

NOROVIRUS, CULTURED.

A 48 YEAR MYSTERY SOLVED

Dr. Mary Estes and her Lab at Baylor College of Medicine have successfully cultured human norovirus in intestinal cells.

Scientists have been trying to culture the virus since the first norovirus outbreak was described in 1968.

The lack of an *in vitro* culture system has long been considered the single greatest barrier to norovirus research.

HISTORY OF NOROVIRUS RESEARCH

1929

RUMORED

Dr. John Zahorsky, a pediatrician, gives the name "winter vomiting disease" to a common childhood illness that causes vomiting, diarrhea, and a fever.

1972

VISUALIZED

The Norwalk virus is first seen by Dr. Albert Kapikian and his team at NIH using immune electron microscopy (IEM).

1992

CREATED

Empty shells of norovirus proteins (capsids) are artificially created by the Estes Lab. These virus-like particles are not infectious and enable studies of the capsid.

1968

DESCRIBED

An elementary school in Norwalk, OH experiences an outbreak of "winter vomiting disease." A virus is suspected.

1990

CLONED

The Norwalk virus genome is cloned, paving the way for an era of molecular studies.

2016

CULTURED

Human noroviruses are successfully cultured by Dr. Mary Estes and her team at Baylor College of Medicine.

READ THE ARTICLE

K. Ettayebi *et al.*, *Science* 10.1126/science.aaf5211 (2016).

WHAT IS NOROVIRUS?

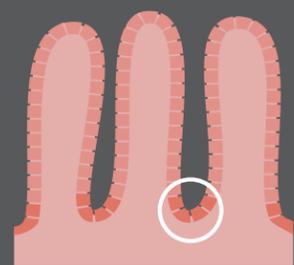
- It is a tiny ($\approx 27\text{nm}$), spherical virus belonging to the *Caliciviridae* family.
- It is the most common cause of diarrhea in the world and the most common cause of foodborne illness in the United States.
- An estimated 1 in 15 Americans experience the virus each year, amounting to around 20 million cases.



HOW DOES THE CULTURE SYSTEM WORK?

Viruses need host cells to replicate. Human noroviruses replicate in the epithelial cells that line our gut. (This was confirmed by the fact that the culture system works.) Intestinal crypts, which contain stem cells, create these epithelial cells in our bodies every day, and are rapidly dividing.

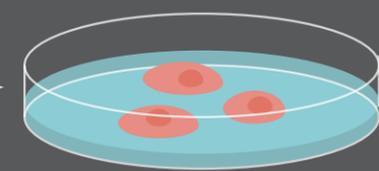
- 1 The researchers followed new technology developed by Drs. Sato and Clevers in the Netherlands and made new epithelial cell cultures from crypts from adult human intestinal tissue (biopsy samples or samples from gastric bypass surgeries). These tissues were medical waste and would have been discarded.



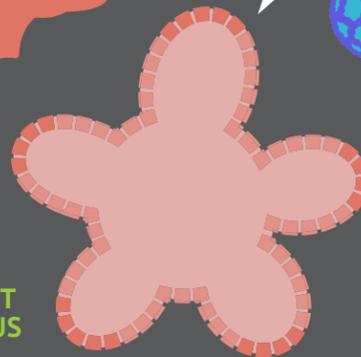
CRYPTS IN THE INTESTINE

- 2 Under the right conditions, the stem cells in the crypts multiply and form the surface (epithelial) layer of our gut, only in miniature and in a dish, to become Human Intestinal Enteroids (HIEs) or "miniguts." They function like the tissue they came from, and can be used indefinitely.

CELLS FROM ISOLATED CRYPTS



GROWN MINIGUT READY FOR VIRUS



Through careful experimentation, the Estes team found a key addition to the media greatly increased virus yields. It was human bile. We produce bile in our livers and secrete it into our gut to help digest food. They found that some norovirus types, like GII.3, need bile to replicate, while it only enhances replication of other noroviruses, like GII.4.

The bile is affecting the cells, not the virus itself, and pig bile works as a substitute for human bile.

- 3 These "miniguts" were inoculated with human norovirus, and after 72 hours, the researchers were seeing far more viral genetic material than they initially added (a 1,000-fold increase), indicating the virus was infecting and multiplying in the cells.



GENERAL VIROLOGY

We can dig deeper into how noroviruses work and what makes them so good at making us sick. This knowledge may shine light on other viruses.

FOOD AND ENVIRONMENTAL VIROLOGY

This is the first step towards directly testing inactivation methods against the human norovirus to know if they are effective. This could lead to better disinfectants, prevention strategies, and safer food and water.

CLINICAL MEDICINE

Being able to work with the actual virus will be a boon to vaccine research, and we will better understand what the virus does in the body, potentially leading to more targeted treatments.

EPIDEMIOLOGY

We can better understand how the virus evolves, spreads, and how it affects populations. This can lead to better risk management strategies that protect public health around the world.