

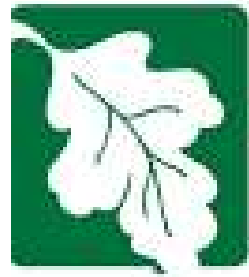
Viruses Who knew ?

Project Overview

***Determining the effectiveness of a
standard stone trench in removing
viruses***

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Barnstable County Department of Health and Environment
Massachusetts Alternative Septic System Test Center

Where did the study come from?



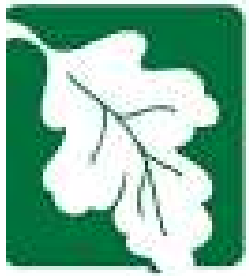
MassDEP

Commonwealth of Massachusetts

Department of Environmental Protection

Title 5 Working Group

Determined need to validate the present set back requirements between bottom of soil absorption system and groundwater



MassDEP

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Department of Environmental Protection

Study design

Determined with working group in consultations with DEP staff including Dr. Oscar Pancorbo Division and Station Director, Wall Experiment Station

First

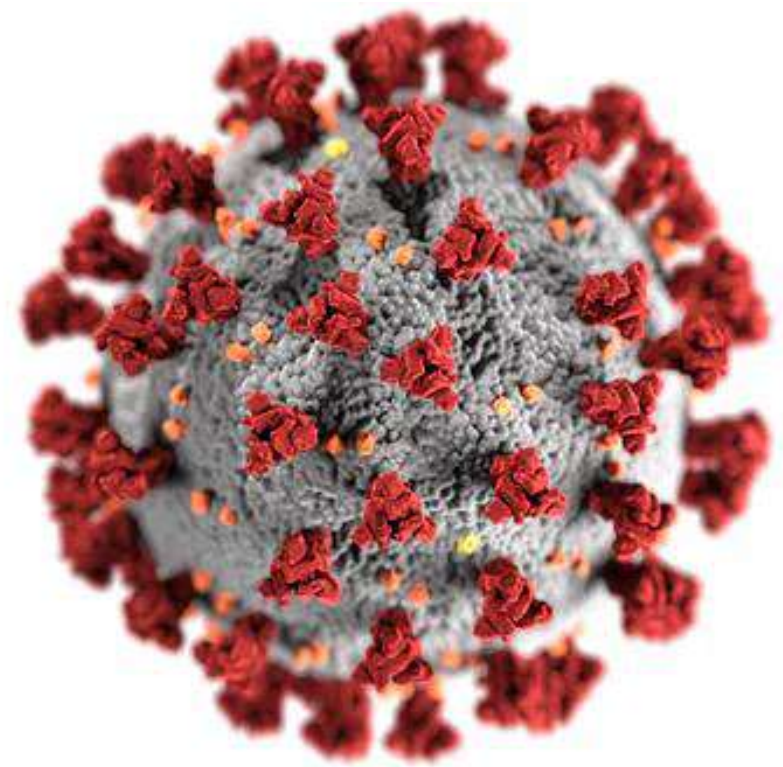
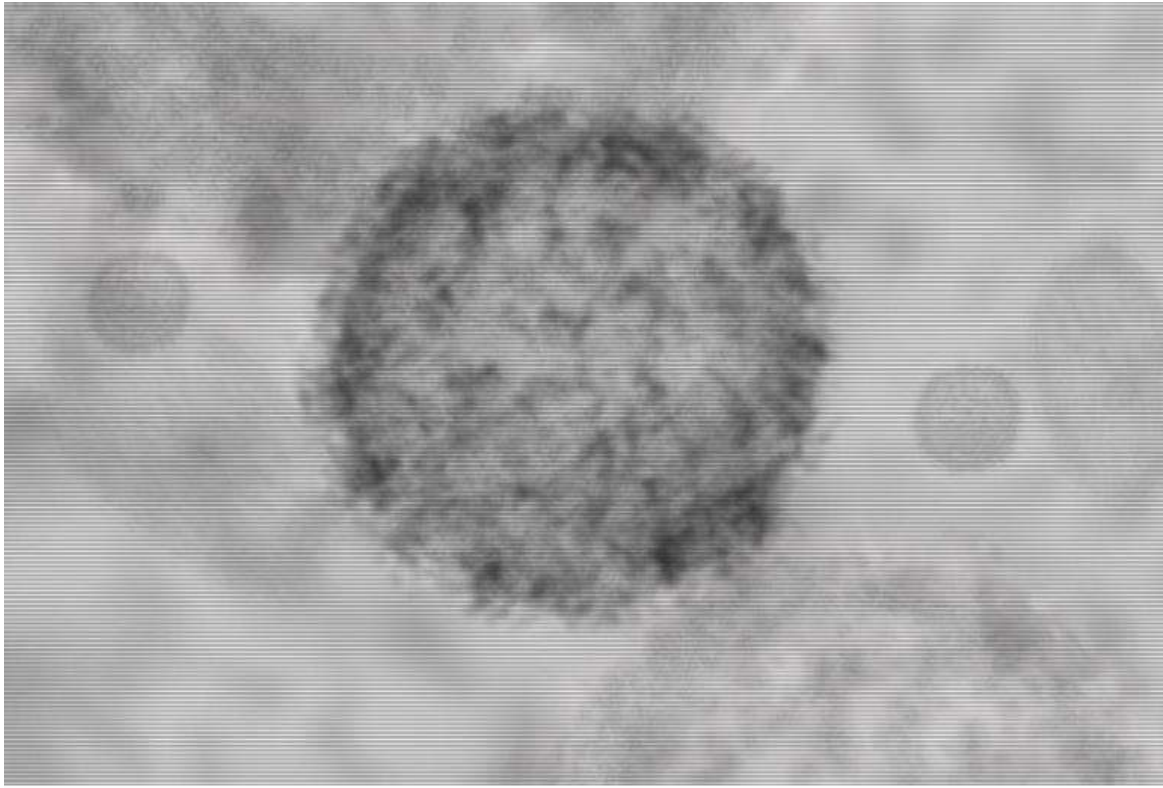
A few facts about viruses



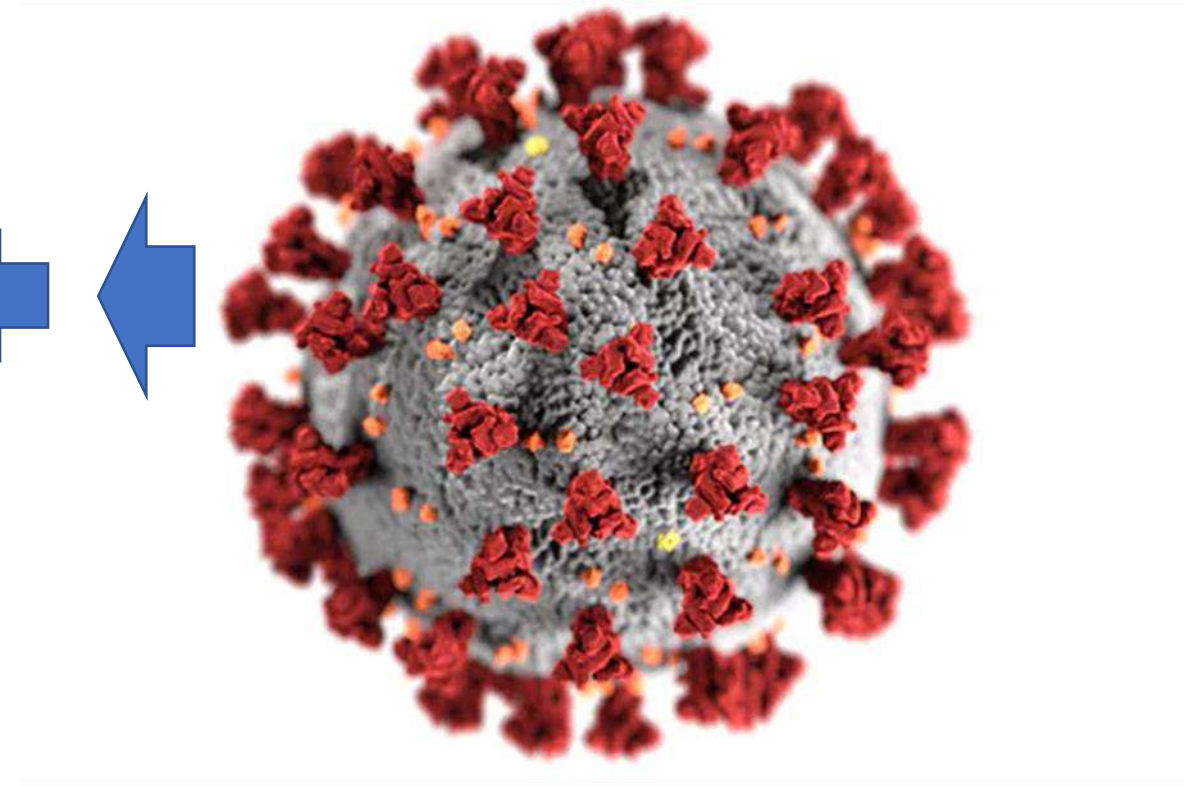
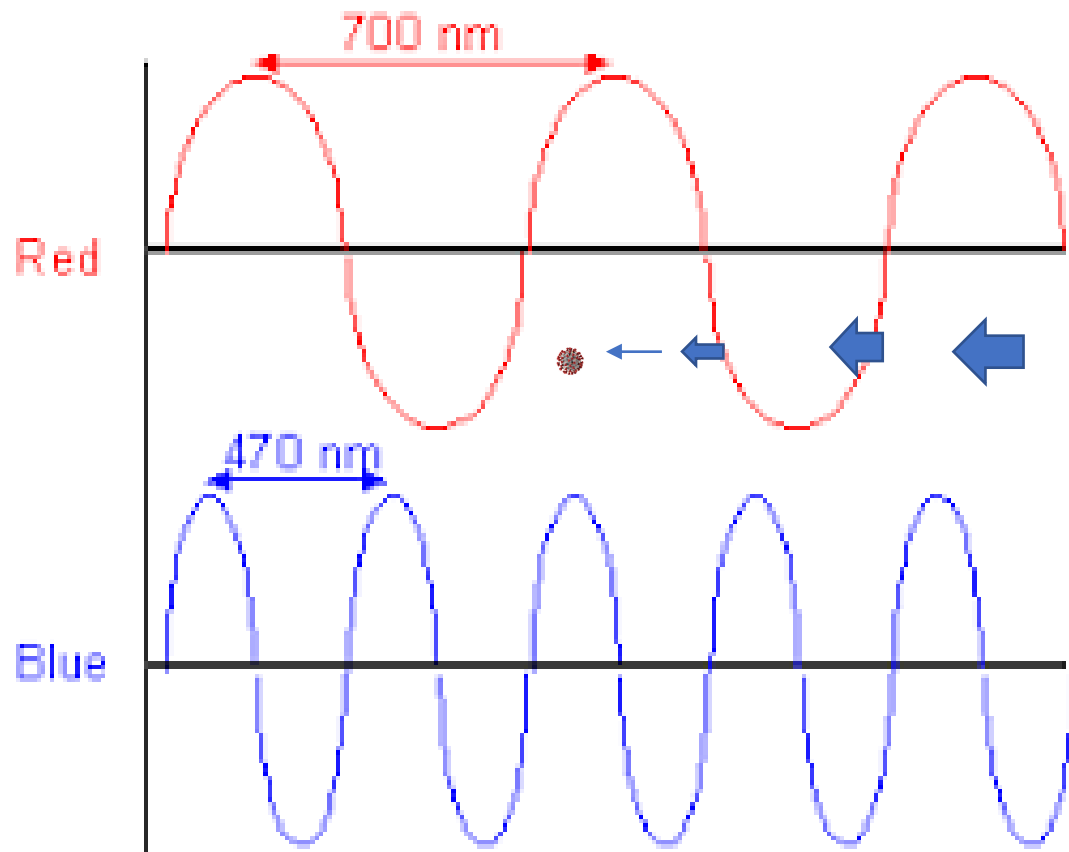
A cluster of numerous green, spherical virus particles, likely poliovirus, is shown against a textured purple background. The particles are small and uniform in size, with a slightly fuzzy or granular surface texture.

They're small

Duuuhhh.....



- Indelibly burned into the mind of nearly every person on the planet resides the colorful illustration (although “colors” used are subjective since at 50 nanometers in size, visible light is far too long in wavelength to be sensitive to the COVID-19 virus; it just passes through it.)



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IMAGINE

One 200 nanometer
virus



x 22,000 magnification



One 0.2-millimeter
grain of sand

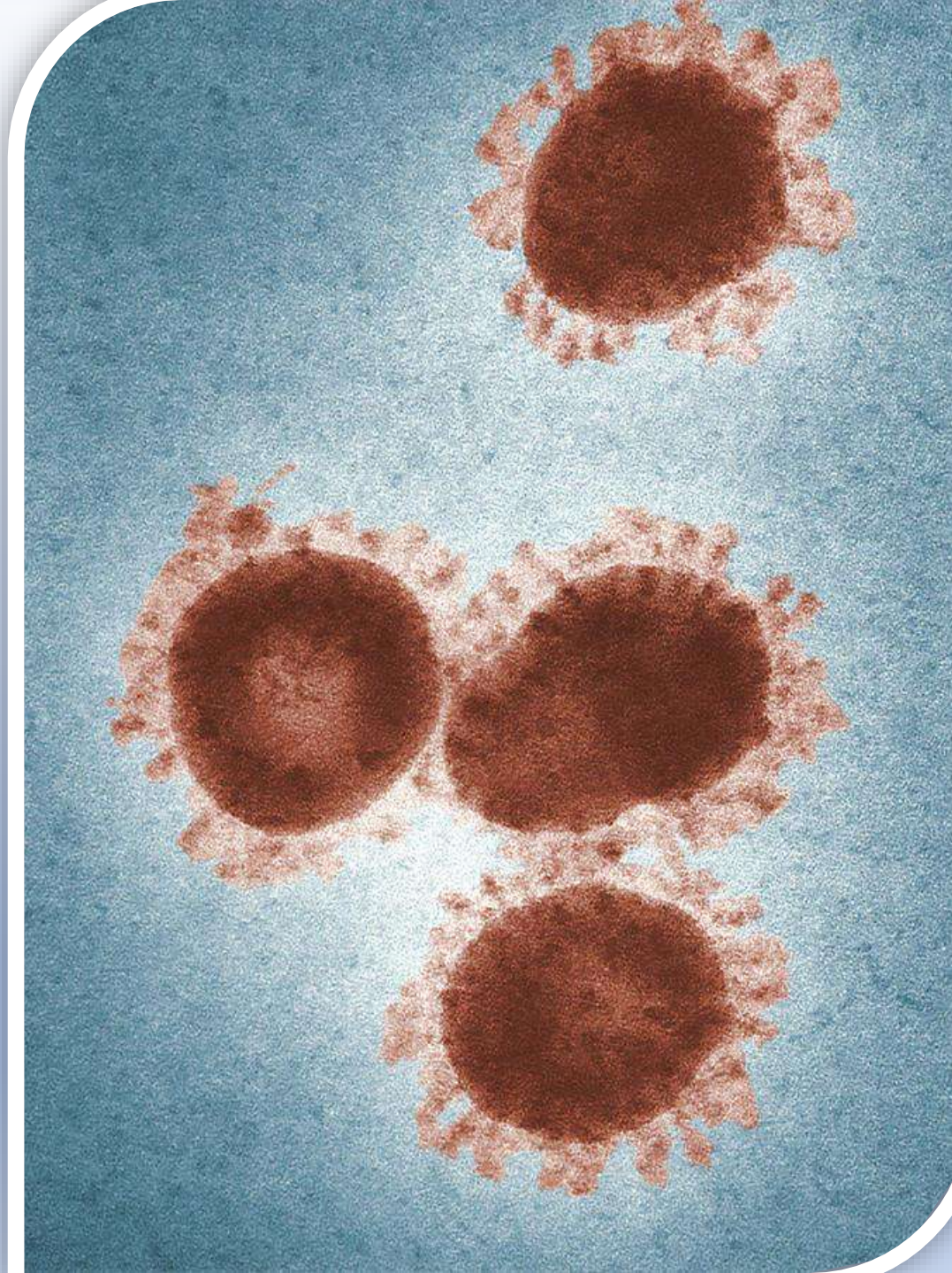


x 22,000 magnification

15 feet

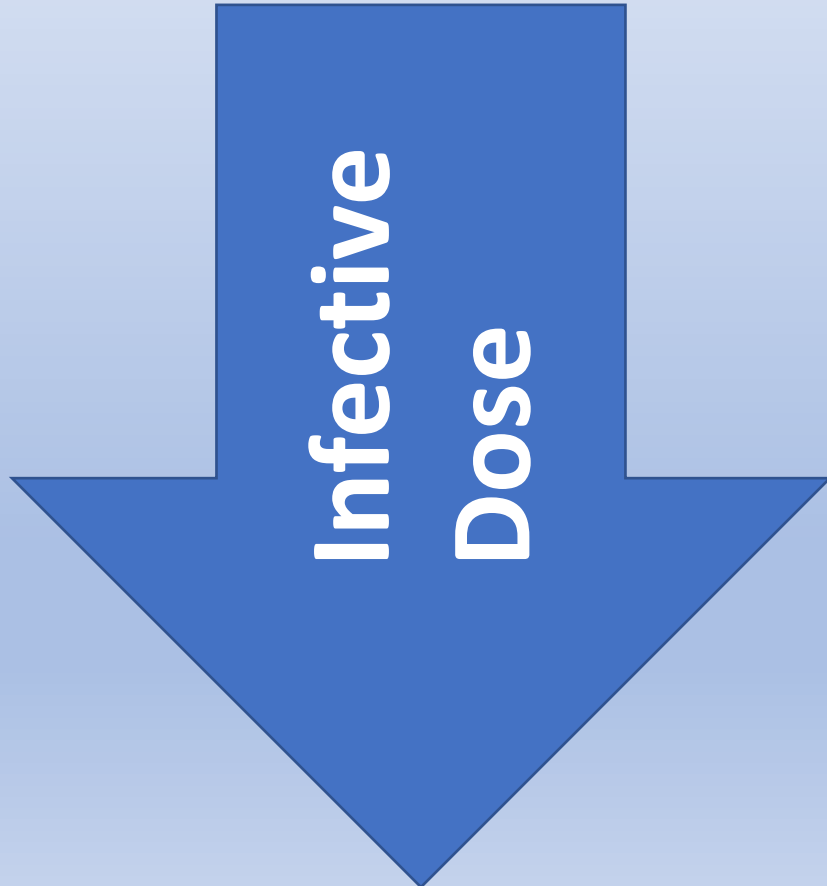


**Viruses generally have
low infective doses**



In general

< 100 virus particles



$10^5 - 10^{11}$ per gram of fecal matter

Resulting in



Or the more familiar



Viruses are many..

150+

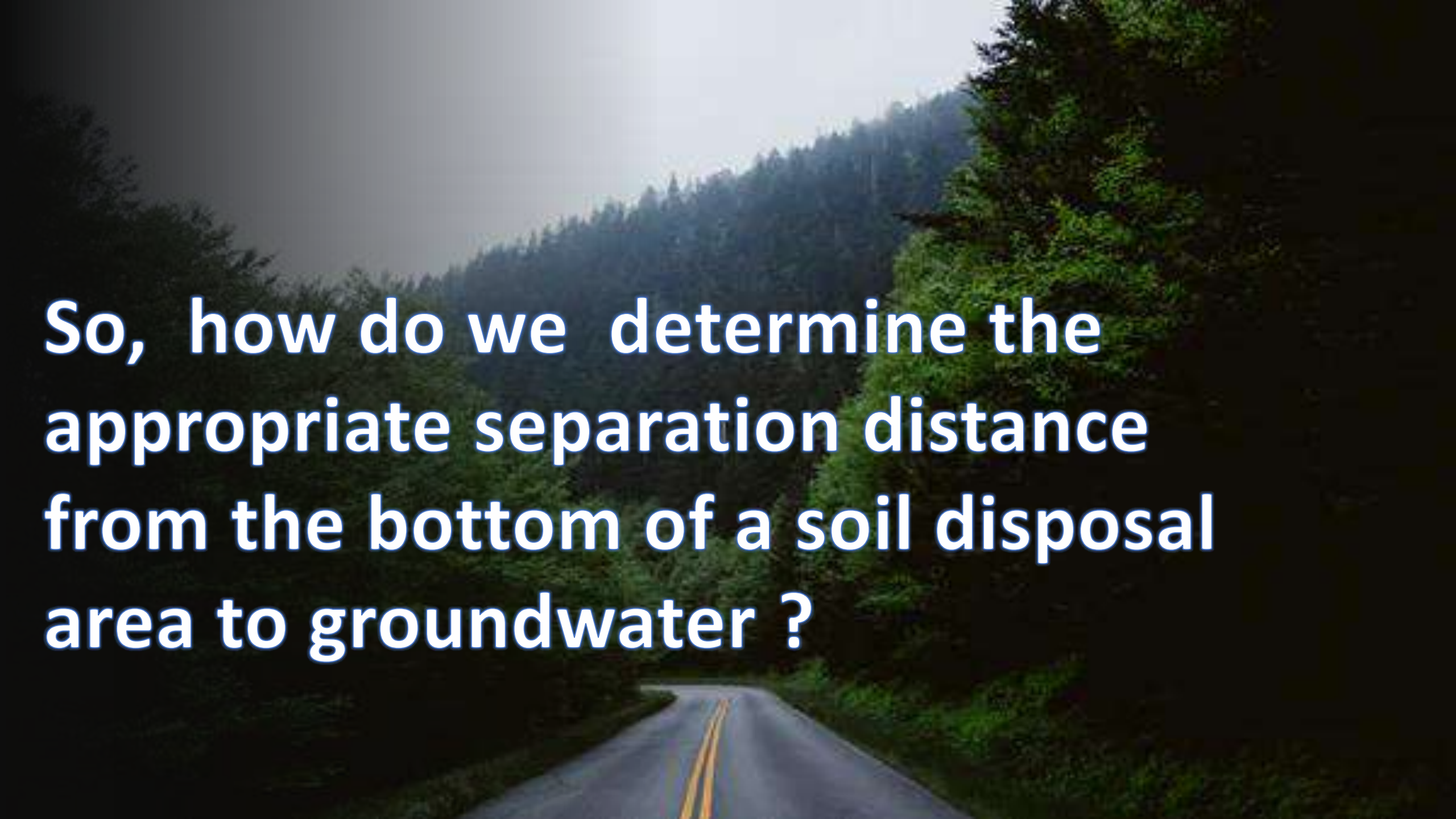


Picornaviridae (poliovirus, enterovirus, coxsackievirus, hepatitis A virus, and echovirus),
Caliciviridae (norovirus, calicivirus, astrovirus, and sapovirus), Reoviridae (reovirus and rotavirus), Adenoviridae (adenovirus), and Coronaviridae (coronavirus).

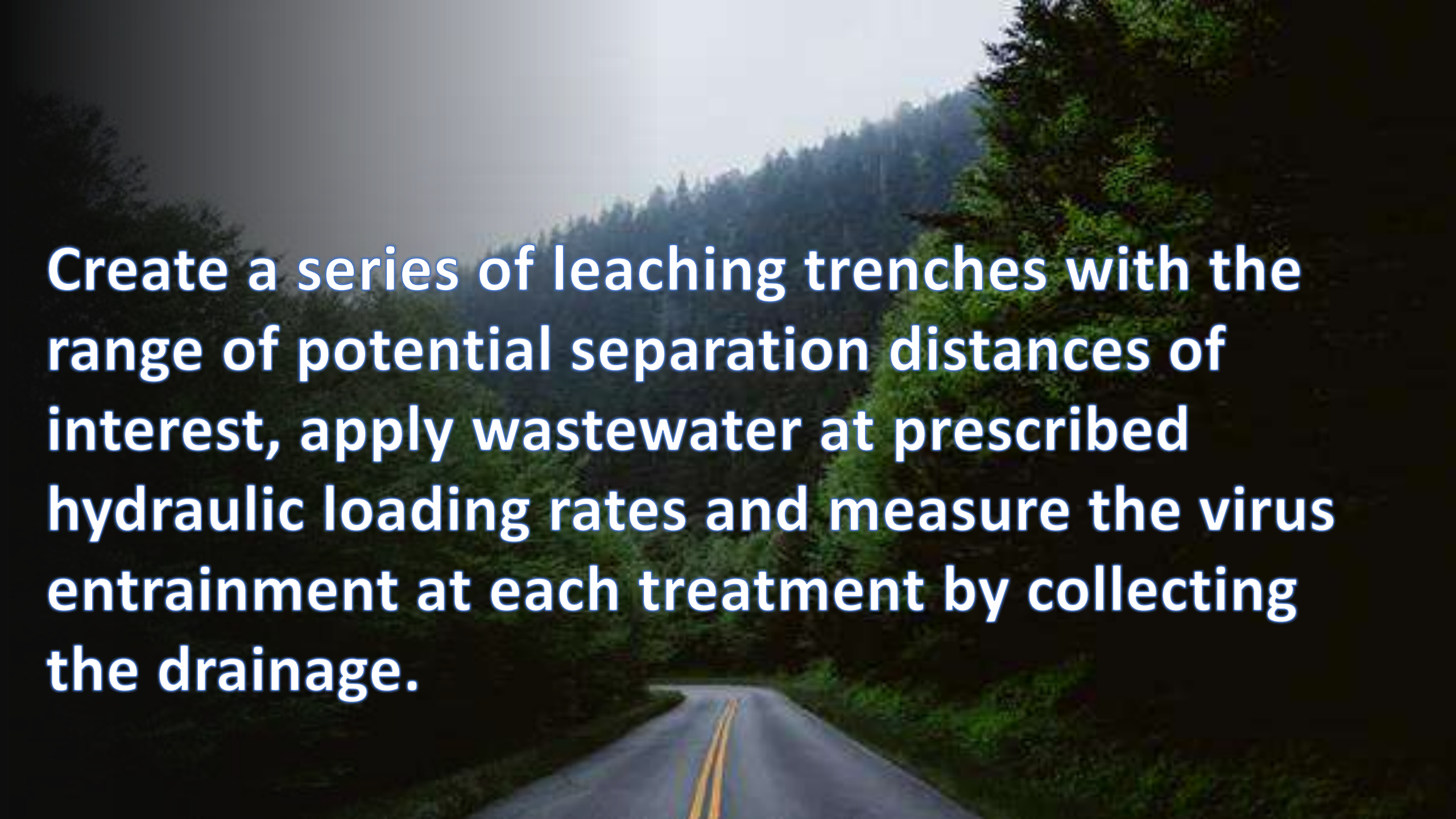
Just to name a few...

Viruses are NOT cute



A photograph of a two-lane asphalt road with yellow double lines, curving through a dense forest. The scene is shrouded in thick fog, obscuring the distant hills and trees. The lighting is dim, creating a somber and mysterious atmosphere. The road leads the viewer's eye into the misty woods.

So, how do we determine the appropriate separation distance from the bottom of a soil disposal area to groundwater ?

A photograph of a two-lane asphalt road with yellow double lines, curving through a dense forest. The scene is shrouded in a thick fog or mist, obscuring the distant trees and the horizon. The trees are mostly evergreens, and the overall atmosphere is quiet and somewhat mysterious.

Create a series of leaching trenches with the range of potential separation distances of interest, apply wastewater at prescribed hydraulic loading rates and measure the virus entrainment at each treatment by collecting the drainage.

But which of the 100+ viruses
to measure?

Phage viruses – harmless
analogues to pathogenic viruses

After all, we
don't want to
end up like this.



Coliphage

If you think it's just a phage you're going through.....

It's more likely a phage going through **you**

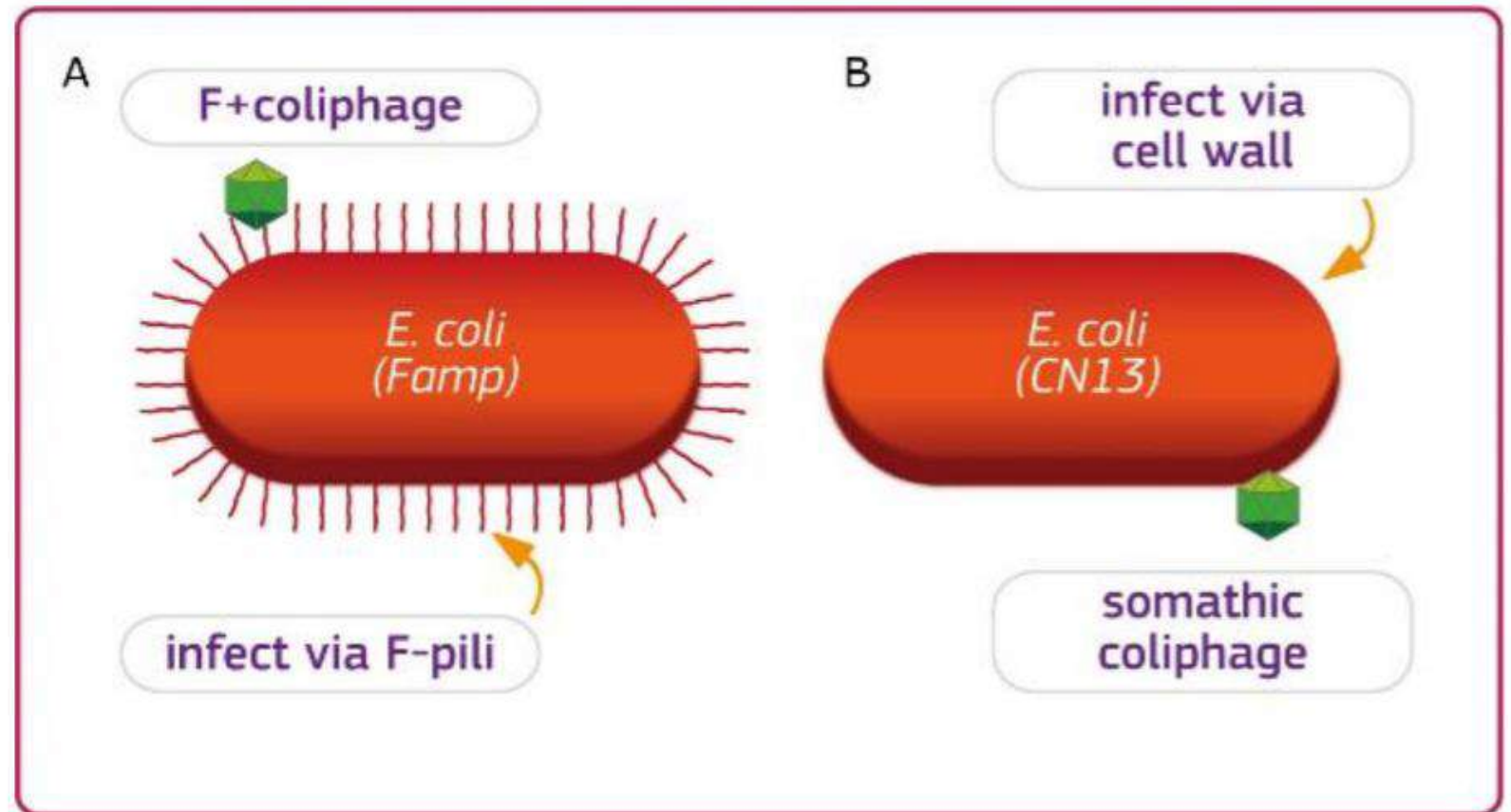
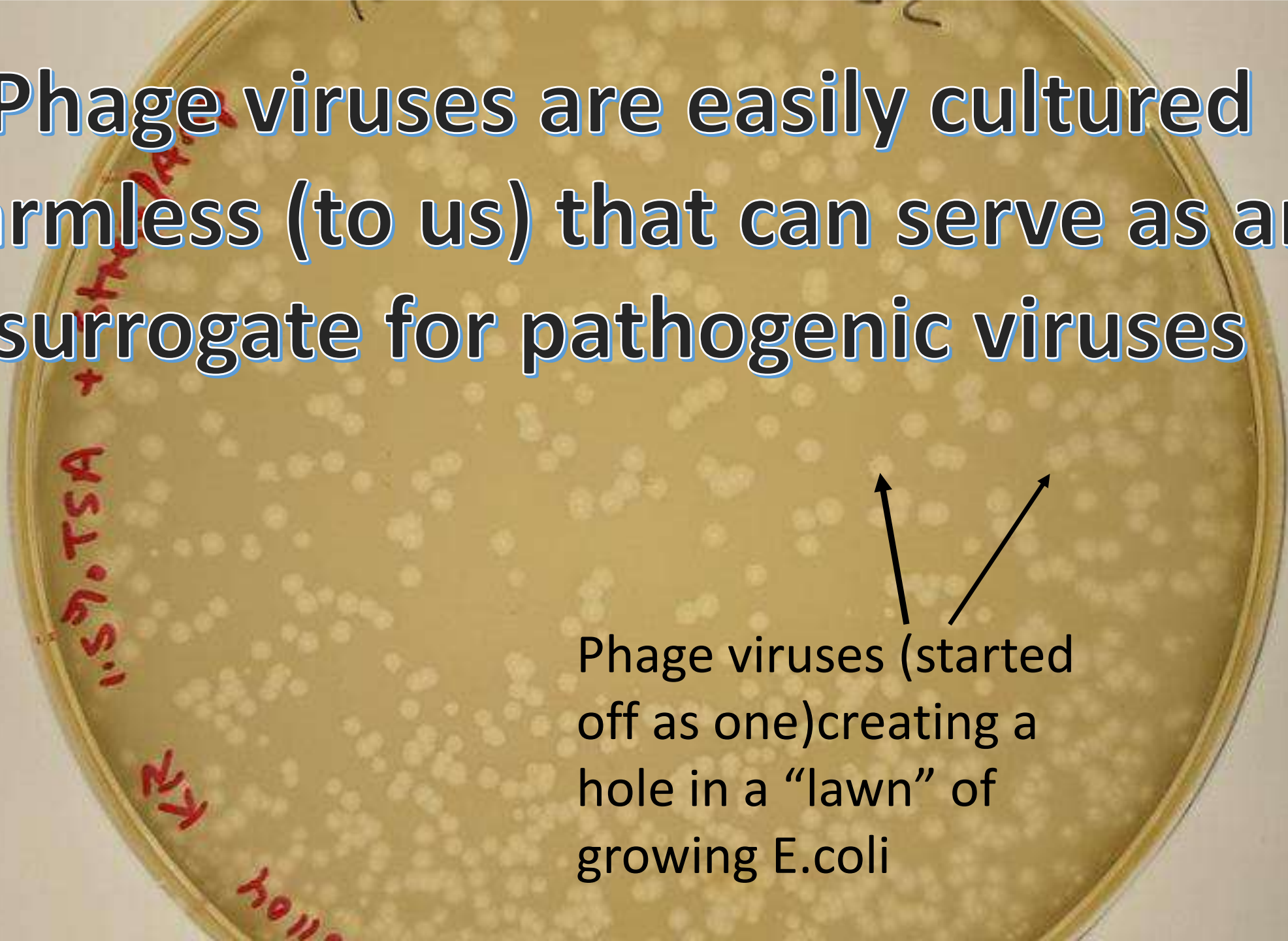


Figure 5. Schematic representation of F-specific (or male-specific) coliphages, somatic coliphages and their host cells. (A) F-specific coliphages infect host cells (e.g. *E. coli* _{Famp}, *Salmonella typhimurium* WG49) through the sex pili encoded by the F-plasmid. (B) Host strains of somatic coliphages include *E. coli* (e.g. *E. coli* CN13) and related species which are infected through the cell wall.

Phage viruses are easily cultured
harmless (to us) that can serve as an
surrogate for pathogenic viruses



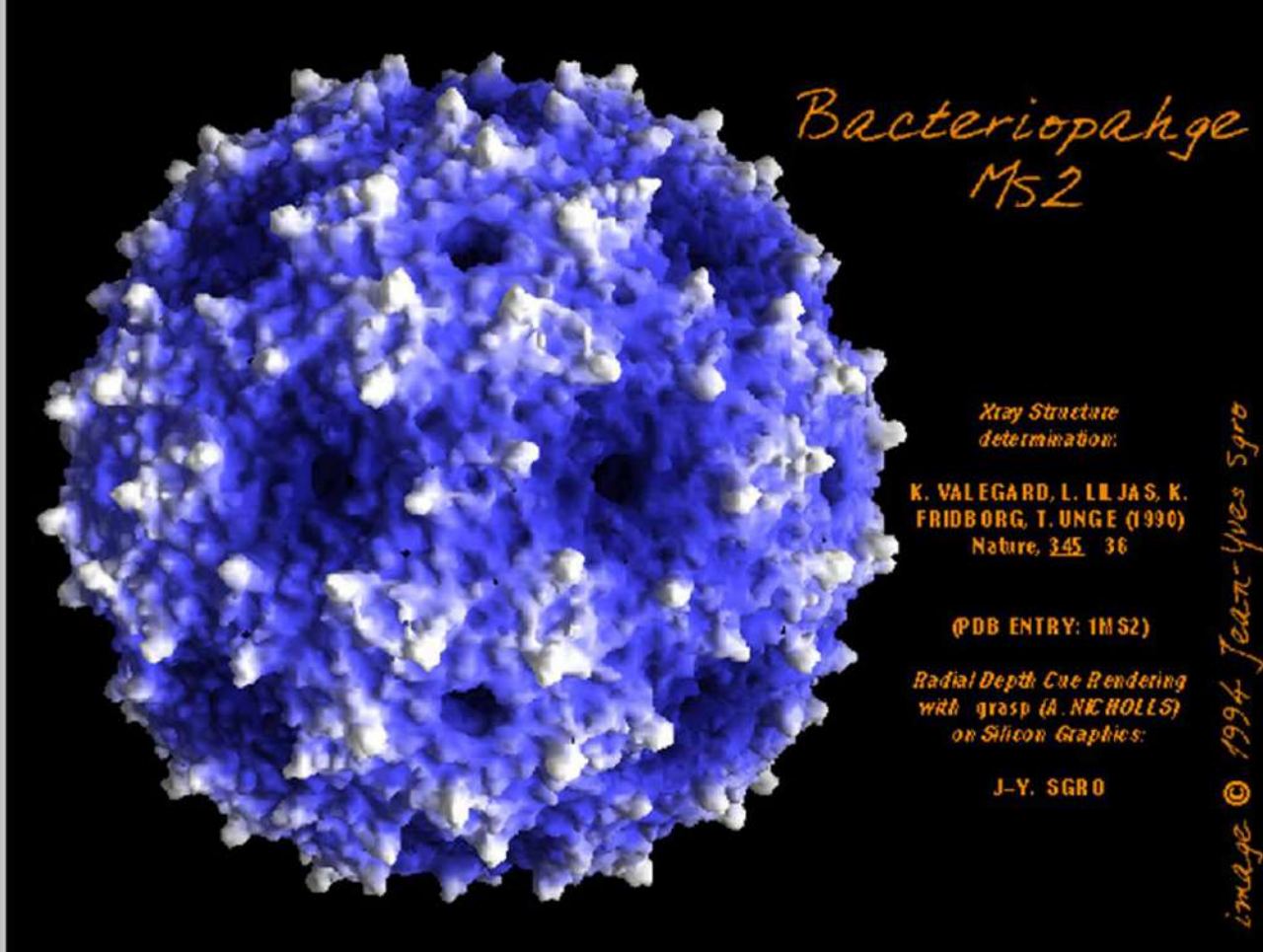
A petri dish containing a bacterial lawn of E. coli. Numerous small, clear, circular plaques are visible across the surface, representing areas where phage viruses have lysed the bacteria. Two black arrows point from the text below to two of these plaques. Red handwritten text is visible along the left edge of the dish, including '1:57. TSA', '1:57. TSA', and '1:57. TSA'.

Phage viruses (started
off as one) creating a
hole in a “lawn” of
growing E.coli



**Grubs creating a hole in
a lawn**





*Bacteriophage
MS2*

*X-ray Structure
determination:*

*K. VALEGARD, L. LILJAS, K.
FRIDBERG, T. UNGE (1990)
Nature, 345 36*

(PDB ENTRY: 1MS2)

*Radial Depth Cue Rendering
with grasp (A. NICHOLLS)
on Silicon Graphics:*

J.-Y. SGR0

image © 1994 Jean-Yves Sgro

Massachusetts Department of Environmental Protection

Virus Entrainment Study

Study Detail

VIRUSES

- Male Specific Phages (MS-2)
- Somatic Phages
- Various animal (human) viruses

BACTERIA

- Escherichia coli
- Enterococcus sp.
- Fecal Coliform

Massachusetts Department of Environmental Protection

Virus Entrainment Study

Study Detail

Gravity Fed

2-FT x 5 replicates

3-FT x 5 replicates

4-FT x 5 replicates

5-FT x 5 replicates

**Pressure
dosed**

2-FT x 5 replicates

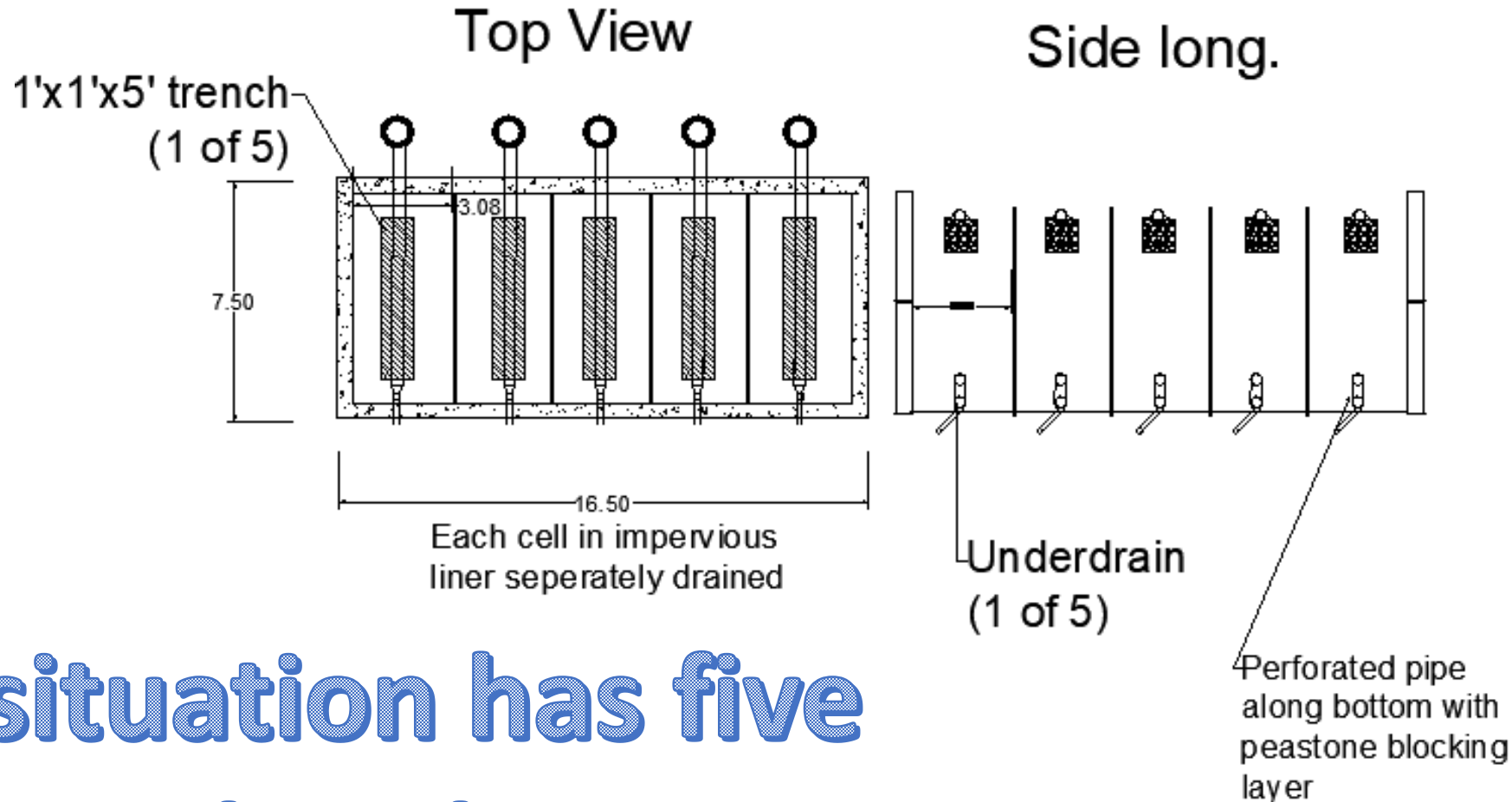
3-FT x 5 replicates

4-FT x 5 replicates

Total 35 replicates

Each Treatment

5,000 Gallon Precast Septic Tank Shim
to contain test cell replicates and brought to ground surface



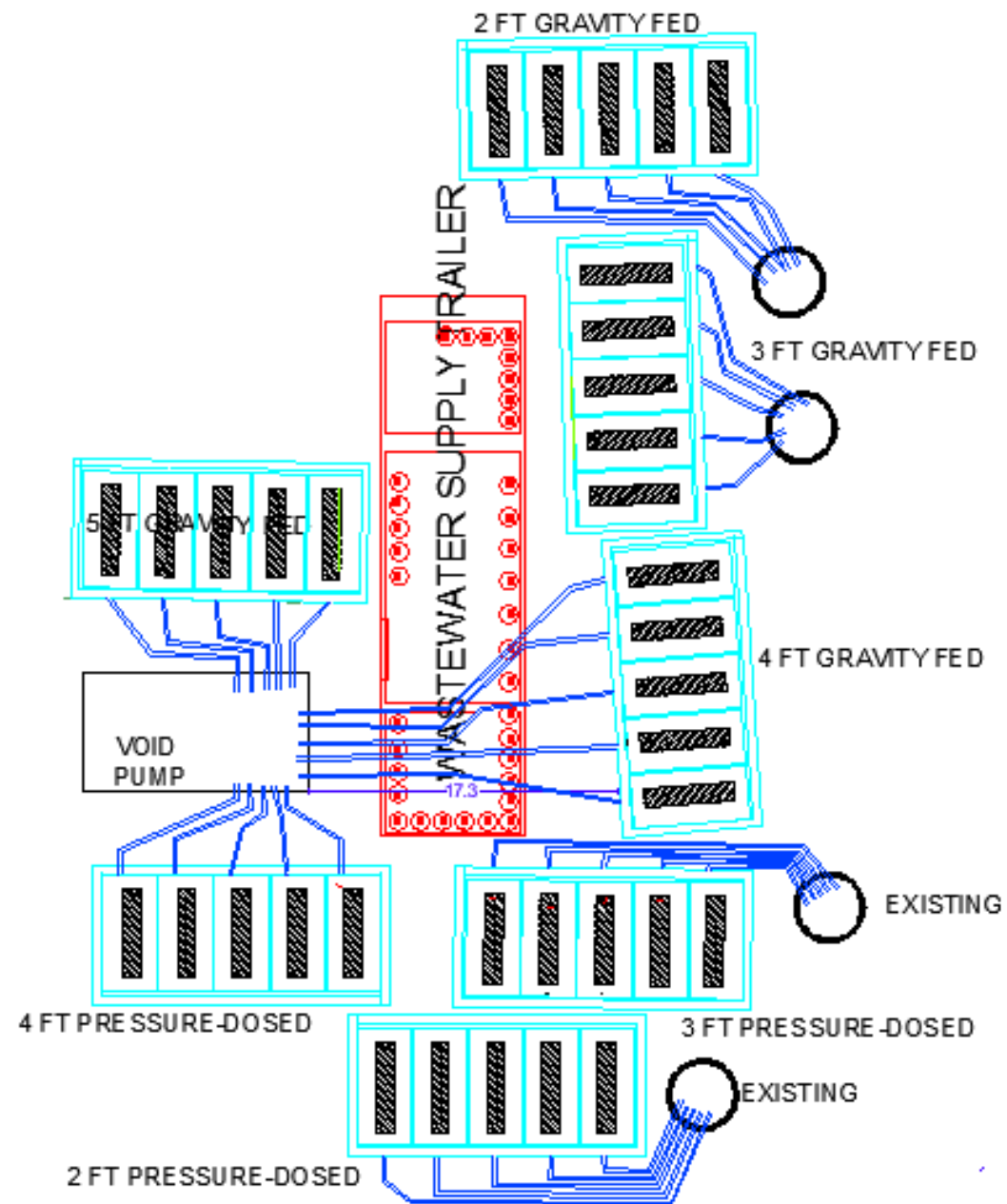
Each situation has five
identical replicates

Each
situation has
five identical
replicates

5



The site

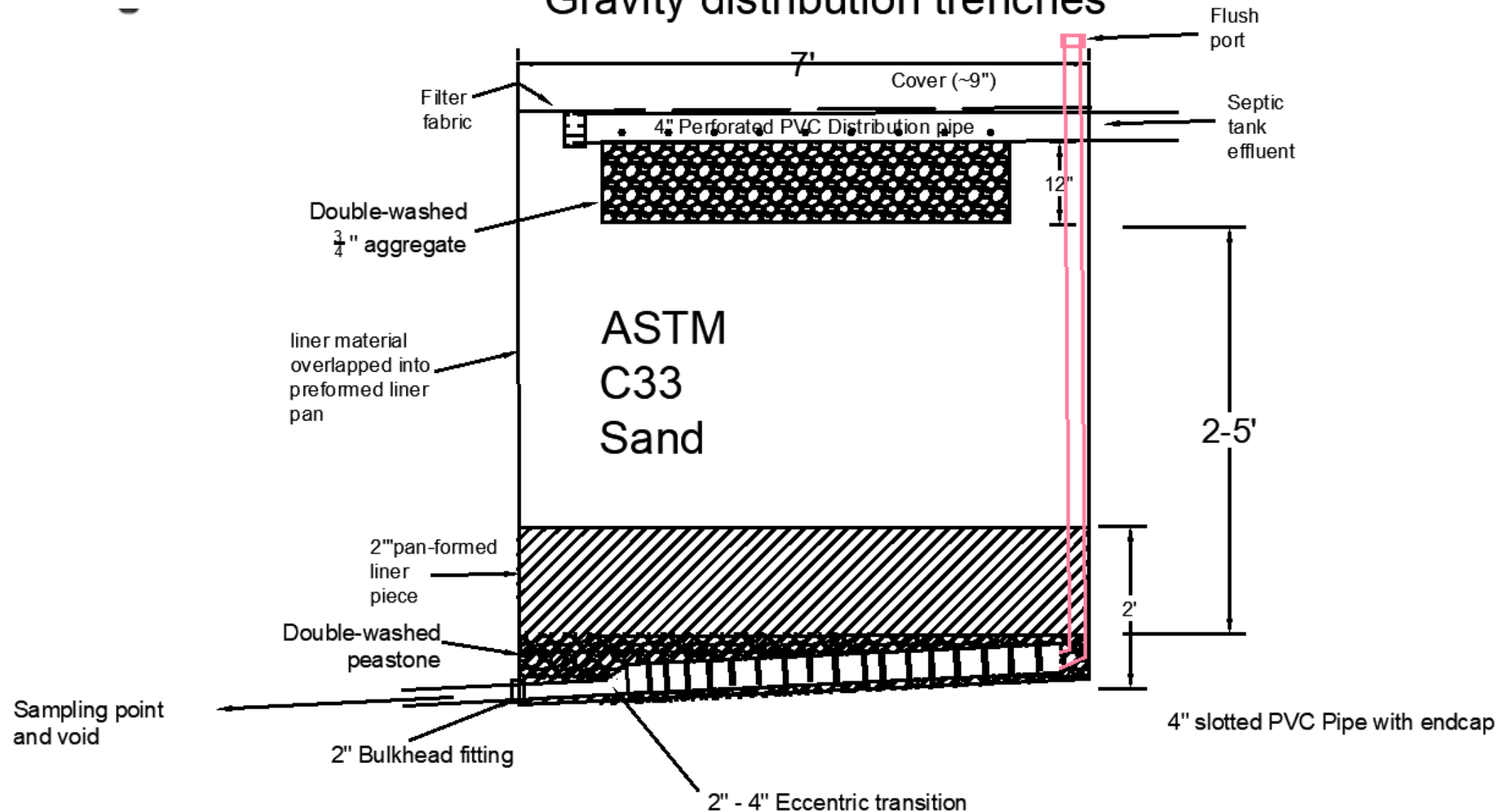


7 Treatments



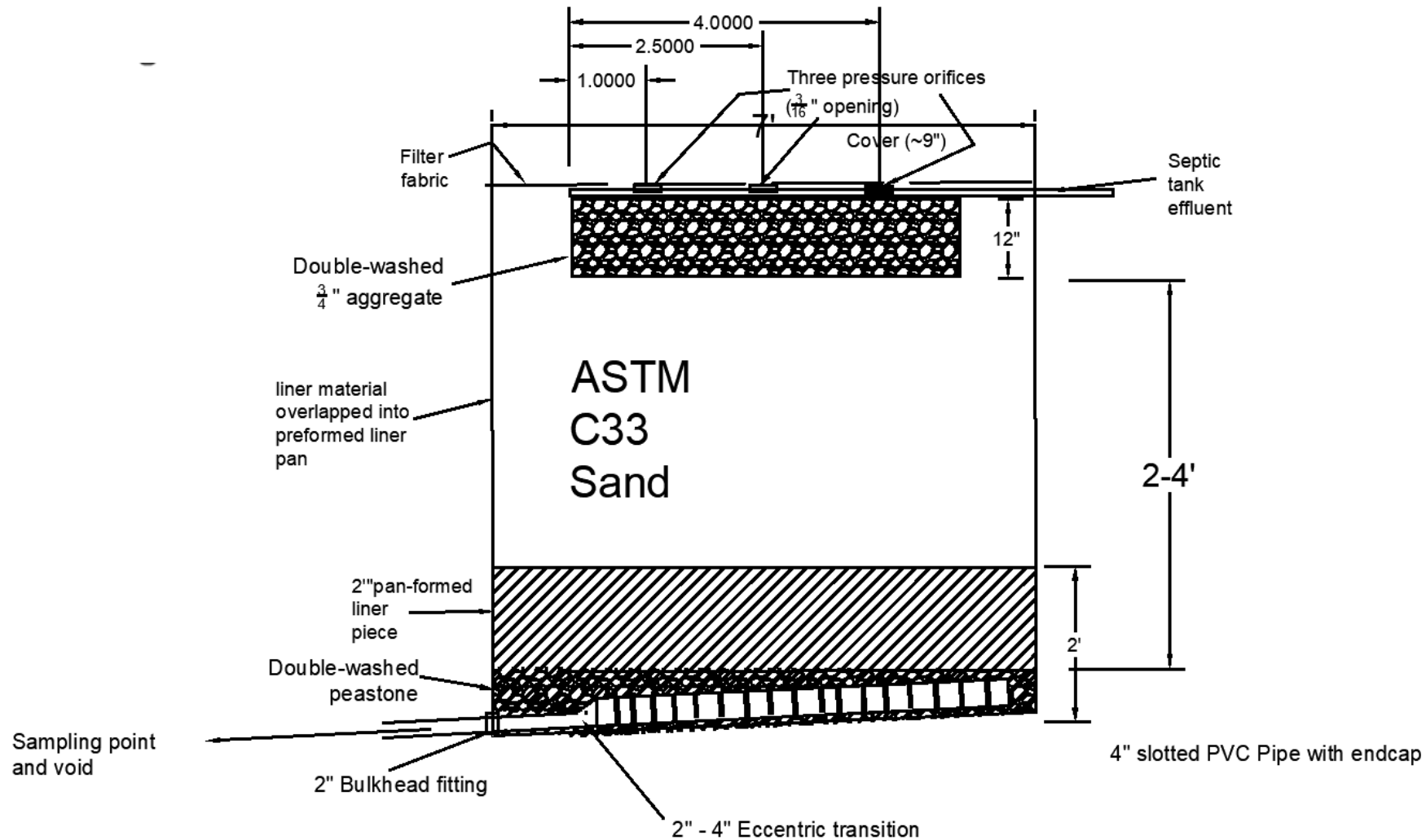
Sample replicate - gravity

Gravity distribution trenches



Sample replicate - LPD

Pressure distribution trenches





Progress to date..... (December 2020)

- All test cells created, partially piped.



Pressure dose end cleanout

Ponding observation port

Under drain flush port

One of fifteen

A photograph of a laboratory setting. In the center, a person with white hair and glasses, wearing a white lab coat and purple gloves, is working at a lab bench. The bench is cluttered with various laboratory equipment, including pipettes, bottles, and containers. In the foreground, several clear petri dishes are arranged on a dark surface. The background shows a white refrigerator, a window with a view of the outdoors, and wooden cabinets. The overall lighting is somewhat dim, and the image has a slightly grainy texture.

Progress to date.....

Laboratory constructed, analyst hired, methodology for virus assays
MS-2 and somatic coliphage verified and initial trials completed.



Progress to
date.....
(December 2020)

35 Dosing mechanisms
partially constructed

Watch this space

Just the beginning

What's the most important point to remember?

The Isoelectric point