

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

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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

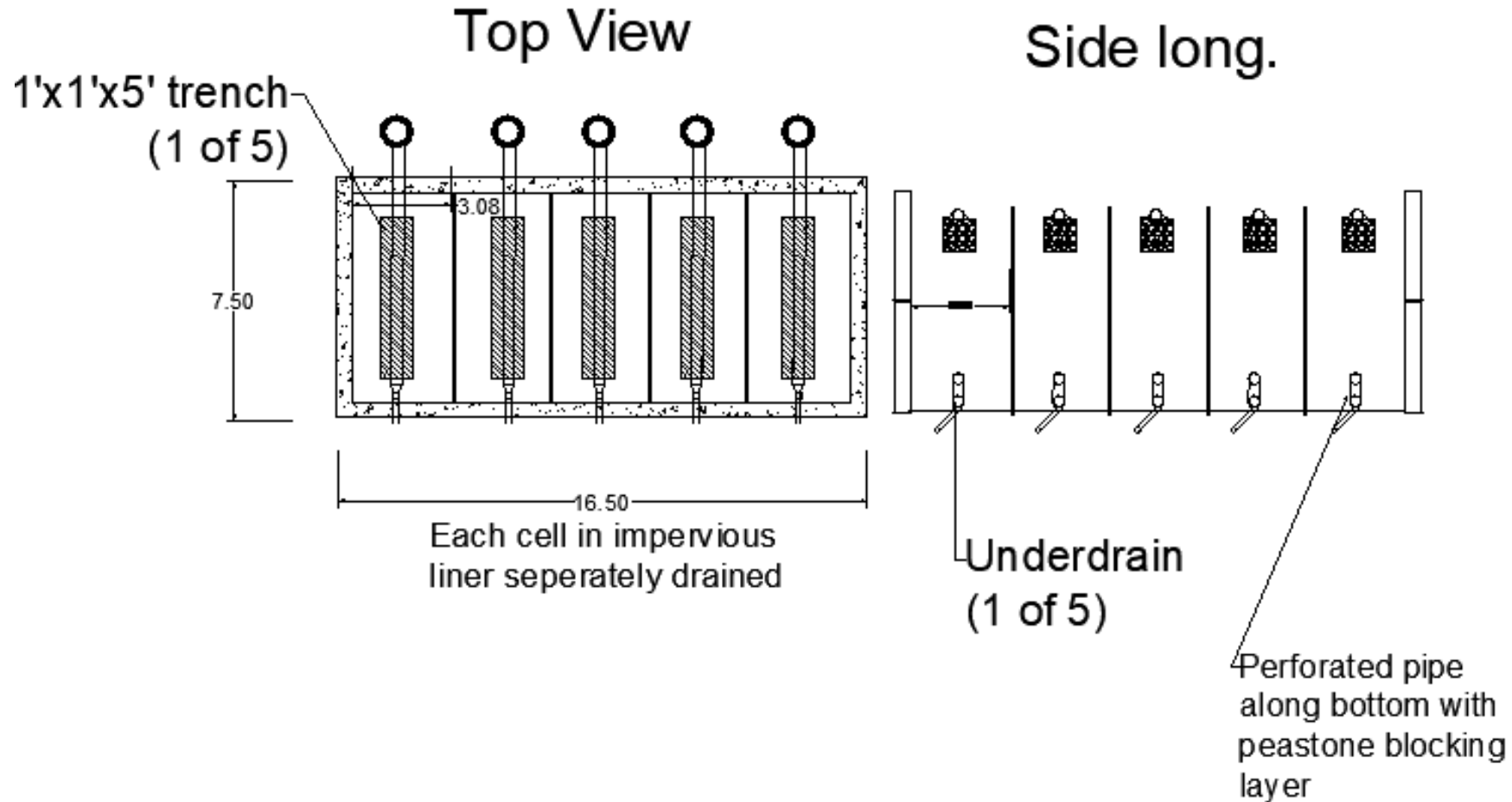
3-FT x 5 replicates

4-FT x 5 replicates

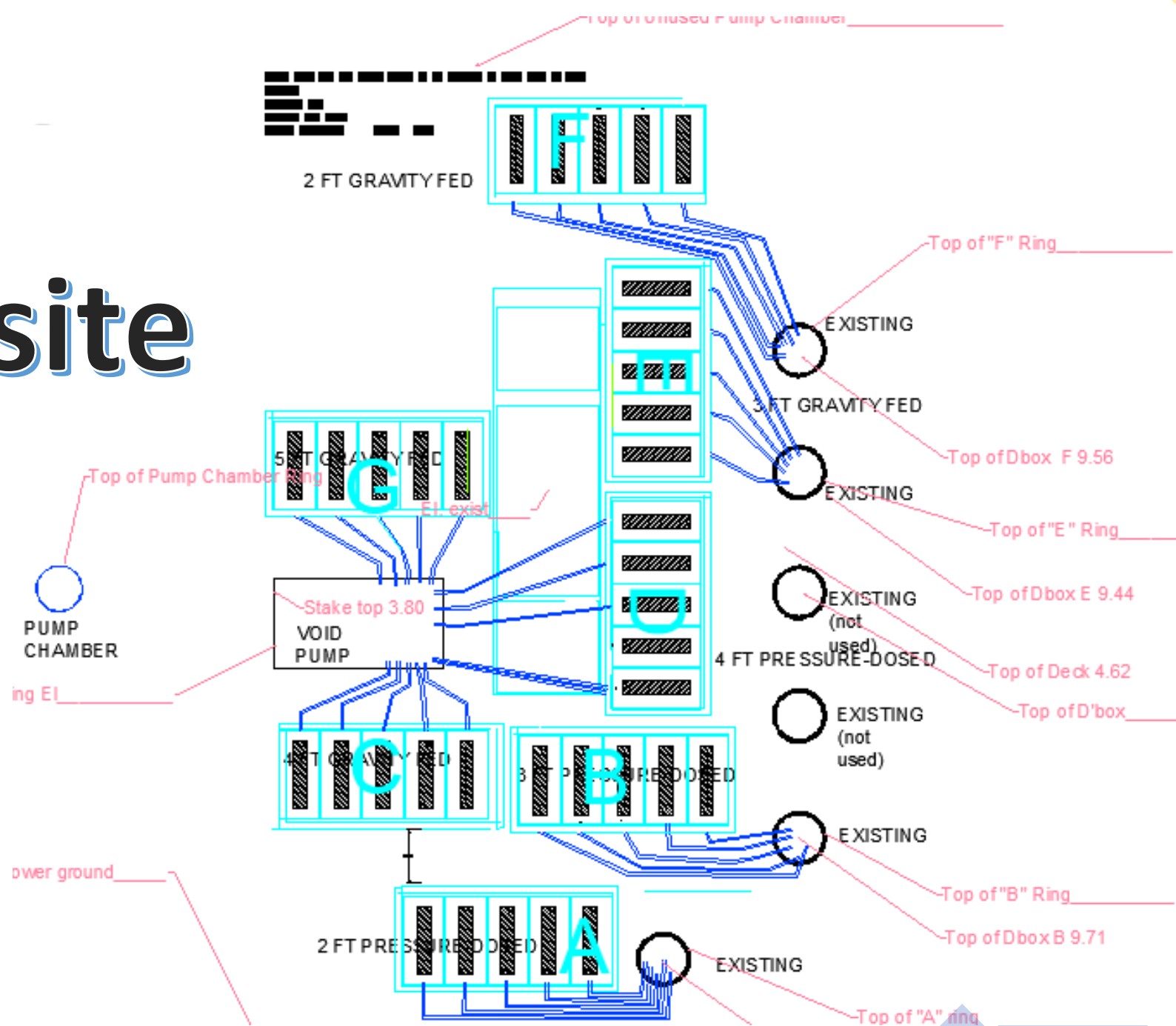
Total 35 replicates

Each Treatment

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

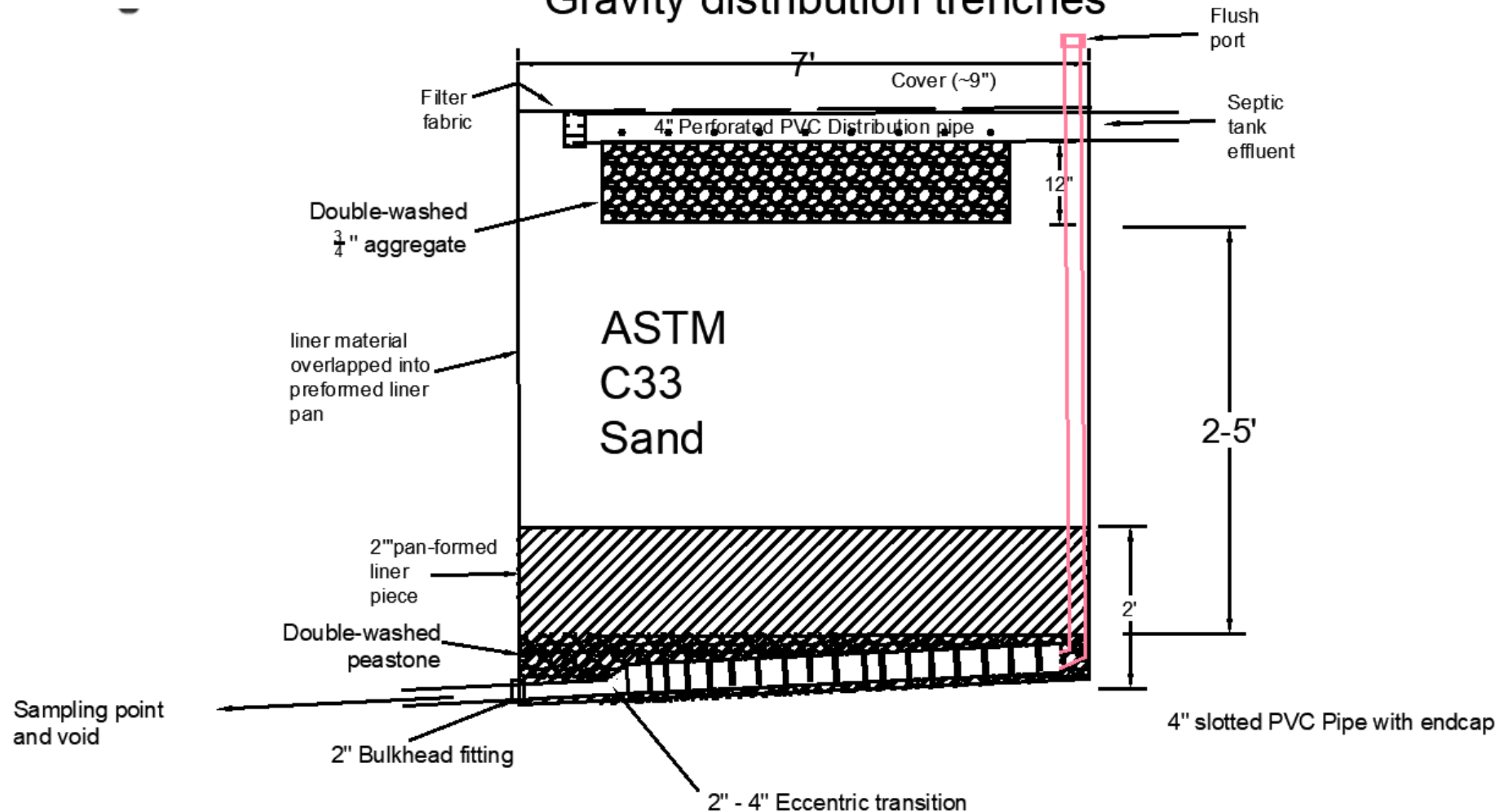


The site



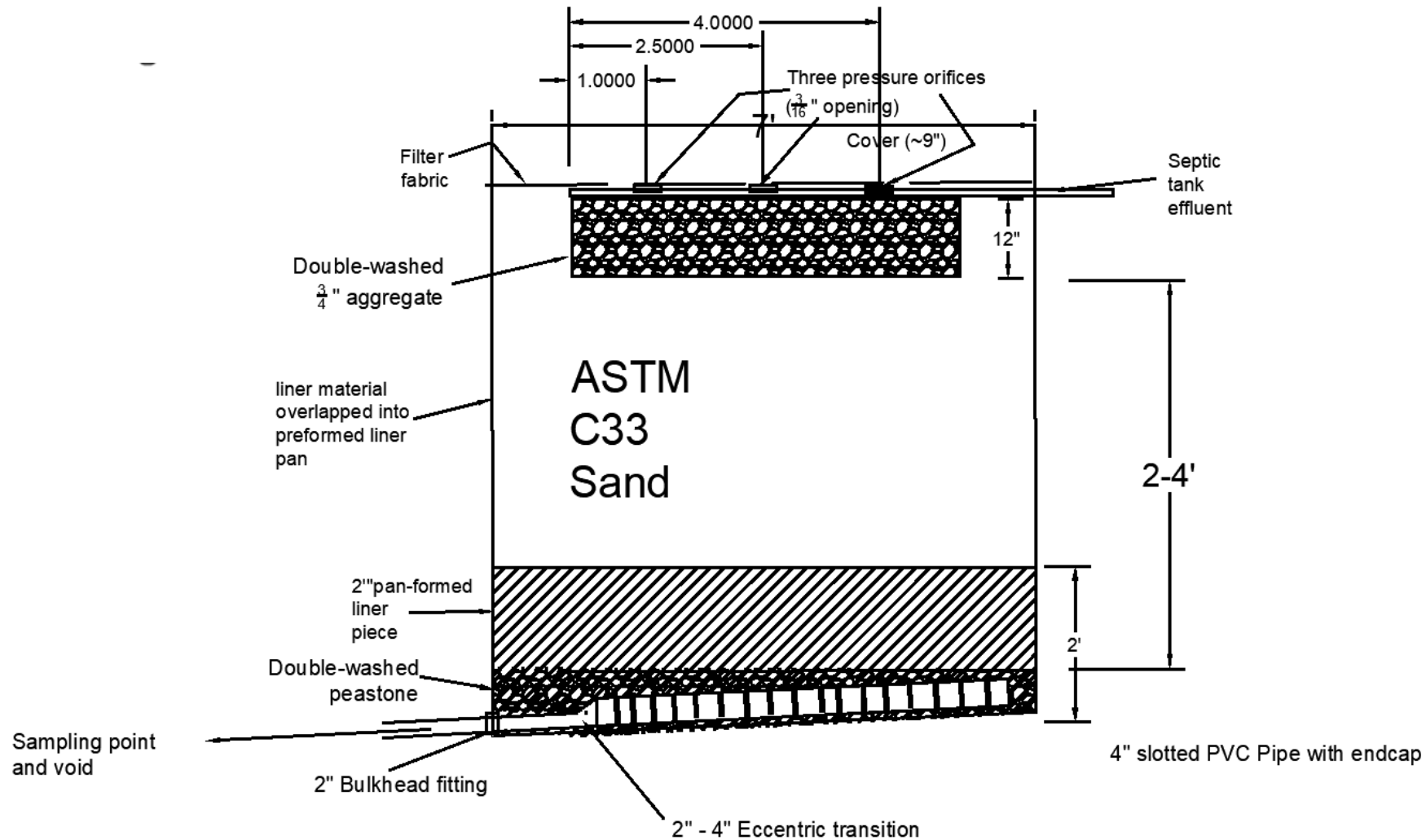
Sample replicate - gravity

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 of 200 µl glycerol
800 µl host]

in 5ml freezer vials

↓
Freeze @ -80°C if possible
↓
DeFrost to use
as working
stocks

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 @ 2.5 ml
antibiotic

↓
Incubate 4 h @ 37°C

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

Coliphage Stock Prep

Make two 5ml tubes of 1X TSB

↓
Add loop of host bacteria from
isolated colony on 1.5% TSB agar
plate
to each tube/each host

↓
Incubate 16-24 h @ 36°C

↓
Transfer overnight culture to
6 tubes @ 250 ml 1X TSB
@ antibiotic

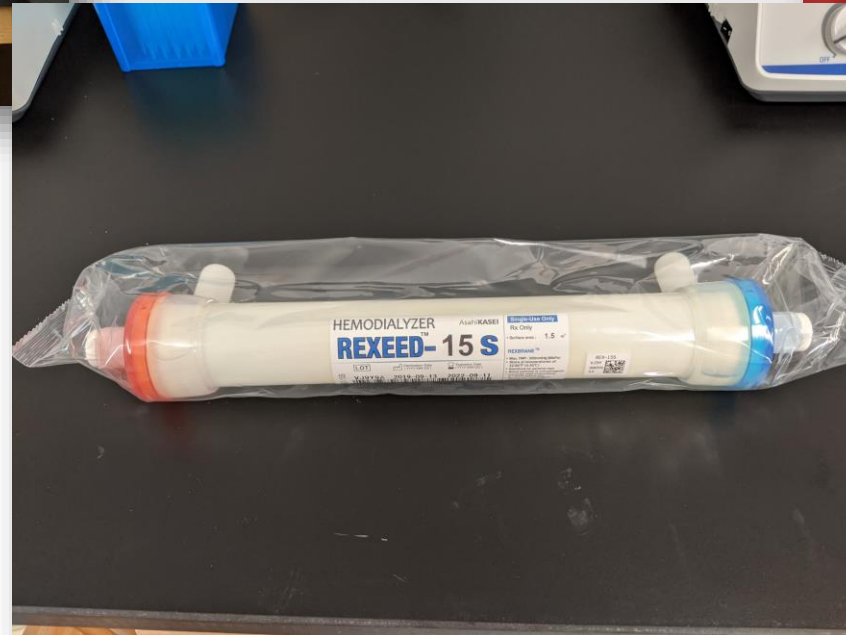
↓
Incubate 4 h @ 36°C → sort out
log phase

↓
Rehydrate phage stock solution
one ml TSB → each

↓
Add one ml of stock phage to
phage host log phase host

↓
Incubate @ 36°C overnight (16-24 h)
Centrifuge @ 3500g for 10 min
Filter supernatant into 100 ml vials

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

