

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

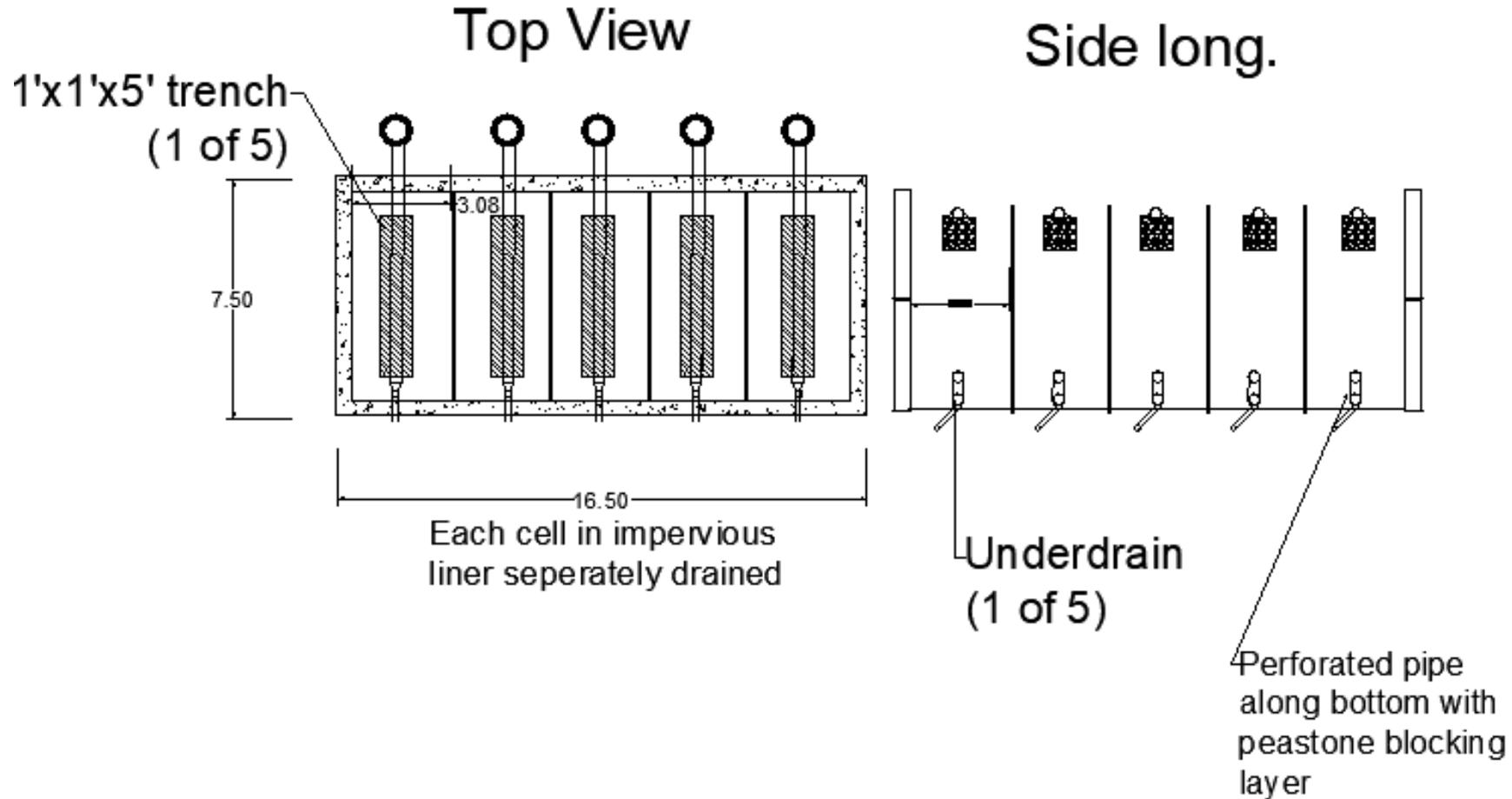
3-FT x 5 replicates

4-FT x 5 replicates

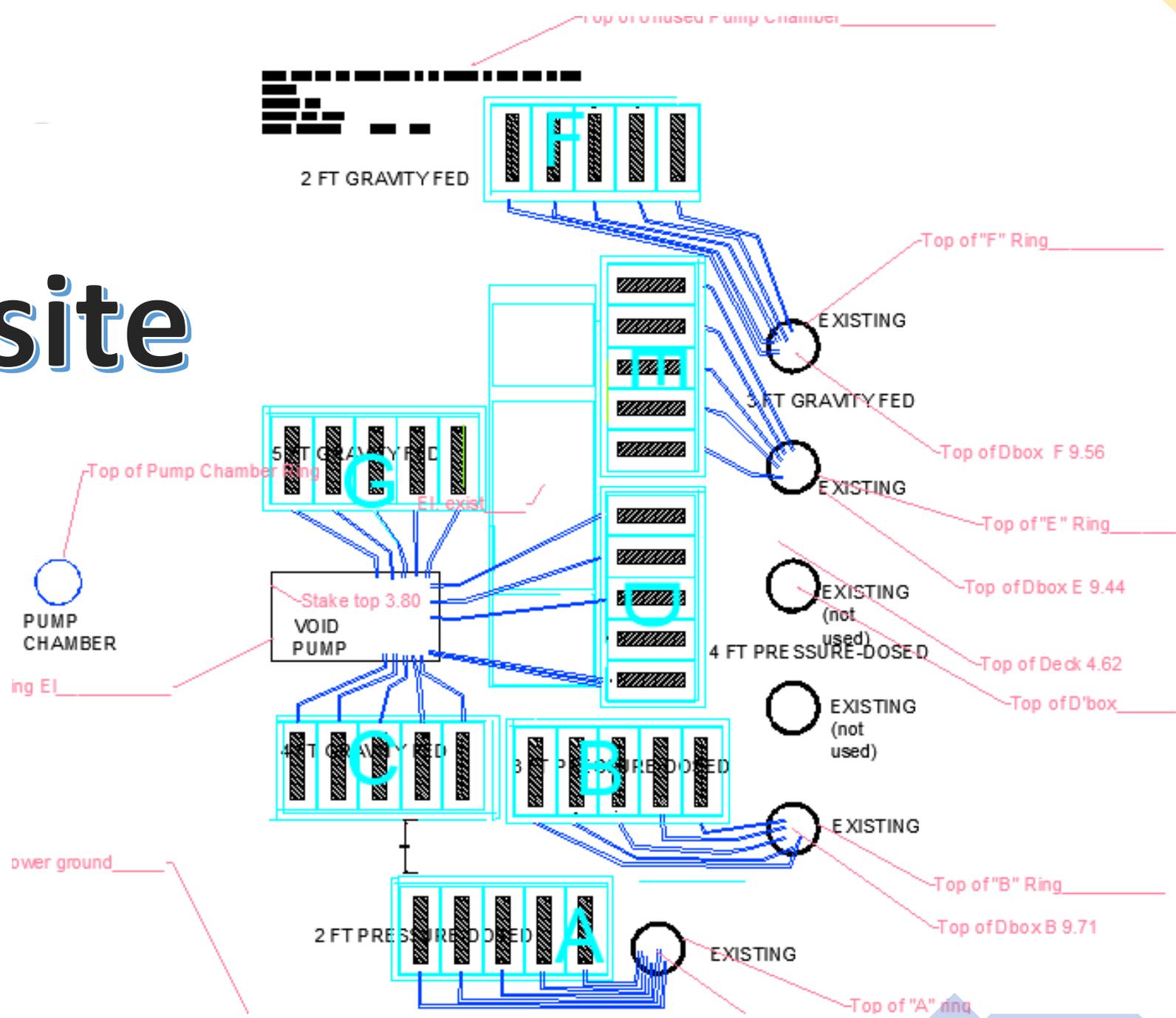
Total 35 replicates

Each Treatment

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

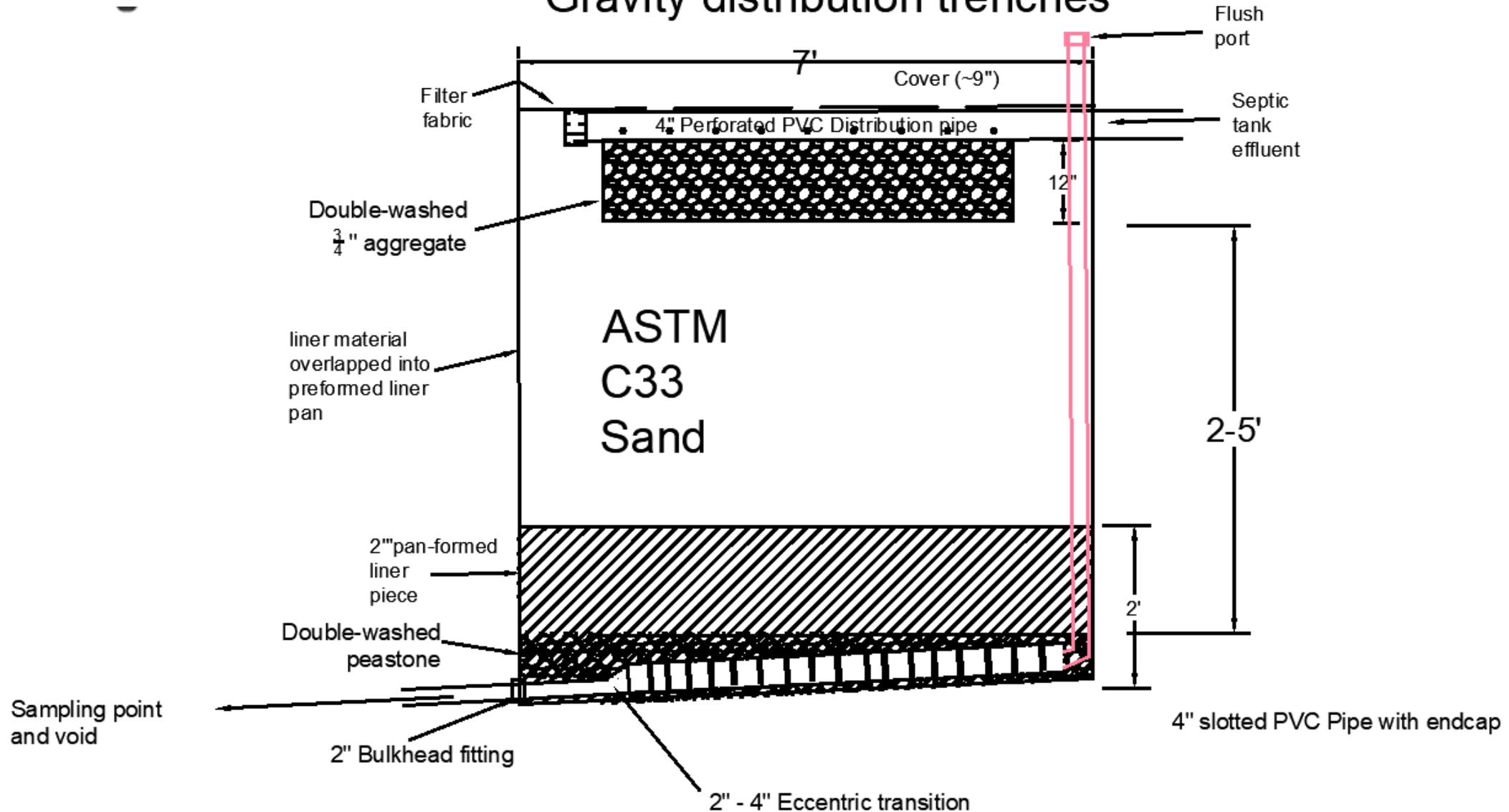


The site



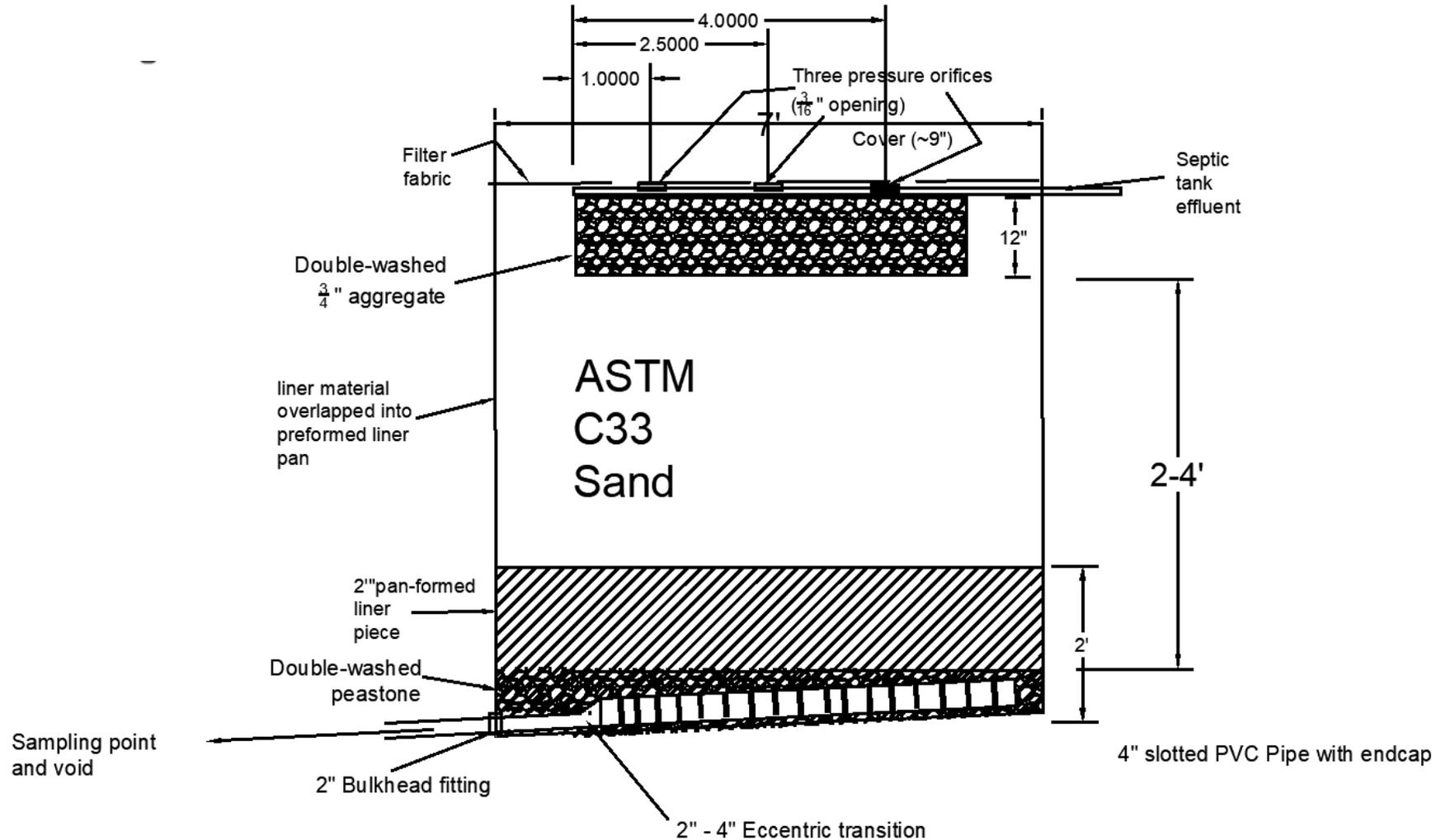
Sample replicate - gravity

Gravity distribution trenches



Sample replicate - LPD

Pressure distribution trenches



Virus Analysis

Prep Steps (viral QA/QC prep)

Host bacteria - ATCC pure cultures

Establish frozen stocks: Streak onto plates w 1.5% TSB agar @ antibiotic (15g agar/liter TSB)

Incubate overnight @ 36°C

Pick a colony

Inoculate into TSB in tube @ antibiotic vol?

Grow to log-phase

Harvest → mix sterile glycerol w TSB log-phase host [1:4 eg 200 µl glycerol 800 µl host]

in 5ml freezer vials Freeze @ -80°C if possible

De-frost to use as working stock

Overnight cultures:

10 ml stock TSB @ 100 µl antibiotic stock in 15 ml falcon tube

Inoculate w loop of host stock culture

Incubate overnight @ 36°C

Log-phase cultures:

Add 5 ml of overnight to 250 ml TSB @ 25 ml antibiotic

Incubate 4 h @ 37°C

Adjust vol to 4 samples - 100 ml mid-log/sample

Cell phase Stock Prep

Make two 5ml tubes of 1X TSB

Add loop of host bacteria from isolated colony on 15% TSB agar plate

to each tube/each host

Incubate 16-36 h @ 36°C

Transfer overal cell culture to 6 tubes @ 250 µl 1X TSB @ antibiotic

Incubate 4 h @ 36°C → harvest log phase

Rehydrate phage stock culture 1 ml TSB → each

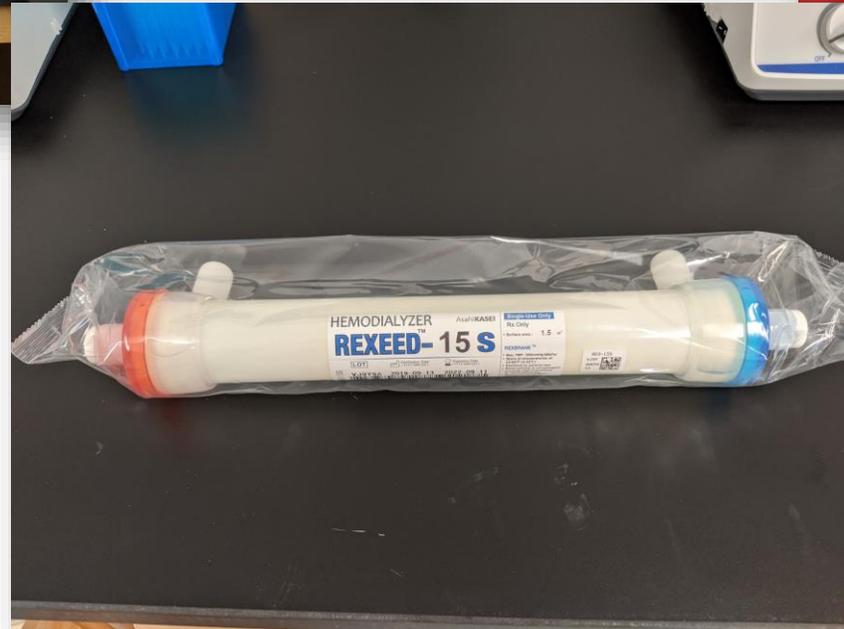
Add one ml of stock phage to phage stock 100 µl phage 100 µl TSB

Incubate @ 36°C overnight (16-24 h)

Centrifuge @ 3500g/10 min

Filter supernatant into 100 µl aliquots

Virus Analysis



Bacterial Analysis



Test Cell Construction



Test Cell Construction



Test Cell Construction



Test Cell Construction



Test Cell Construction



Dosing 35 Test Cells



Laboratory Construction



Laboratory Construction



Laboratory Construction

