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)

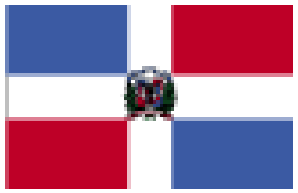
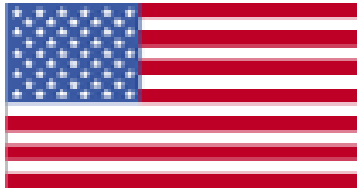
Data analysis

- 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)

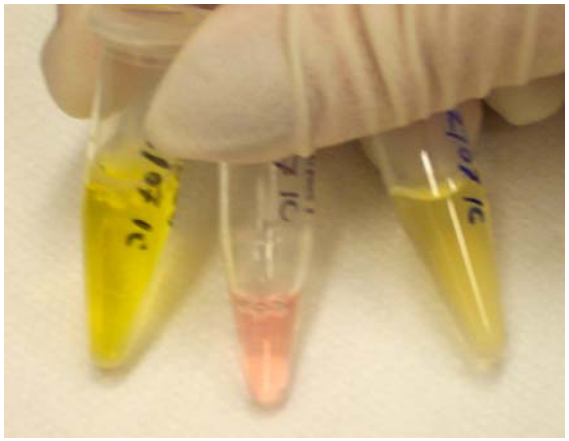
City	Soda	Milk	Bottled water	Boiled water	Filtered non-boiled tap water	Non-filtered non-boiled tap water	All non-boiled tap water	All drinks
Lawrence	2.3	1.9	1.7	1.0	1.1	0.7	1.8	8.8
Lowell	1.8	1.6	1.3	0.6	0.9	1.3	2.2	7.5

- **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

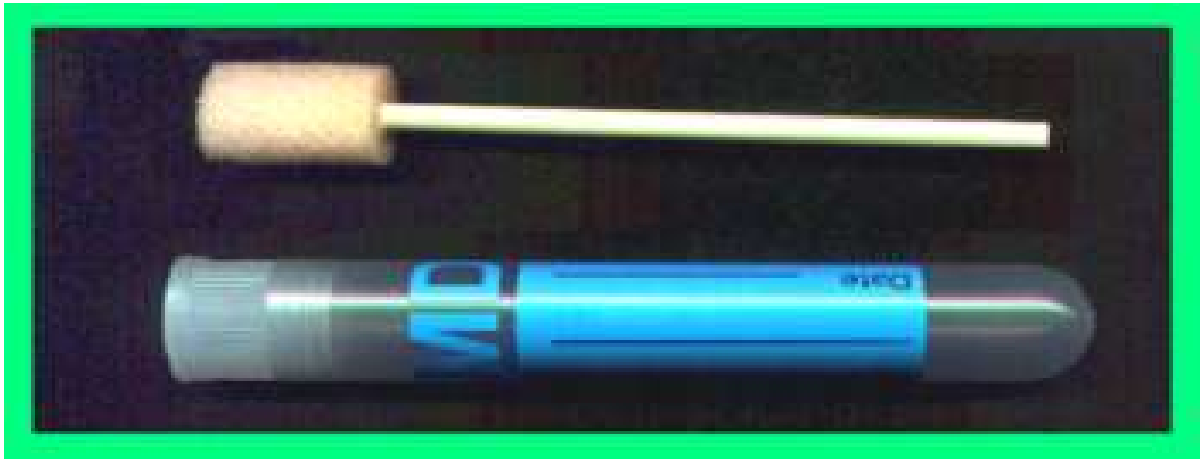
Test and validate novel infection surveillance technique that uses salivary antibody as a biomarker of infection

Salivary antibody – advantages and challenges



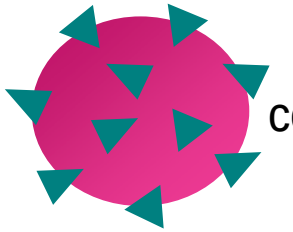
- Advantages:
  - Sampling well tolerated by children
  - Multiple samples are possible
- Challenges:
  - Substantial variability in antibody concentrations
  - Precipitation of antibody-protein complexes
  - Non-specific reactivity
  - Lower concentrations of antibodies than in serum

Saliva sampling

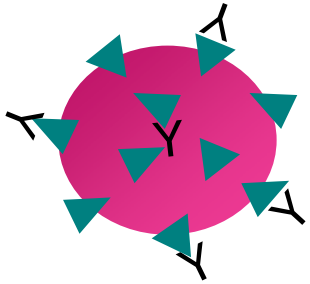


- Oracol™ oral fluid samplers
- Centrifugation to separate saliva from the sponge and debris from saliva
- Storage at -80° C until analysis
- Analysis at EPA using Luminex™ multiplex microbead immunoassay

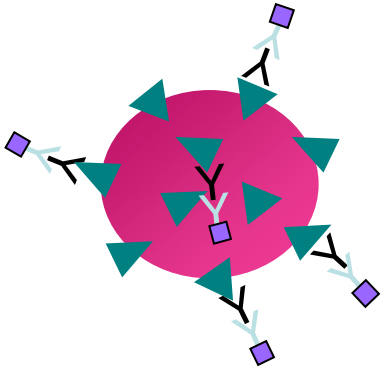
Luminex xMAP microsphere suspension microplate immunoassay



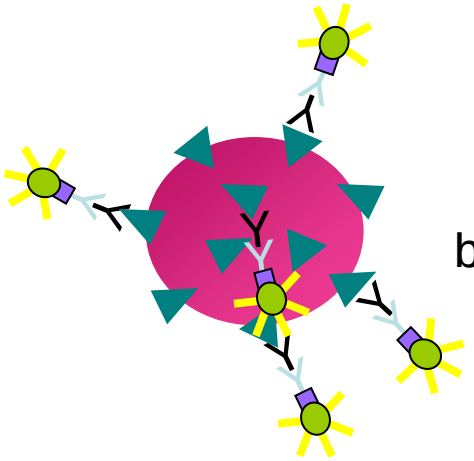
1. Microscopic bead is coupled with one specific protein



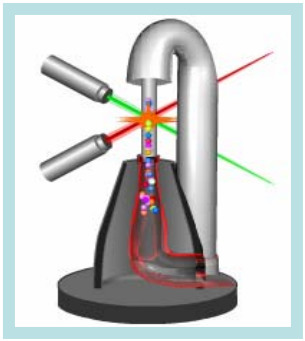
2. Saliva is incubated with beads; salivary antibodies react with protein



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

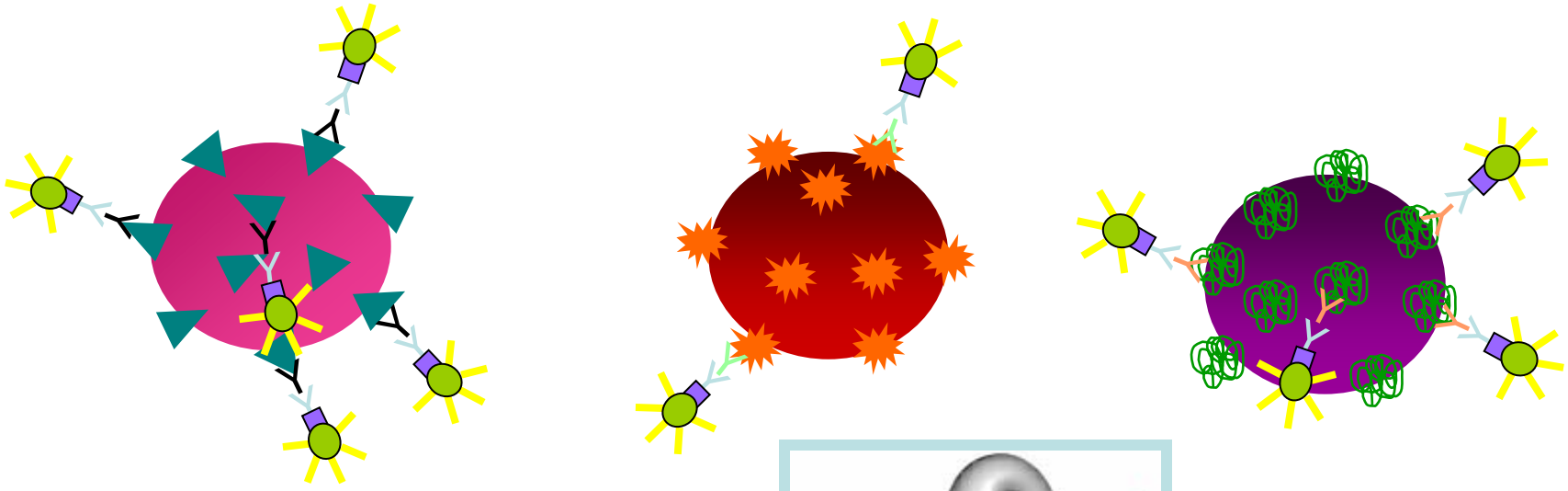


4. SAPE is added to wells to bind biotinylated detection antibodies

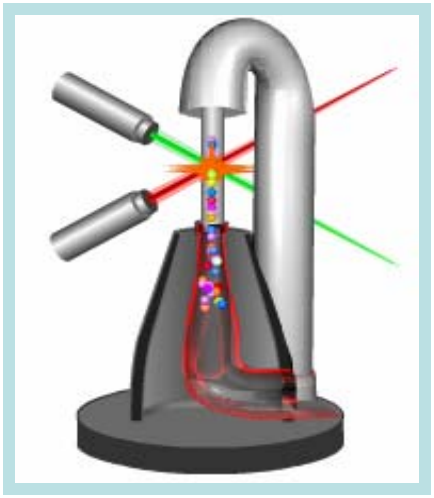


5. Microplates are analyzed using Luminex instrument

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

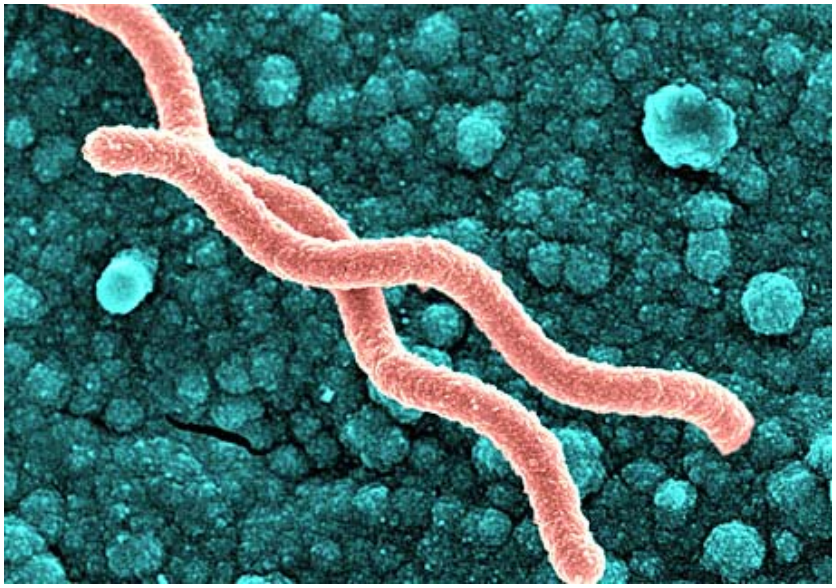
- *Cryptosporidium*
- Noroviruses
- Rotaviruses
- *Helicobacter pylori*
- *Toxoplasma gondii*

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)

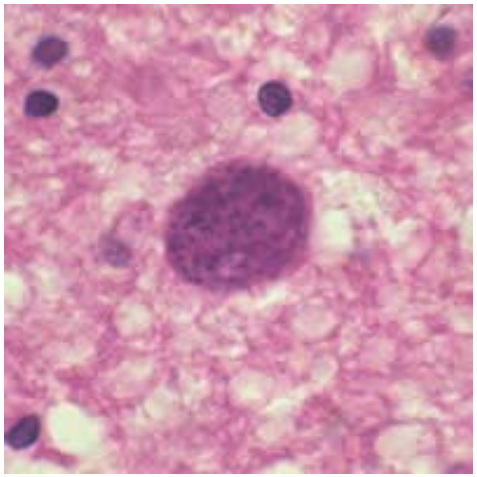


Toxoplasma gondii

- 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

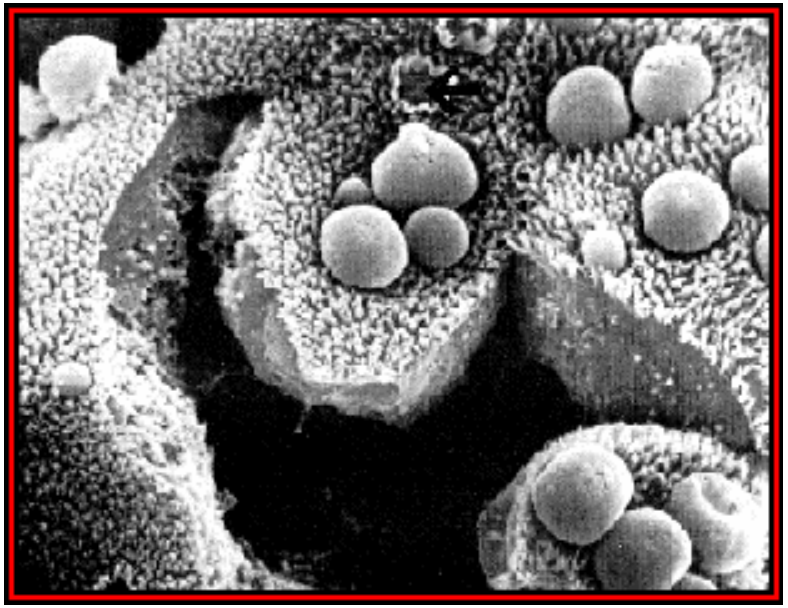


T. gondii cyst in brain tissue

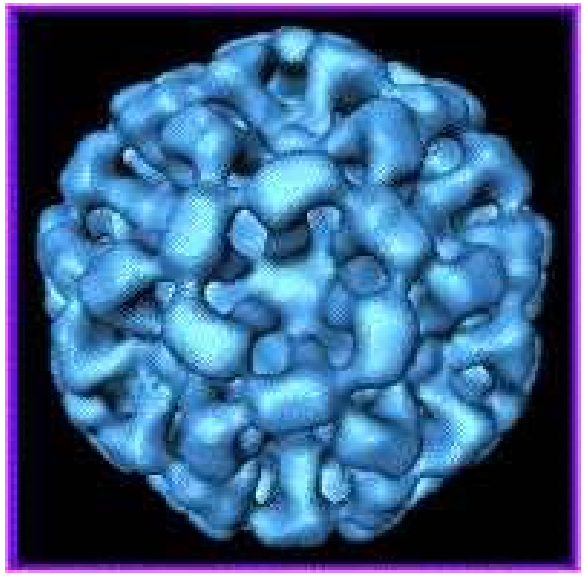
http://www.dpd.cdc.gov

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



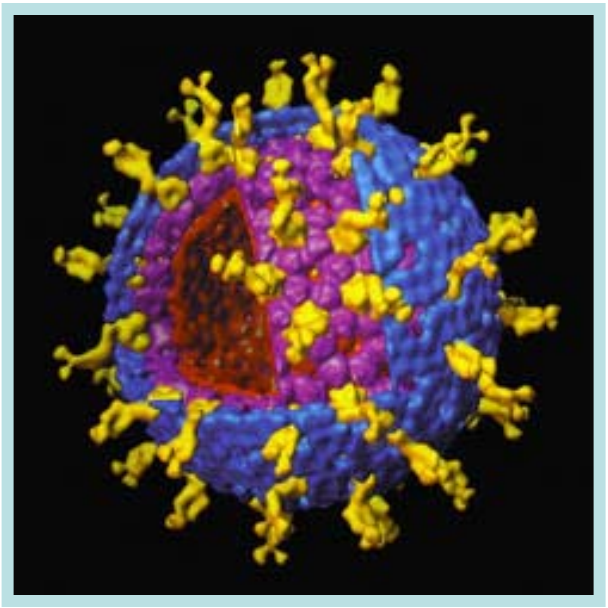
- The major cause of gastroenteritis in adults
- Severely underreported
- Highly infectious, resistant to chlorine
- Identified as cause of drinking water outbreaks
- Typical symptoms include vomiting and diarrhea

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

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

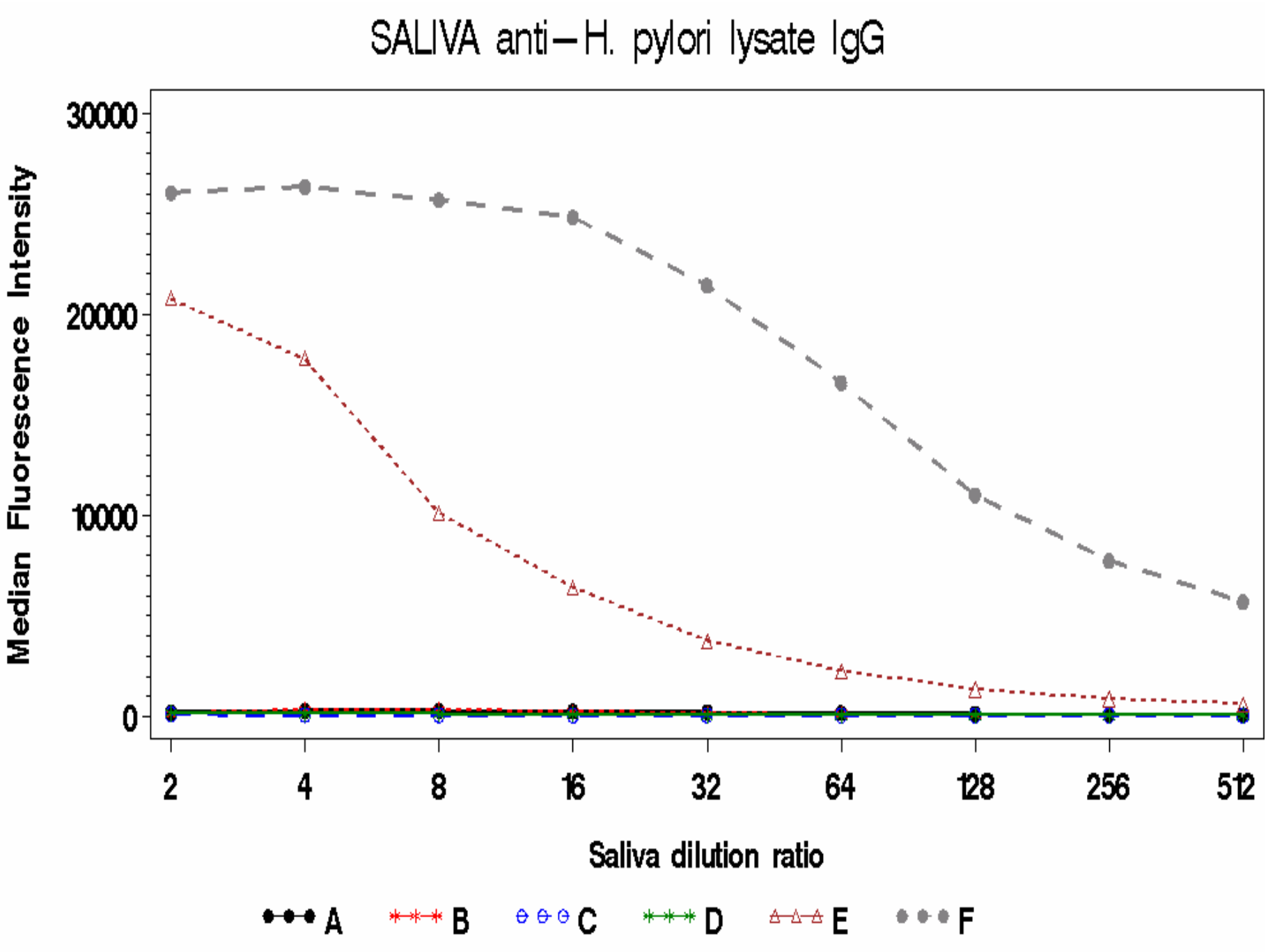
Rotaviruses

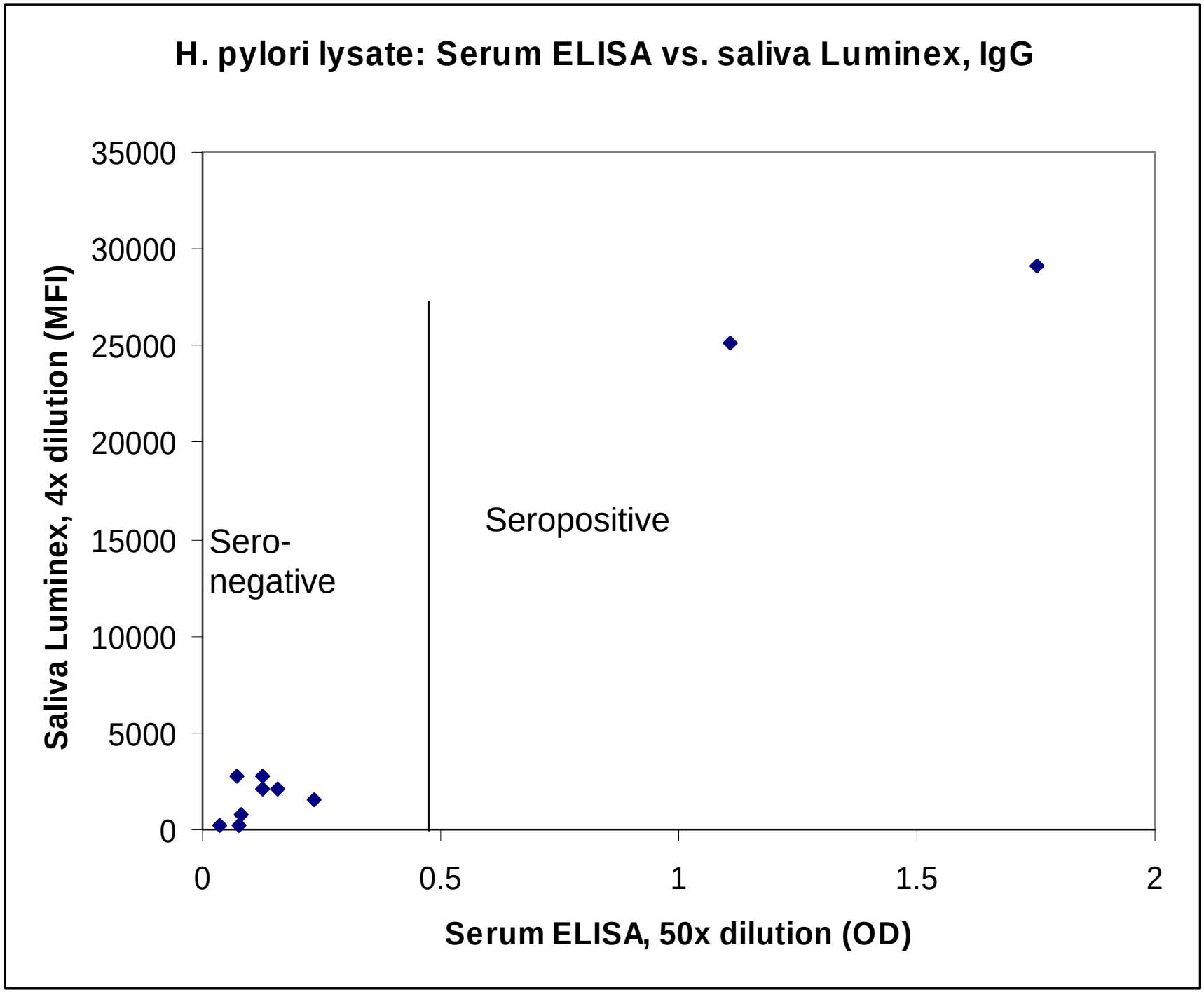
- Major cause of gastroenteritis in children
- Severely underreported
- Detected in surface and ground water
- Purified rotavirus particles procured from the Cincinnati Children's Hospital:
  - DS1 strain:
    - Triple layered particles
    - Double-layered particles
  - WA strain:
    - Triple layered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

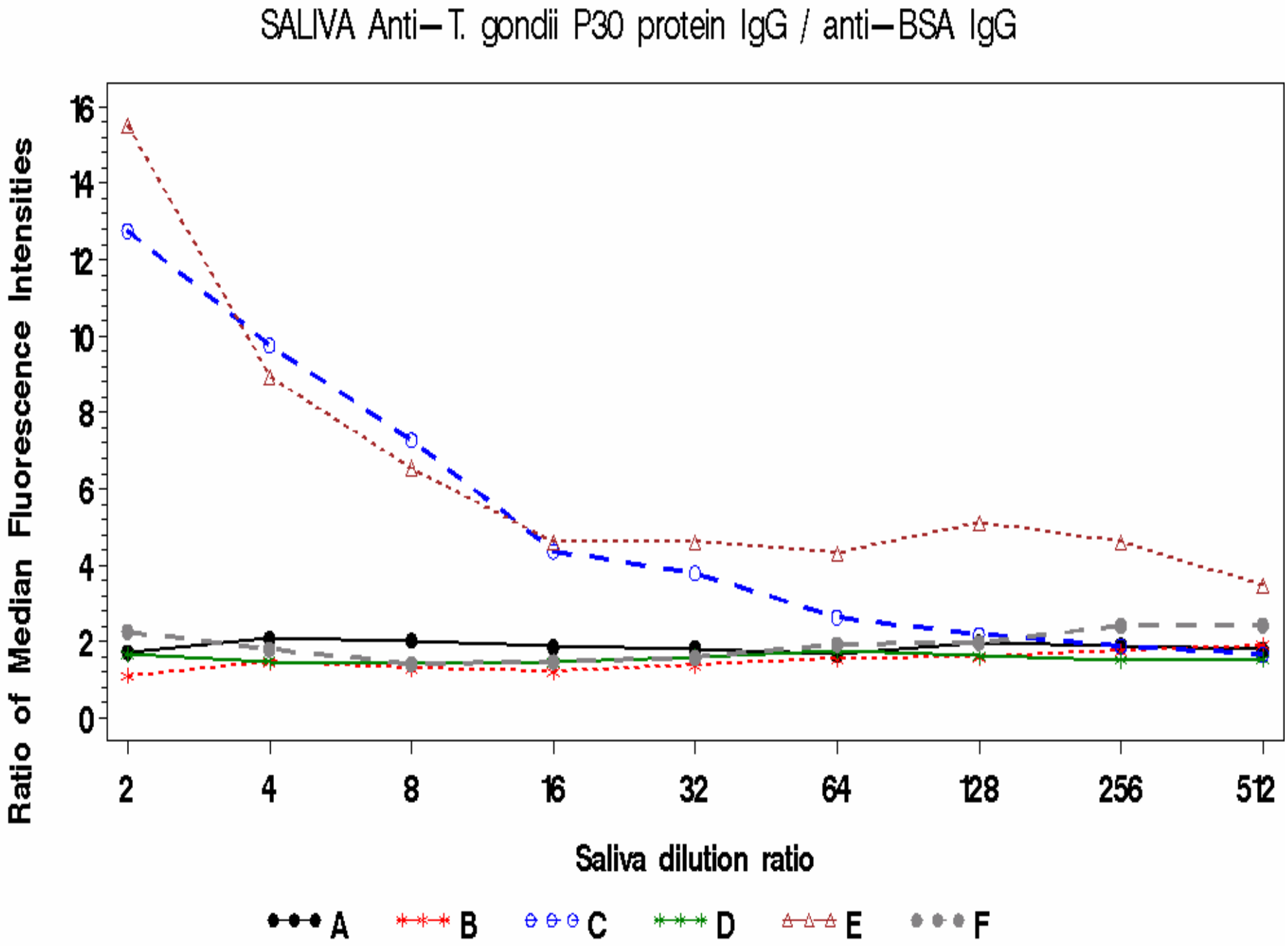


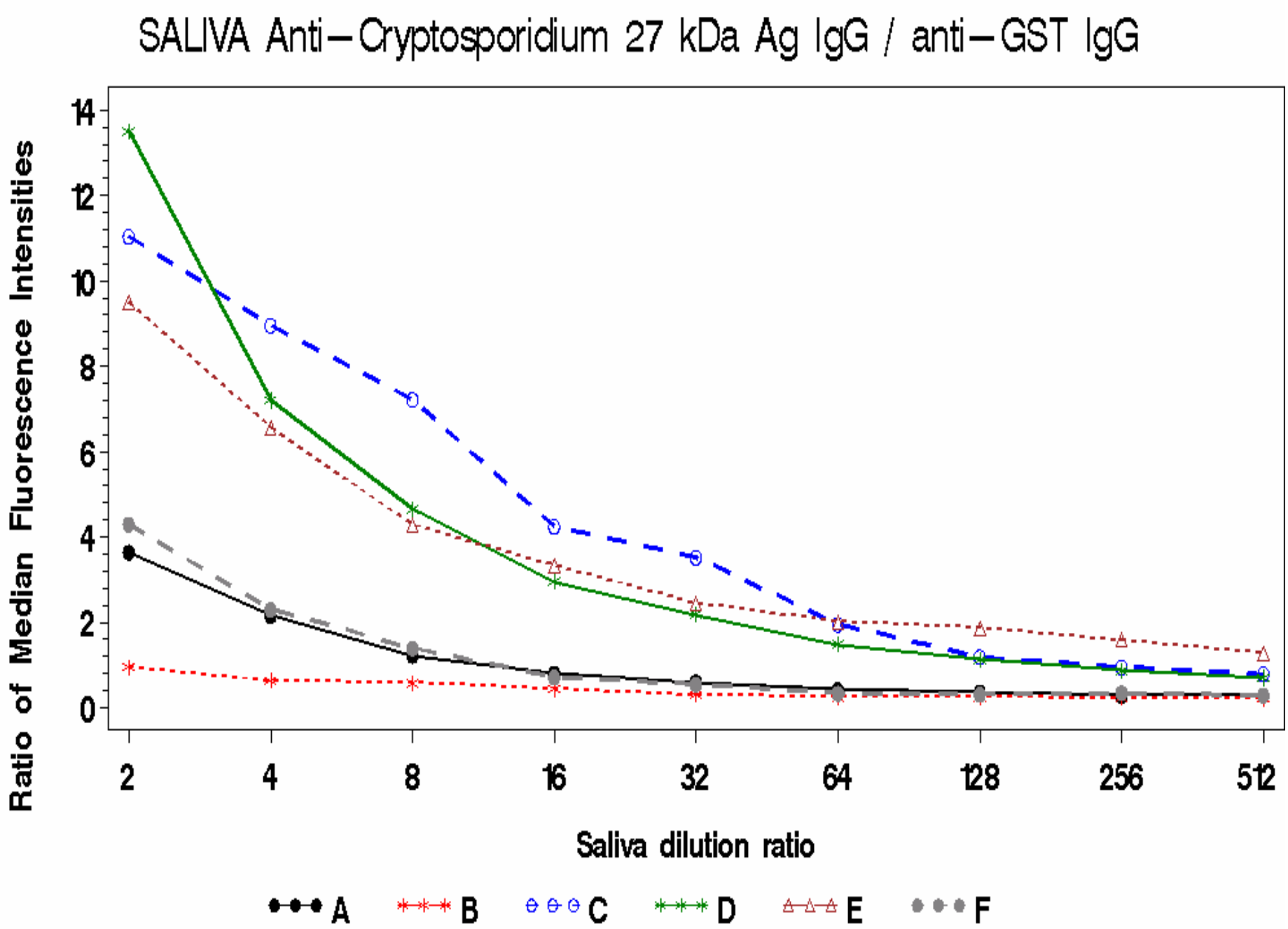
From Maricel Seeger, Buenos Aires / EFE

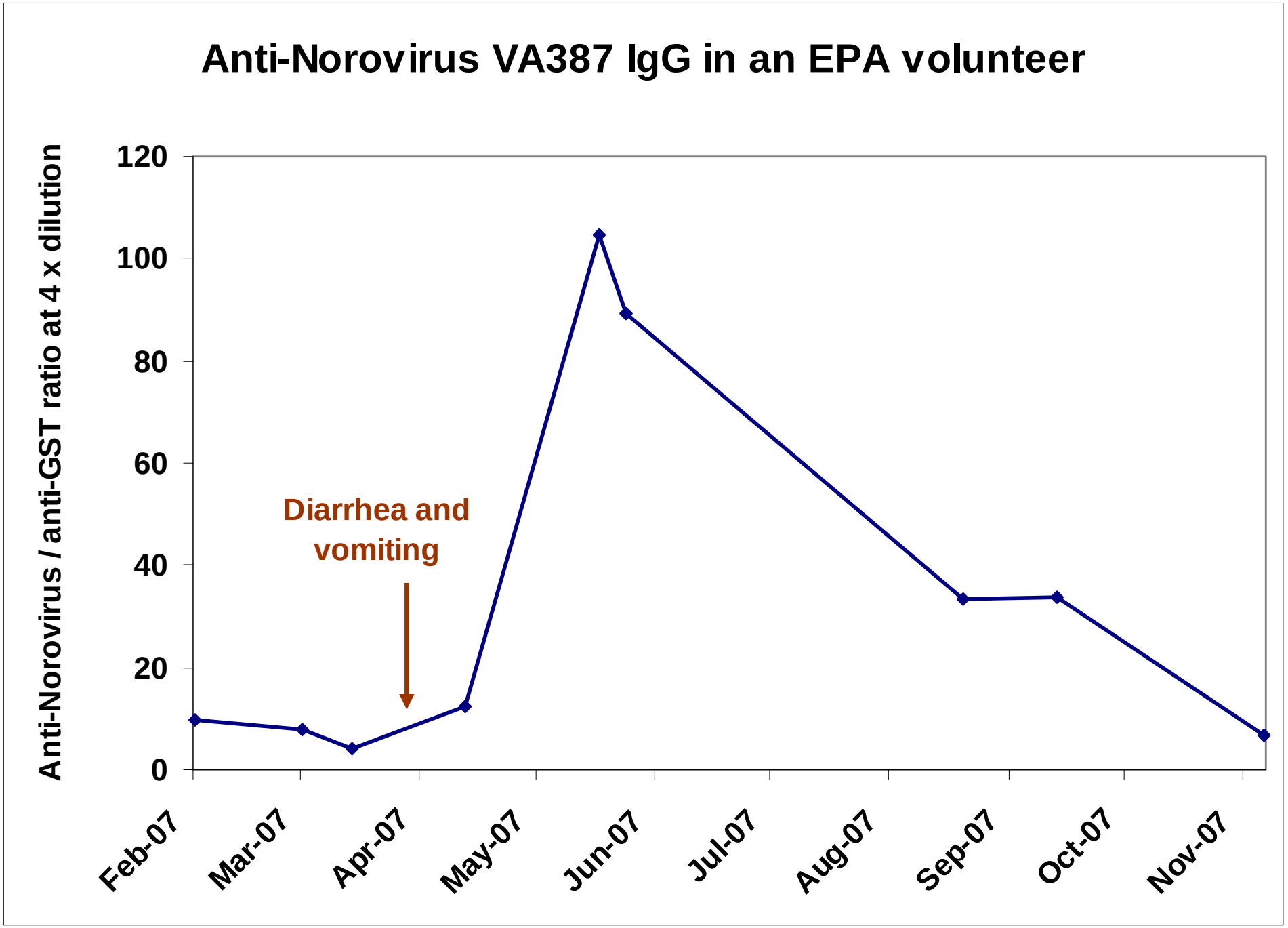
Selected results of assay development and validation


SALIVA anti–H. pylori lysate IgG
Median Fluorescence Intensity
30000
20000
10000
0
2
4
8
16
32
64
128
256
512
Saliva dilution ratio
A
B
C
D
E
F


H. pylori lysate: Serum ELISA vs. saliva Luminex, IgG
Saliva Luminex, 4x dilution (MFI)
35000
30000
25000
20000
15000
10000
5000
0
Sero-
negative
Seropositive
0
0.5
1
1.5
2
Serum ELISA, 50x dilution (OD)

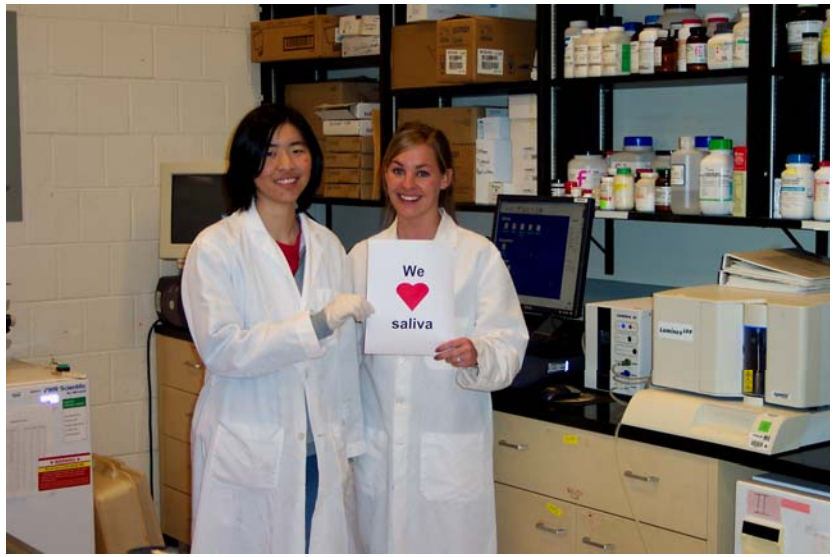

SALIVA Anti–T. gondii P30 protein IgG / anti–BSA IgG
Ratio of Median Fluorescence Intensities
0
2
4
6
8
10
12
14
16
2
4
8
16
32
64
128
256
512
Saliva dilution ratio
A
B
C
D
E
F


SALIVA Anti−Cryptosporidium 27 kDa Ag IgG / anti−GST IgG
Ratio of Median Fluorescence Intensities
0
2
4
6
8
10
12
14
2
4
8
16
32
64
128
256
512
Saliva dilution ratio
A
B
C
D
E
F


Anti-Norovirus VA387 IgG in an EPA volunteer
Anti-Norovirus / anti-GST ratio at 4 x dilution
120
100
80
60
40
20
0
Diarrhea and vomiting
Feb-07
Mar-07
Apr-07
May-07
Jun-07
Jul-07
Aug-07
Sep-07
Oct-07
Nov-07

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