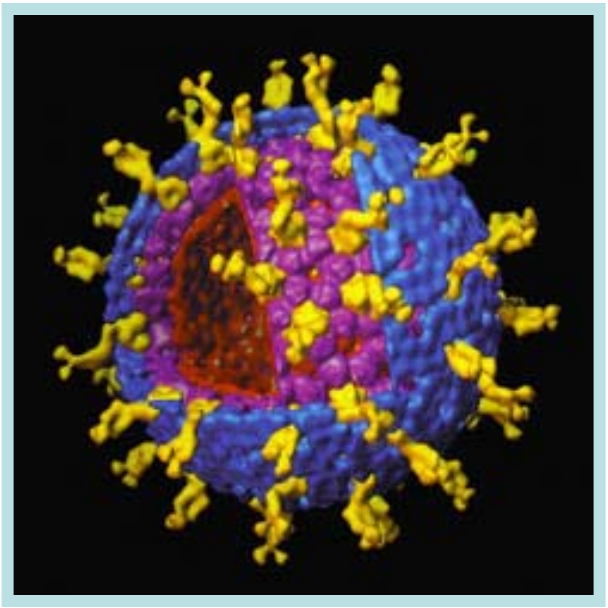
- The major cause of gastroenteritis in adults
- Severely underreported
- Highly infectious, resistant to chlorine
- Identified as cause of drinking water outbreaks
- Typical symptoms include vomiting and diarrhea

**Proteins (provided by the Cincinnati Children's Hospital):**

- Genogroup II strain VA387 recombinant capsid protein
- Genogroup I Norwalk virus recombinant capsid protein

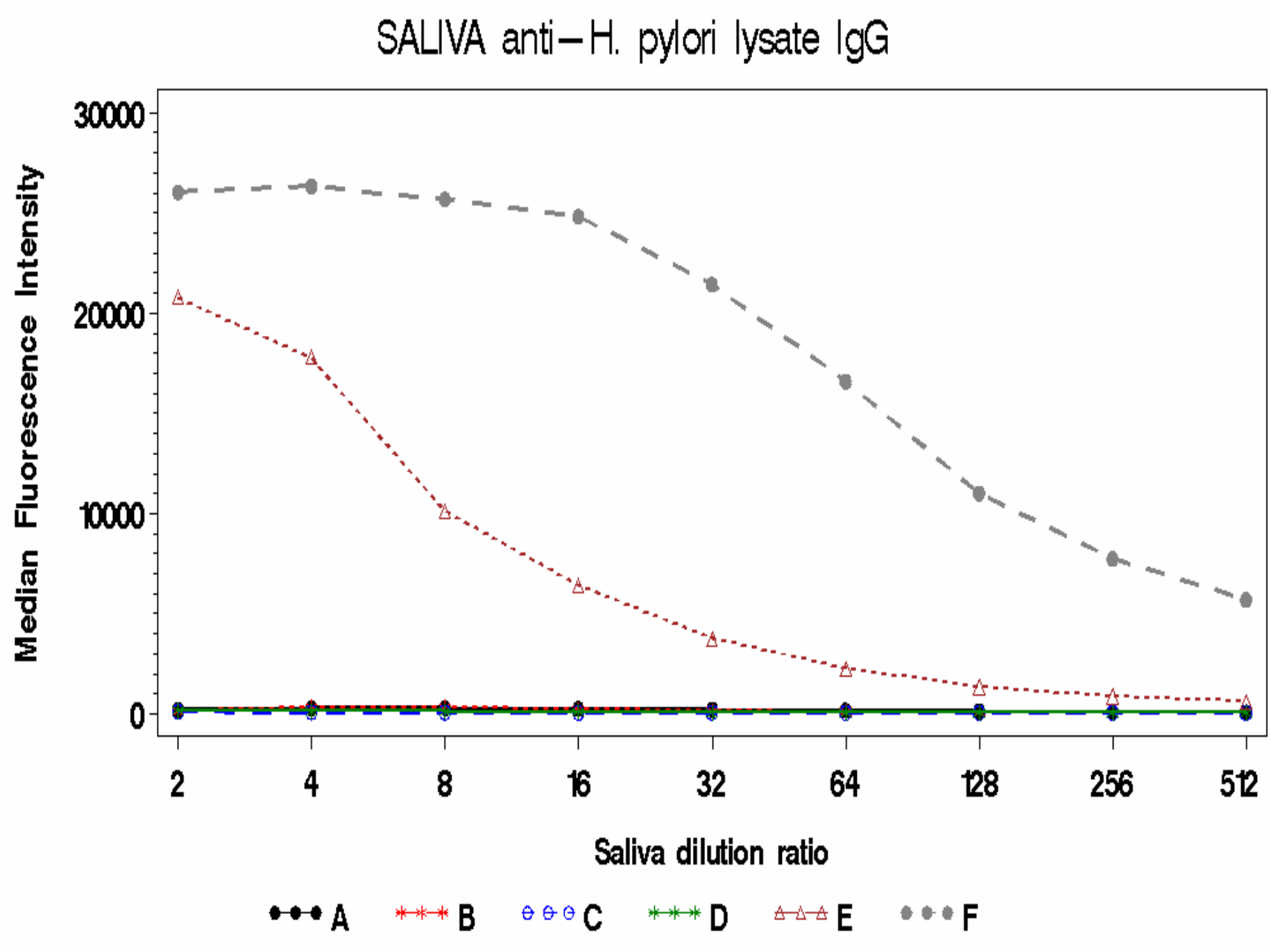
# Rotaviruses

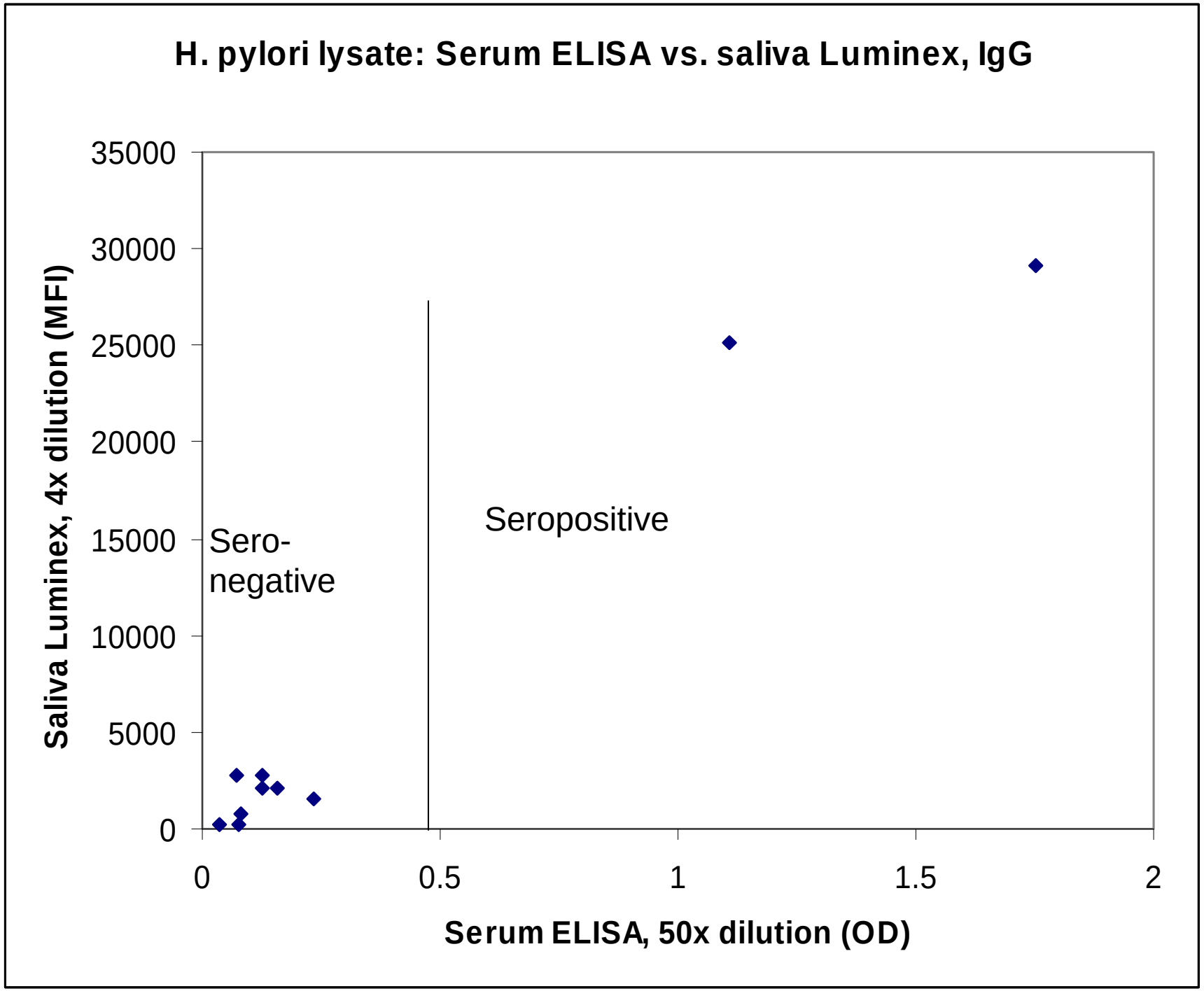
- Major cause of gastroenteritis in children
- Severely underreported
- Detected in surface and ground water
- Purified rotavirus particles procured from the Cincinnati Children's Hospital:
  - DS1 strain:
    - Triple layered particles
    - Double-layered particles
  - WA strain:
    - Triple layered particles
    - Double-layered particles

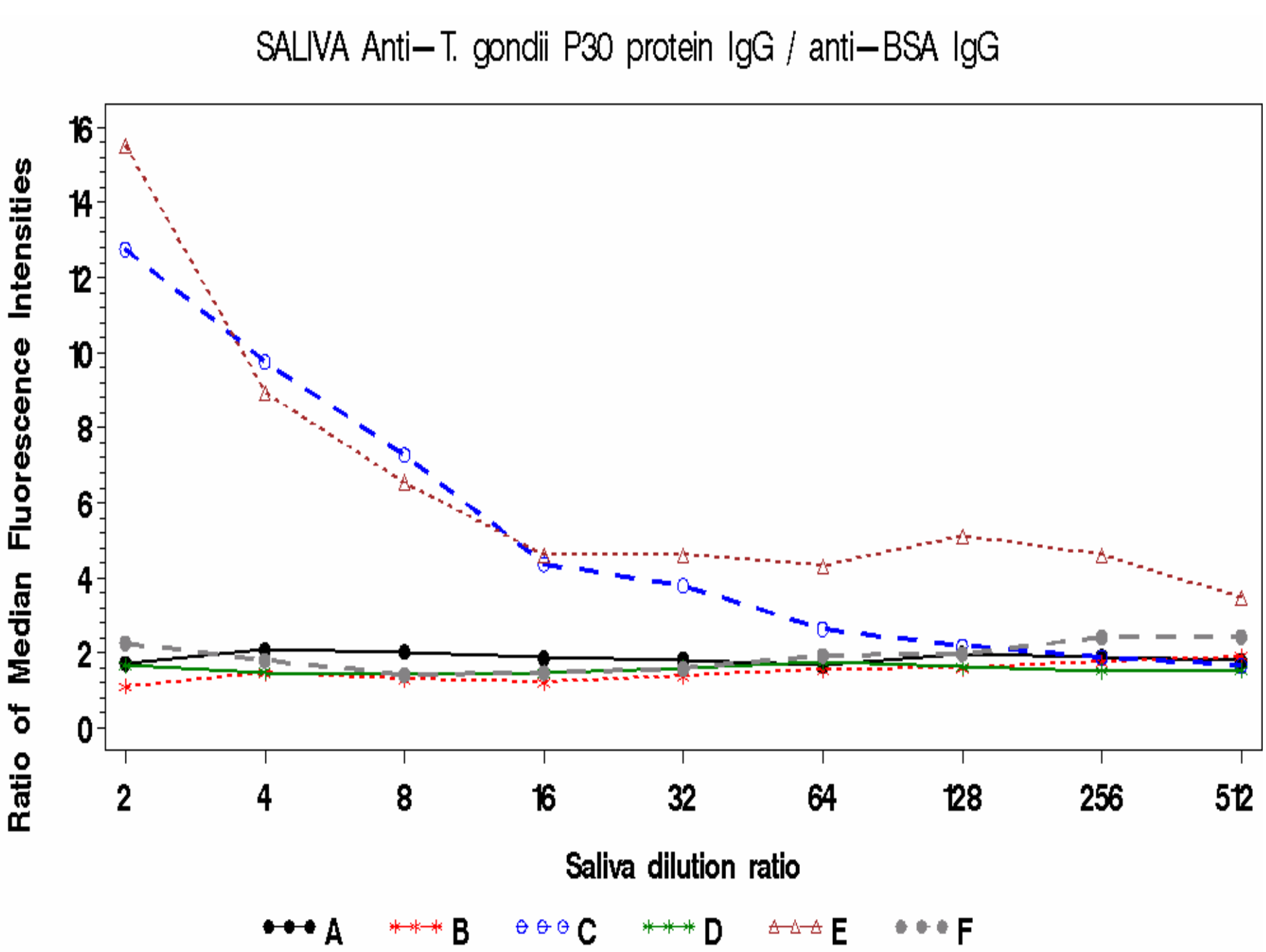


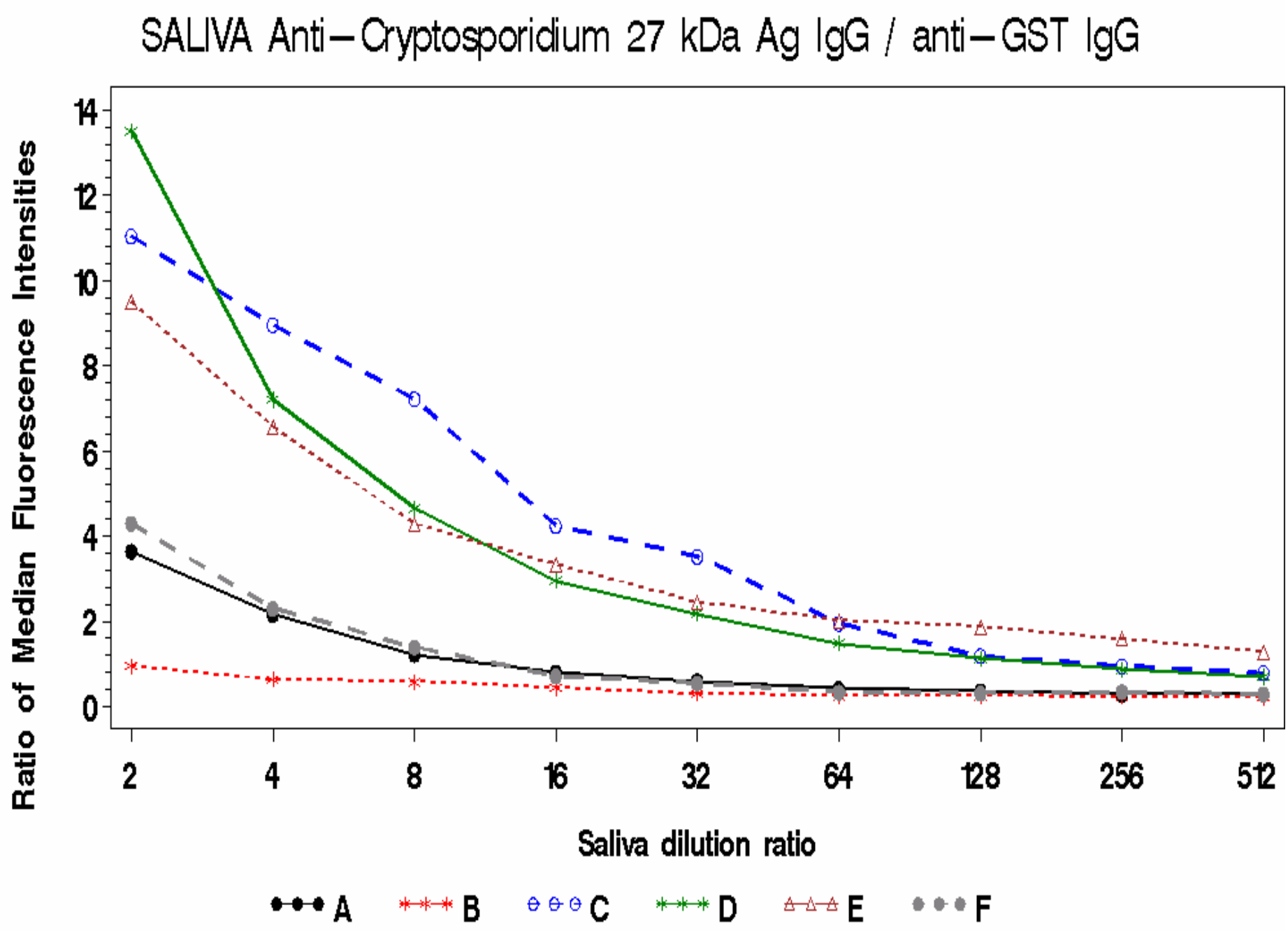
From Maricel Seeger, Buenos Aires / EFE

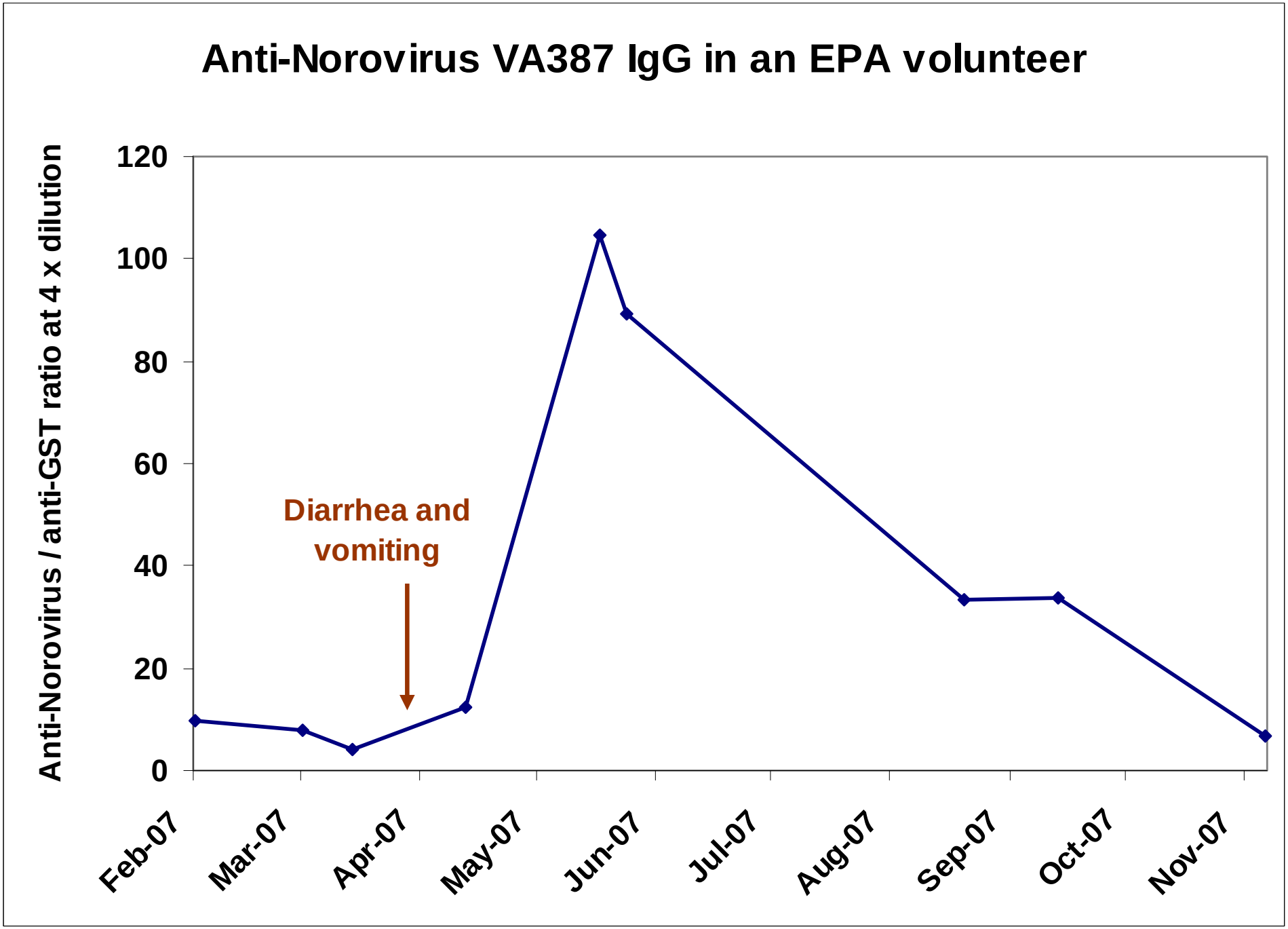
# Selected results of assay development and validation





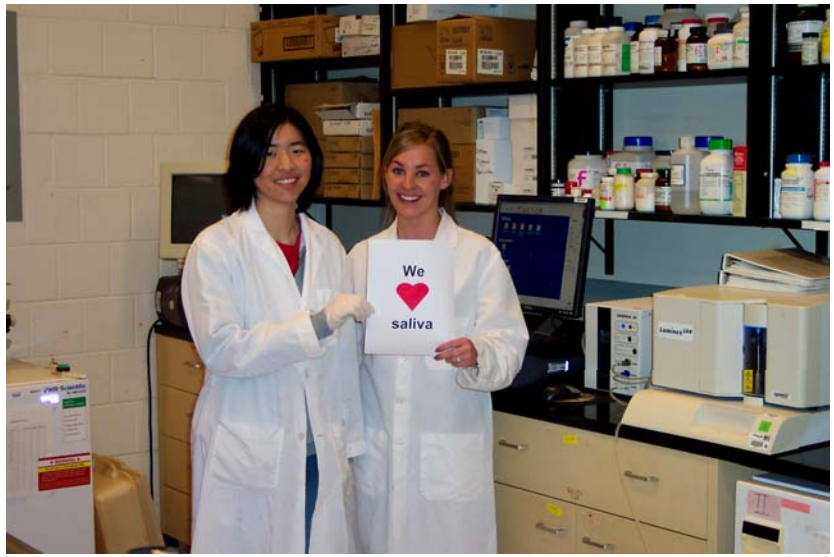






# Study status and future directions

- Objective 1
  - Completed development of assays for all target pathogens
  - Developed multiple internal controls
- Objective 2 is underway
  - Detected immunoconversions to norovirus
- More antigens can be added to multiplex assay at relatively low cost
  - *Giardia lamblia* (acquiring recombinant giardins from CDC)
  - Recreational water pathogens
  - Biofilm-associated pathogens



# Acknowledgments


- NCEA, NERL and OW TSC colleagues who donated their saliva and blood samples
- CDC for providing transformed *E. coli* culture expressing recombinant *C. parvum* protein
- Cincinnati Children's Hospital for providing purified norovirus proteins
- EPA colleagues who provided support and consultations