

3-D Cultures Help Identify, Screen Noroviruses

Scientists develop successful *in vitro* cell culturing

Results: Pacific Northwest National Laboratory researchers and collaborators have shown for the first time that human noroviruses can infect and replicate in a three-dimensional model of human small intestinal tissue. Previous attempts in two-dimensional cultures have failed.

Why it matters: Recent outbreaks of human noroviruses have plagued cruise ships, restaurants, nursing homes and schools. Noroviruses cause severe gastroenteritis that typically lasts 24 to 48 hours. According to one of the researchers, Charles Gerba, a newly emergent strain of norovirus that appeared in the United States this winter appears to be more serious and deadly compared to previous outbreaks. Because of the lack of suitable tissue culture or animal models, studies to track their environmental sources and their pathogenesis in humans and identify emergent strains have been difficult.



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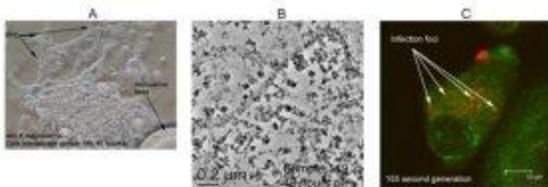


Figure 1. Human norovirus infection of 3-D small intestinal cells. A) Demonstration of host cellular cytopathic effect, B) transmission electron micrograph showing numerous particles that are the correct size for human noroviruses, C) fluorescent in situ hybridization demonstrating the presence of viral RNA. [Enlarged View](#)

Methods: The research team grew the tissue cells on collagen-coated microcarrier beads under physiological fluid shear conditions in bioreactors. They used microscopy, polymerase chain reaction and fluorescent hybridization to find evidence of norovirus infection. Evidence of infection and replication of the two principal viral groups that infect humans was demonstrated for multiple passages of viruses through cell culture (Figure 1). Their results, published in *Emerging Infectious Diseases*, demonstrate that the highly differentiated 3-D cell culture model can support the natural growth of human noroviruses, whereas previous attempts using differentiated monolayer cultures failed. The tools highlighted in the paper are now being used on biosecurity-relevant model systems such as tularemia (rabbit fever).

According to lead author Tim Straub, "Scientists have been trying to get an infectivity assay for the human noroviruses going for about 30 years now, and we are truly the first. Our study shows that selecting the appropriate cell line, growing the cells as 3-D aggregates and infecting them when they are fully differentiated is key for successful *in vitro* cell culturing of human noroviruses."

Next steps: Future research will include further testing of a broader panel of genetically diverse human noroviruses to obtain a better understanding of norovirus. The goal is to develop improved prevention methods.

Source: Straub TM, K Höner zu Bentrup, P Orosz Coghlan, A Dohnalkova, BK Mayer, RA Bartholomew et al. 2007. "[In vitro cell culture infectivity assay for human noroviruses.](#)" *Emerging Infectious Diseases* 13(3):396-403.

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