

Request for Approval under the “Generic Clearance for Citizen Science and Crowdsourcing Projects” (OMB Control Number: 2080-0083)

TITLE OF INFORMATION COLLECTION: Using Citizen Science to Improve Drinking Water Epidemiology Studies

PURPOSE: This project showcases the simplicity of an innovative saliva test and improves the way epidemiology studies are designed using citizen science. Families with school age children will report incidences of gastrointestinal disease to school nurses and/or science teachers to facilitate follow-up stool and saliva tests by EPA in impacted school districts. Drinking water, stool and saliva samples will be collected by researchers and analyzed by Interamerican University, EPA Office of Research and Development (ORD) and Region 2 scientists. The project will allow citizens to investigate the incidence and type of gastrointestinal illness in rural communities in Puerto Rico without municipal (PRASA) drinking water treatment plants. This will better characterize and inform public health concerns related to drinking water treatment processes.

NEED AND AUTHORITY FOR COLLECTION: InterAmerican University received an Institutional Review Board (IRB) for Protection of Human Subjects in Research on January 26, 2018. On April 5, 2018 this project received approval from EPA’s Human Subjects Research Review Office for use of Human Subjects Research according to the requirements of EPA Order 1000.17 Change A1 (Policy and Procedures on Protection of Human Research Subjects) and can confirm that this study complies with EPA Regulation 40 CFR 26 (Protection of Human Subjects).

USES OF RESULTING DATA: Results from all aspects of the epidemiology study will be compiled and interpreted for publication in peer-reviewed journal articles. The resulting data may provide a direct link between community health and drinking water treatment using citizen science in underserved communities in rural Puerto Rico.

DATA COLLECTION METHODS: School nurses, science teachers, and/or researchers from EPA Region 2 and InterAmerican University will recruit and enroll local families with at least one child in the 4th to 6th grade. An adult family member or guardian will complete one baseline survey to gather limited demographic data and information about risks related to waterborne illness (*i.e.* water usage and sanitation). At approximately one-month intervals, for a total of 3 months, an adult family member or guardian will complete a health survey on symptoms experienced by the child participant. Completed surveys will be returned to school nurses and science teachers by the child participant or family member. Study staff from InterAmerican University will collect and compile surveys from schools. EPA and InterAmerican University will analyze stool, saliva and drinking water samples. Compiled survey information and analytical results will be interpreted to prepare a summary report that includes all aspects of the epidemiology study.

PARTICIPANT UNIVERSE:

Category of Respondent	No. of Respondents	Number of responses per respondent	Participation Time per response	Burden Hours
Family members	500	4 (1 baseline + 3 health surveys)	15 minutes	500 hours
Totals	500			500 hours

AGENCY COST: The estimated cost to the Federal government is \$70,000 for project design and implementation. \$30,000 of sampling supplies will be purchased by EPA (\$15,000/year). Four EPA employees are collaborating on this project. It is estimated that each spends about 20% of their time (0.20 FTEs each) on this citizen science project.

STATISTICAL ANALYSIS:

The objective of statistical analysis is to assess and compare associations between water quality and infections with specific potentially waterborne pathogens. Anticipated survey results will satisfy the survey objectives.

Two approaches to statistical analysis of assay data will be used. For acute infections which have a relatively short incubation period and produce short-term immunity, such as noroviruses, *Campylobacter spp.* and *Cryptosporidium spp.*, immunoconversion will be used as an indicator of incident infection as described previously (Griffin et al., 2015). Immunoconversion will be defined as at least four-fold increase in salivary antibody response between consecutive samples. Additional criteria may be used to improve the specificity of the immunoconversion tests, such as age-specific cut-off values derived from regressing antibody data on age using penalized splines as described previously (Egorov et al., 2010), and at least three-fold increase in third sample (S3, collected 2 months after baseline) compared to baseline (S1) sample (Wade et al., manuscript in review). For chronic infections, *H. pylori* and *T. gondii*, analysis will focus on identifying chronically infected individuals. DNA-based molecular methods will also be conducted to determine the bacterial diversity and the presence of waterborne pathogens in the stool samples.

Analysis of associations between water quality and acute infections will be conducted using logistic regression models. Analysis will be repeated for asymptomatic infections (immunoconversion, no symptoms) and symptomatic infections (immunoconversion with symptoms).

For chronic infections, demographic, socioeconomic and behavioral risk factors for infections will be explored. In addition, potential impacts of chronic infections on antibody responses to incident acute infections will be explored.

DATA QUALITY ASSESSMENT PROCEDURES:

All samples from the same individual will be assayed at the same time to minimize assay variability. Samples from at least 20% of study participants will be assayed in duplicate. Controls (human samples positive to pathogens included in this study) as well as negative controls (blanks) will be assayed on each 96 well microplate. All analytical errors, such as insufficient number of Luminex beads (less than 50 beads of each type) acquired by the Luminex device, will be documented. All samples associated with errors as well as all other samples from the same individuals will be re-analyzed on a new plate. Plates with unusually high antibody responses to controls (GST or total IgG) will be identified using analysis of distributions of plate-specific responses at the end of the study. Plates with antibody responses to internal control antigens above the mean plus two standard deviations (outliers) will be re-analyzed.

DNA extractions will be conducted using established protocol and following manufacturer's instructions. Sequencing of the 16S rRNA will be used following the procedures outlined in Caporaso et al (2011) and as modified by Kapoor et al (2016). qPCR assays will follow the steps described in Kapoor et al (2015). Standards (dsDNA) will be used for quantifying gene copies and no template controls will be used to determine the presence of cross-contamination.

ADMINISTRATION OF THE INSTRUMENT: (Check all that apply)

Web-based or Social Media

In-person

Telephone

Mail

[] Other, Explain

INSTRUMENT: Instrument script is attached below. Final online product will include mandatory OMB control number, expiration date, and burden statement.

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REFERENCES:

- Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci USA* 108:4516-4522
- Egorov, A.I., T.L.M. Montouri, L. Ascolillo, H.D. Ward, D.A. Levy, R.D. Morris, E.N. Naumova, J.K. Griffiths. 2010. Recent diarrhea is associated with elevated salivary IgG responses to *Cryptosporidium* in residents of an eastern Massachusetts community. *Infect.*, 38(2): 117-23.
- Griffin, S.M., R.R. Converse, J.S. Leon, T.J. Wade, X. Jiang, C.L. Moe, A.I. Egorov. 2015. Application of salivary antibody immunoassays for the detection of incident infections with Norwalk virus in a group of volunteers. *J. Immunol. Methods*, 424: 53-63.
- Kapoor, V., Elk M., Li X., Impellitteri C.A., Santo Domingo J.W. 2016. Effects of Cr (III) and Cr (VI) on nitrification inhibition as determined by SOUR, function-specific gene expression and 16S rRNA sequence analysis of wastewater nitrifying enrichments. *Chemosphere* 147:361-367.
- Kapoor, V., Ryu H, Pitkanen T, Wendell D, and Santo Domingo JW. 2015. Distribution of human-specific Bacteroidales and fecal indicators in an urban watershed impacted by sewage pollution using RNA and DNA based quantitative PCR assays. *Applied and Environmental Microbiology*. 81:91-99.
- Wade, T.J., S.A.J. Augustine, S.M. Griffin, E.A. Sams, K.H. Oshima, A.I. Egorov, A.P. Dufour. 2017. Asymptomatic norovirus infection associated with swimming at a tropical beach. *PLOS ONE*, submitted.

ID# _____
(home / survey number)

Self-Report Health Questionnaire & Risk Factor Survey

Complete one per student

Name of Interviewer: _____

Date of Interview: _____

Place of Interview: _____

Name of Interviewee: _____

Home Address: _____

Interviewee's information

1. Age: _____
2. Sex: 1- Male 2- Female



Household information

3. How many people live in the house? _____

Please complete the following table for each person that lives at this address:

Name	Relation to the Head of the Household	Age	Sex	Occupation/Student

ID# _____
(home / survey number)

1. What is the occupation of the head of the household?
2. What is the highest educational degree achieved?

No School Elementary or Less Middle School
 High School University Post Graduate

3. How long has your family lived at this location?

a. If less than 10 years, where did you move from? (address)

4. How many bedrooms are there in the house?

5. Do you own the house?

Yes No, the home is rented
 Living with friends Living with family
 Other (explain) _____

Water usage

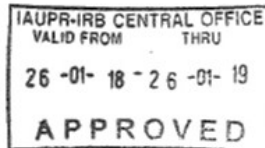
6. How often do you drink from the tap without boiling the water?

Always Most of the time
 Sometimes Never

7. Do you treat the water before drinking it? Yes No

a. If yes, please explain how.

with filter by boiling
 with chlorine other method



ID# _____
(home / survey number)

8. Do you have any problems with your drinking water? Yes No

If yes, which of the following happened?

- bad odor bad taste
 cloudy color
 other (explain) _____

9. Do you drink water at school? Yes No

If yes, which?

- drinking fountain bottled water
 tap water other

10. Do you drink water from other sources? Yes No

If yes, which?

- bottled water river water
 rain water other

Sanitation

11. Does your home have access to any of the following?

- toilet discharging into a sewage system toilet discharging into a septic tank
 latrine toilet discharging into a river or land
 other (explain) _____

12. If you have septic tank, how often is emptied?

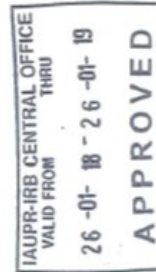
- never once a year or less
 more than once a year

13. Are there problems with your septic tank? Yes No

- leaks bad odor
 other (explain) _____

14. Do you own any of these pets or farm animals?

- dogs cats pigs
 chickens ducks cows



ID# _____ (home / survey number)

Daily Report Card Study ID Number _____

Participant: _____

First Name _____

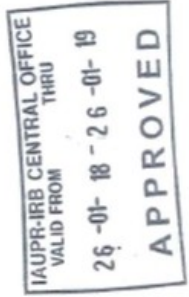
Last Name _____

Date: / /

Please note any symptoms experienced during trips you made for each day of the week.

Day	Had any symptoms?										Were you traveling?			
	Diarrhea			Diarrhea w/ blood		Vomiting (feeling sick)	Stomach ache/pain or cramping	Nausea (feeling sick)	Headache	Fever or chills	Cough, nasal congestion, sore throat, or infection	No symptoms	Abroad (outside the US)	Did not travel
Monday	1	2	3+	1	2	3+	1	2	3+					
Tuesday														
Wednesday														
Thursday														
Friday														
Saturday														
Sunday														

If you experience diarrhea or vomiting this week, please complete questions 1 through 12 of the Extended Daily Record



ID# _____
(home / survey number)

Consent Form

If you want to be part of the health study, please print your name and **school name** and then sign and date in the boxes shown below. Parents or guardians must sign on behalf of children under the age of twelve (12). If you are under 21 years of age, please sign the document and have your parent or guardian sign the document as a witness to your signature. If you (or a family member or friend) would like more information about this study, please do not hesitate to contact Graciela Ramirez Toro at 787-264-1912 ext. 7630, 7631.

I confirm that I read the information sheet on this study and I have the opportunity to ask questions. I agree to take part in the health study.

Name of Student Participant	Signature	Date	School Name
	X _____		
Name of Student Participant's Parent (If student is less than 21 years old)	Signature	Date	
	X _____		

