capsid

the official newsletter of NoroCORE

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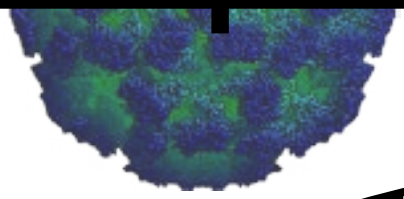

the goal

"...working together to reduce viral food borne illness"

NoroCORE, the USDA-NIFA Food Virology Collaborative, is a food safety initiative that focuses on outreach, research, and education in the field of food virology.

NoroCORE's ultimate goal is to reduce the burden of food borne disease associated with viruses, particularly noroviruses.

contents

Update on Efforts in Prevention and Control

Dr Lee-Ann Jaykus, Scientific Director

The Prevention and Control Core is one of the four NoroCORE research functions. Its purpose is to improve our understanding of the occurrence and behavior of enteric viruses in the food safety continuum so as to inform development of scientifically justifiable control measures. Over the last four years, the NoroCORE scientific team has made some important contributions to prevention and control, and I would like to highlight some of those here.

Several groups are engaged in studies to look for evidence of human norovirus (NoV) environmental contamination in public restrooms (NCSU); restaurant restrooms (Clemson, OSU, Rutgers, and CDC); and in association with outbreaks (such as in nursing homes) (Emory and CDC). Others are investigating the prevalence of NoV contamination in the fresh produce production environment (Emory, USDA-ARS). Although final results are forthcoming, some positive samples have been confirmed. It looks like the prevalence of contamination in non-outbreak settings is low, but it is not zero. Studies like this provide confirmation of the lengthy persistence of NoV in the environment, and the need for rigorous cleaning and disinfection.

A second major activity of the Prevention and Control core is to develop and evaluate methods to prevent viral contamination in foods and their production/processing/preparation environments. For the molluscan shellfish and fresh produce sectors, NoroCORE extension and outreach personnel have developed fact sheets and worked to incorporate relevant enteric virus information into upcoming mandated national training curricula. In response to increasing concern about the role of vomitus in NoV transmission (as an aside, the NCSU team recently provided experimental evidence supporting virus aerosolization during a simulated vomiting event), Clemson collaborators are producing "editable" vomit clean-up guidelines. These should be especially useful for small restaurants having limited food safety resources.

Falling in the middle of prevention and control are compounds that can be used to inactivate enteric viruses if they are present on hands, surfaces, and/or foods. A number of products assume that anti-bacterial efficacy equates to anti-viral efficacy, but we know that this is not the case. Although human NoV cannot be cultivated in vitro, there are ways in which we can infer virus inactivation using non-culture based laboratory techniques or cultivable virus surrogates. These methods have been used, for instance, to evaluate the efficacy of a variety of produce washes; commonly used surface disinfectants (e.g., chlorine, ethanol, quaternary ammonium compounds); and hand sanitizers. While the bad news is that most of these compounds have limited efficacy against human NoV, at least at manufacturer recommended concentrations, some newer ones are more effective.

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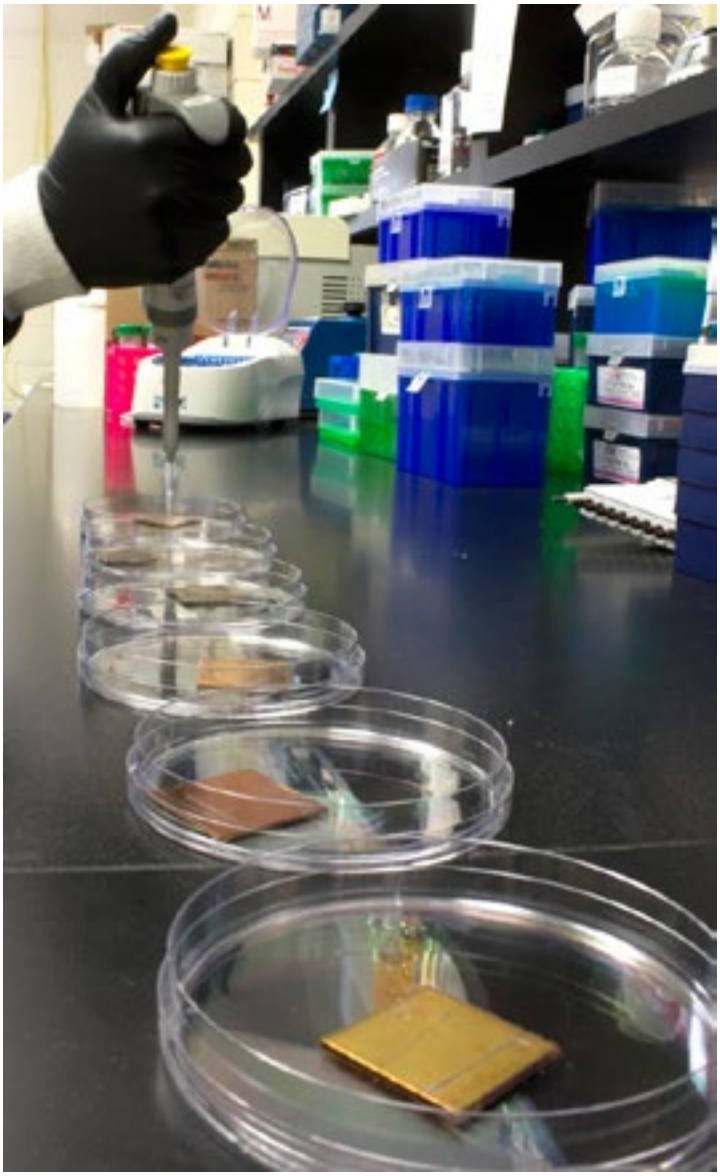
Destruction of human noroviruses on copper alloy surfaces

Publication: Manuel, CS, Moore, MD, Jaykus, LA. 2015. Destruction of the Capsid and Genome of GII.4 Human Norovirus Occurs During Exposure to Metal Alloys Containing Copper. Appl. Environ. Microbiol. [Epub ahead of print].

We are proud of all of our NoroCORE students, and here we highlight a recent study performed by Clyde Manuel and Matthew Moore in the Jaykus lab at NCSU, on the effects of copper alloys on the human norovirus. This work was also just featured by the American Society for Microbiology, and has received significant media attention around the world.



Application of samples to alloys with different copper contents.

Objective & Rationale:

Copper's unique antimicrobial properties have been known for centuries. (Its first use as a sterilizing agent was recorded over 4,000 years ago!) In the past few years, there has been renewed interest and research into copper's ability to degrade pathogens. You may have heard about the prospect of using copper doorknobs in hospitals to prevent infections. Clyde and Matthew's work marks the first time copper's impacts on the human norovirus have been studied.

Methods:

- They used small sheets of five different copper alloys that had 61 to 100% copper content, as well as stainless steel as a control, which is also the classic metal of choice in medical facilities.
- Samples of human stool containing a GII.4 norovirus (the type most often responsible for norovirus outbreaks worldwide) or GII.4 virus-like particles (VLPs) were deposited on the different metals. (VLPs are structurally identical to the virus capsid, which is the outer protein coating that protects the viral genome, but they lack the genome inside. VLPs are particularly useful for norovirus research since we cannot yet culture the actual virus.)
- The researchers then looked at virus survival at set intervals (0, 15, 30, 60, 120, and 240 minutes).
- They used RT-qPCR to assess the integrity of the viral genome, and several methods to assess capsid integrity (specifically RT-qPCR, transmission electron microscopy, Western blot, and a histo-blood group antigen (HBGA) binding assay).

Results:

Based on their findings, it appears that both the viral genome and its capsid are being damaged in the face of copper, also rendering the virus unable to bind to our HBGA receptors. When the viral particles in the stool samples were exposed to pure copper for an hour, there was approximately a 3-4 log10 reduction in the number of viral genomes detected. (To put it into perspective, that may not be as effective as a strong bleach solution, but there are disinfectants out there that are thought to be similarly effective, or even less effective.) Increasing the copper content led to greater destruction of the viral particles, while virtually no change was seen on the stainless steel after four hours.

One really neat finding is that when viewed on transmission electron microscopy, the VLPs went from being evenly scattered across the viewing area at the beginning, to being clumped together after about an hour, ultimately becoming an indiscernible field of debris at four hours, while nothing changed on the stainless steel (See figure).

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New NoroCORE Graduate Fellows!

We have a new batch of bright scientists who recently received our Graduate Fellowships, which include a stipend and funding towards education. Here they are!

Benjamin Anderson is currently pursuing a Ph.D. in Food Science and Technology at the University of Georgia under the direction of Dr. Jennifer Cannon. Ben obtained his undergraduate degree in human development and gerontology at UGA, as well as an MPH focused on environmental toxicology at the UGA School of Public Health. Prior to beginning his PhD, Ben worked for over 3 years at the Gwinnett County Health Department in Georgia. His current research objectives seek to investigate the efficacy of natural antimicrobial compounds against norovirus and hepatitis A virus. In addition, he is involved in a collaborative project among UGA, the CDC, and the Georgia Department of Public Health to assess the extent of environmental contamination that occurs during norovirus outbreaks at long-term care facilities. Ben has been described as "tremendously dedicated, intelligent and passionate about his work" by his advisor.

Joyce Sweeney is a Ph.D. student at Georgia State University working under the advisement of Dr. Suri Iyer. She obtained her undergraduate degree in Mathematics at Vanderbilt University, upon which she was gainfully employed in the financial sector. Realizing her true passions were in science, she quit her job to pursue a career in research. Her current interests include the detection and culture of noroviruses. The overall objective of her project is to utilize HBGA glycans for norovirus detection and growth. This work will lead to the development of microarrays using characterized glycopolymers for detection and differentiation of norovirus strains. Joyce has been described as a very hard worker whose talents will be very useful to move the field of norovirus research forward.

Zachary Marsh is pursuing a Master's of Public Health in Epidemiology at Emory University under the advisement of Dr. Juan Leon. Prior to coming to Emory, he received a Bachelor of Science in Biology at Arkansas State University. His research involves the development of a quantitative microbial risk assessment (QMRA) model to evaluate the risk of human norovirus and hepatitis A virus contamination of produce. The model will assess risk factors at three stages – production, processing, and shipping – combined with dose-response information for norovirus and hepatitis A virus, to provide a full picture of the potential impact of produce contamination due to foodborne viruses. Zachary was described by his advisors as the ideal "student that NoroCORE looks to further develop," as well as a "passionate scientist and public health practitioner who strives to make a meaningful difference."

Natalie Seymour is a Master's student at North Carolina State University working under the direction of Dr. Ben Chapman. She received her undergraduate degree at NCSU in Food Science. Her overall interests include the prevention and control of foodborne illnesses at the retail and foodservice level. Her research involves the collection and analysis of third-party audit data to evaluate and understand food safety non-compliances in grocery store food handling. In turn, she is helping to create behavior-based interventions that are targeted at food handlers. Natalie's advisor states that she "shows great potential for an influential career as a food safety professional," and has demonstrated a strong work ethic and a high standard of excellence in her work.

A big congratulations to you all, and we look forward to seeing you continue to grow in your careers and as individuals!

Collaborator

Dr. Angela Fraser
Associate Professor, Food Safety Specialist
Department of Food, Nutrition, and Packaging Sciences
Clemson University

"It has been great to work with so many experts in the field, and working with them has truly helped me to hone my skills as a researcher. These connections and collaborations have made all of the hard work we've been doing extremely worthwhile."

Dr. Fraser grew up in Michigan, and received her B.S. in Dietetics in 1984 and an M.S. in Institutional Administration in 1987 from Michigan State University. Shortly after completing her M.S., she worked as an Environmental Health Specialist (EHS) conducting regulatory inspections and food safety training at facilities, as well as assisting in disease outbreak investigations. It was during this time she realized that many food safety trainings were not effective at changing behaviors, which led her to want to learn how to develop better educational interventions. In 1995, she completed a Ph.D. in Food Science at Michigan State University, with an emphasis on food safety education. During her Ph.D. program she also worked as a Food Safety Extension Specialist for MSU Extension. Dr. Fraser was on the faculty at NCSU as an Associate Professor and Extension Specialist until she joined the Department of Food Science and Nutrition at Clemson in 2008.

In her 6 years as an EHS, Dr. Fraser conducted over 1,000 regulatory inspections in a wide array of retail/foodservice facilities. While she felt that many of the workers at these facilities had good intentions, the complex regulatory jargon they were being given to follow resulted in some important food safety messages being lost.

Dr. Fraser *"wanted to learn how to train and educate those in the industry given how economically significant this industry is"* and that has been the guiding purpose of her career. Most of her work centers on developing educational interventions targeting institutional and commercial food service operations. At present, Dr. Fraser has a staff of two project coordinators, five graduate assistants, and an undergraduate research assistant with whom she works very closely. She is a very hands-on faculty mentor, which she sees as essential to the growth of her students. As she is a "paper" scientist and not a laboratory scientist, she spends a significant amount of her time writing and analyzing data sets. In addition, she teaches four courses (three undergraduate courses and one graduate course). She loves teaching, particularly when students "get" a concept: *"I love it when the light comes on, and being able to effectively reach people from all backgrounds—that is why I am here."*

When asked why she wanted to become part of the NoroCORE Collaborative, Dr. Fraser explained that she had a lot of respect for Dr. Lee-Ann Jaykus, whom she met while at NCSU. She was also excited to be part of such an accomplished group of researchers, and has felt that getting to know so many people has opened doors for collaboration and sharing expertise. Dr. Fraser and her Clemson colleague Dr. Xiuping Jiang, have been involved in such a collaboration with Procter & Gamble, which is highlighted on page 6.

Angie's charge to young scientists is that it is important to collaborate, and not just with other academics, but also with industry and government professionals. This is the best way to holistically and comprehensively understand a problem so that they can solve it in a manner that reaches beyond the laboratory. She says it is also imperative to always be open to working with individuals who have diverse backgrounds and expertise as this can lead to more creative solutions.

Dr. Charles Pettigrew
Principal Scientist
Procter & Gamble

Dr. Jeff Anderson
Public Health & Sanitation Scientist
Procter & Gamble Professsional

Dr. Jen Shields
R&D Microbiologist
Procter & Gamble

"We have high hopes that this collaboration will enable us to deliver products and resolutions that deliver meaningful disease risk reductions to our consumers." - *Dr. Charles Pettigrew*

Dr. Charles Pettigrew is a South Carolina native who attended Erskine College as an undergraduate. He then completed a Master's in Microbiology at Clemson University, followed by a Ph.D. in Microbiology from the University of Tennessee. His lasting focus has been the study of microbial ecology and ecosystems, and as he says, *"My goal is to use microbial science and the data we generate to help people."*

Dr. Jeff Anderson received his Ph.D. in Analytical Chemistry from the University of Arizona, and then began working for Procter & Gamble (P&G). During his 16 year tenure at Procter & Gamble, Jeff has worked on the development and manufacturing of sanitation and cleaning products (such as Tide®, Dawn®, Cascade®, Mr. Clean®, Spic and Span®, among others) and managed production laboratories in the United States, Europe, Middle East and Africa. For the last 9 years he has been in P&G Professional, the away-from-home division, that serves healthcare, restaurants, and other facilities with professional-level cleaning products and services. He leads their Applied Science group, which deals with developing sanitation and personal hygiene programs to help mitigate public health risk factors. According to Dr. Anderson, *"We're not just interested in developing high performing products, but also we're developing and delivering tools, services, and knowledge that deliver meaningful benefits to professional sanitation and hygiene programs."*

Dr. Jen Shields rreceived her Bachelor's degree in Environmental Science from the University of Chapel Hill (UNC) in 2005. She then worked for the Environmental Protection Agency (EPA) for a year in Pensacola, FL studying bacteriophages in water. She completed a Master's at the University of Georgia studying water quality in estuarine environments, followed by a Ph.D. at UNC focusing on water quality, before switching gears to food

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Collaboration in Action!

Clemson and Procter & Gamble: training new scientists and helping in the community

Every collaboration is unique, and it is interesting to see how they evolve. Here, our interviewees from the Noro Link explain their experiences of working together to further sanitation research, education, and motivation, as well as help out in the community.

How did this collaboration develop?

Dr. Anderson: Chuck and I have been collaborating on various public health related projects for several years because of our interest in chemistry and microbiology relating to food contact surfaces and other surfaces in away-from-home environments. Our customers are the people running restaurants, healthcare facilities, etc., and our down-the-road visions are to deliver products to the world's consumers to help them control noroviruses and other pathogens in their homes as well as away from their homes.

Dr. Pettigrew: Before NoroCORE existed, Jeff and I had reached out to Dr. Jan Vinjé at the CDC to conduct norovirus research on some of the products that we sell. It is through that collaboration that we were connected with NoroCORE.

Dr. Fraser: Dr. Pettigrew contacted me when NoroCORE was just getting started. He wanted to know what it was and how P&G could become involved. Our groups started having monthly calls, and our collaboration evolved from there.

What is the project?

Dr. Anderson: Over the last 2 years P&G and Clemson have been conducting sanitation studies in a regulated kitchen using various surface measurements to better understand cleaning performance factors, as well as assessing the effectiveness of using various behavioral science strategies to improve the efficacy of kitchen cleaning programs.

Dr. Shields: This year we are focusing the majority of our research on using an education and motivation program that is called the "Clean Zone." It's a fun, interactive program that is intended to improve the sanitation results.

How have NoroCORE students been involved with the project?

Dr. Pettigrew: There have been two so far from Clemson: Thomas Yeargin and David Buckley, who have both done work targeting soft surfaces for their NoroCore research projects. Thomas was a student we had last summer, studying sanitation performance factors such as the cleaning chemical, surface design, mechanical energy (scrubbing), and the sanitation employee. David has been studying the behavioral science of sanitation and performance factors, such as the impacts of a combined education, motivation, and monitoring program. We have also hosted many other students from the labs of Angela and Xiuping, and they have received P&G funded training at the CDC in conjunction with NoroCORE. We have been training Angela's and Xiuping's students in a way that lets them go directly from a scientific laboratory to an actual, functioning kitchen.

Dr. Anderson: For those students, they are in a real, regulated kitchen, in addition to working in a traditional lab setting. It's a powerful experience for them, and mixes multiple sciences (chemistry, physics, behavior, and biology).

Dr. Fraser: We have been thrilled that our students can go there and learn in a real-world environment, and the students also develop contacts with people in industry. We as a group have also been able to see how industry develops products and gets them to market.

What have been some of the other outcomes of the work?

Dr. Pettigrew: The reaffirmation that education, training, and motivation are key for food service employees, which dovetails with Angela's work.

Dr. Anderson: In the food service industry, education and motivation are important as a way to get positive change. One such initiative, which we call "Clean Zone," has been found to successfully motivate employees in restaurants. Also, the design of everything in a facility, such as the floors and drains, and methods of cleaning are important. For example, what is to be done about non-cleanable surfaces in a restaurant?

Why is this work important?

Dr. Anderson: We're doing this for several reasons: to enhance the current product experience for our consumers; help us understand cleaning performance factors; and help us develop new products for consumers. In the end, we are trying to create the best possible customer and consumer experience using several strategies simultaneously.

Dr. Fraser: We are also working on educating the regulators, who are responsible for providing oversight in these settings.

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Prevention and Control Update (...continued)

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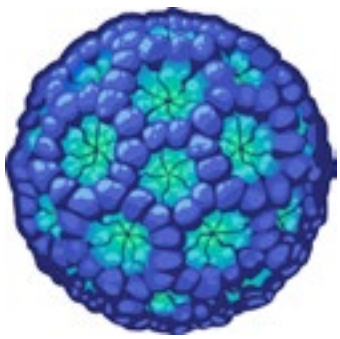
For example, the NCSU team just reported on the anti-NoV efficacy of copper and some of its alloys, which provide up to 3-4 $\log_{10}$ inactivation of human NoV in less than 2 hours. Other emerging technologies (e.g., vaporized disinfectants) show promise in preliminary studies.

Substantial effort has gone into the evaluation of novel technologies to remove and/or inactivate viruses from foods. Many of these studies were leveraged with other USDA-NIFA projects that focused on emerging food processing technologies. For example, high pressure processing, ionizing radiation, pulsed light, and ozone have all been investigated by NoroCORE team members (IFSH, OSU, UGA, UDel). Some technologies perform better than others, and some have more commercial relevance. Those that have been identified to move closer to commercialization are high pressure and pulsed light, and IFSH investigators are working closely with those equipment manufacturers.

So, what does this all mean? Well, first of all, it is important that we not look at any one core function in isolation. For example, over the four years of NoroCORE funding, we have better methods to evaluate inactivation strategies; an improved understanding of virus persistence and resistance; and greater access to advanced reagents and protocols that strengthen the quality of our work. NoroCORE team members have been very successful in engaging industry stakeholders, and relevant questions, which deal with both commercial applicability and protection of public health, are being addressed. What fifteen years ago was a little known food safety problem has now become recognized, characterized, and to a certain degree, contained. While there is more yet to do, we have identified prevention and control strategies that should be both effective and practical. As always, however, an ounce of prevention is worth a pound of cure!



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"The students get experience in the traditional lab setting, but we can get them from the lab to the field in the same day, which is where the magic happens and where the real-world implications are found."

- Dr. Charles Pettigrew

What has been special about this collaboration?

Dr. Fraser: It's nice that it has been multidisciplinary, and everyone on the team has a specific expertise. We've developed a really good working relationship with P&G. They have been so good to our group and supporting our students.

Dr. Anderson: It's been a great collaboration. The Universities and Procter & Gamble are going to get a lot out of it. The students will get a lot out of it, and get experience in the real world, a company lab, and the CDC.

Dr. Shields: This collaboration is unique because of the way it effectively leverages the unique perspectives and skill sets of P&G and Clemson University to aid in preventing the spread of norovirus and other pathogens of concern. With my own background in applied microbiology, I can see how invaluable this project is for bringing students from a lab-based microbiology background together with the chemistry expertise of P&G and the human behavior understanding of both to develop effective strategies for preventing foodborne disease spread in real-world commercial kitchens.

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safety. It was then that she ventured to NC State University for a postdoctoral position in the Jaykus lab.

Now at Procter & Gamble, her focus includes conducting research to understand sanitation risk factors so that meaningful interventions can be developed, along with the sustainable preservation of products. She works with Dr. Anderson and Dr. Pettigrew, looking at sanitation in the away-from-home realm. According to Jen, *"In working with NoroCORE, one of my favorite aspects was the application, a link from research to the world, and see actual impacts. That was also what made me come to P&G."*

announcements

be a part of something cool!

There will be a norovirus going around IAFP, and it's one you actually want to catch! Check our website and social media sites for details.

mark your calendars!

The next Full Collaborative and Stakeholder's Meeting will be held March 2-4, 2016 in Miami, Florida.

The NoroCORE Full Showcase Meeting will be in March of 2017. Date and location TBD.

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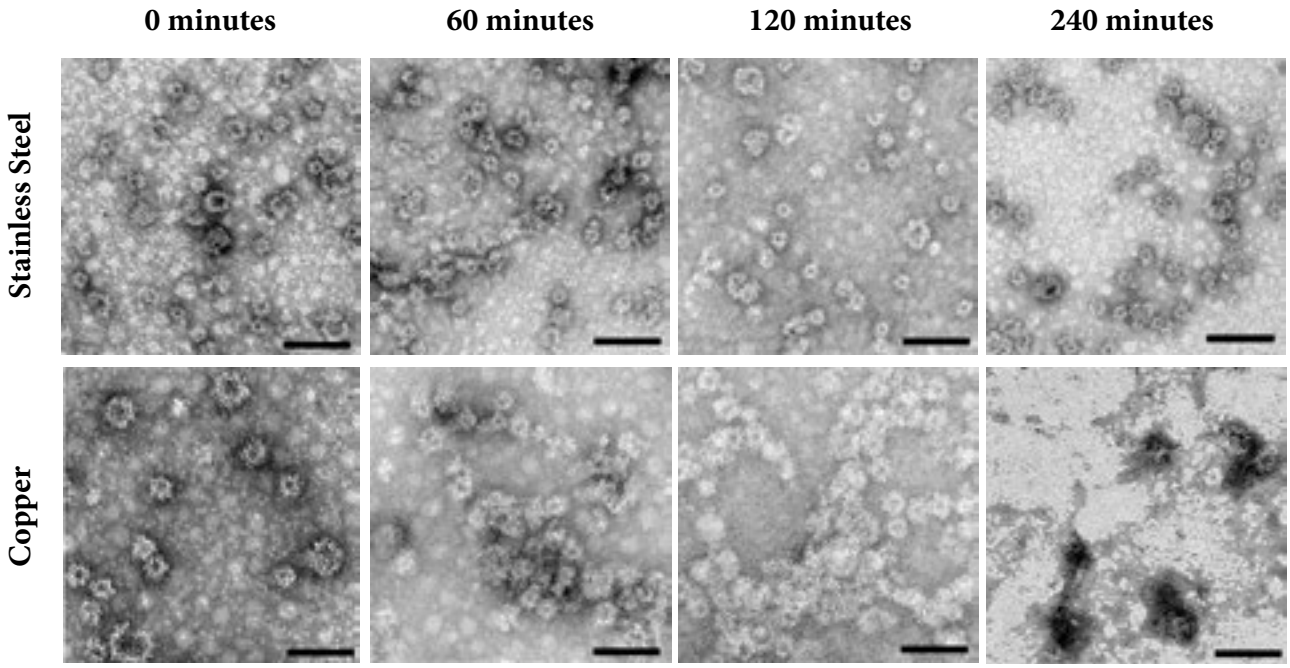

Transmission electron microscopy (TEM) images of norovirus GII.4 VLPs on stainless steel (top row) and copper (bottom row) at 0, 60, 120, and 240 minutes (moving left to right). Scale bar represents 0.1 µm.

Significance:

This research has many potential applications, notably for surfaces we frequently touch, such as doorknobs, handrails, and buttons. In addition to healthcare facilities, copper alloys could be useful in other public and populated areas, such as schools, cruise ships, and eldercare facilities.