Molecular Virus Detection

The viruses in sewage belong to a diverse range of taxonomic groups that have different genetic and phenotypic properties. These viruses either can infect humans or the aquatic life when introduced to the receiving waters. They are nano in size, known to persist, and have lower infectious does than other microorganisms. These viruses can be single stranded RNA viruses (enteroviruses, caliciviruses, Hepatitis A and E, astroviruses and coronaviruses), double stranded segmented RNA viruses (reoviruses and rotaviruses), bisegmented double stranded RNA viruses (picobirnaviruses, single stranded DNA viruses (parvoviruses) or double stranded DNA viruses (adenoviruses). Also sewage polluted waters may contain bacteriophages which are conventionally used for indication of pollution.

The Rose WQEMM laboratory is capable of identifying and quantifying of enteric viruses that are relatively important for current public health issues. Molecular methods such as polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), quantitative polymerase chain reaction (q-PCR) and quantitative-reverse transcription polymerase chain reaction (q-RT-PCR) are being used for identification and quantification of these viruses in a pollution suspected water body.

Virus Assays available in the Rose WQEMM Laboratory

- Human Adenoviruses (Type 40 and Type 41)
- Human Enteroviruses
- Norovirus Genotype I and II
- Bovine Enteroviruses (1,2 and 4-8)
- Hepatitis A virus

Virus Cell Culture

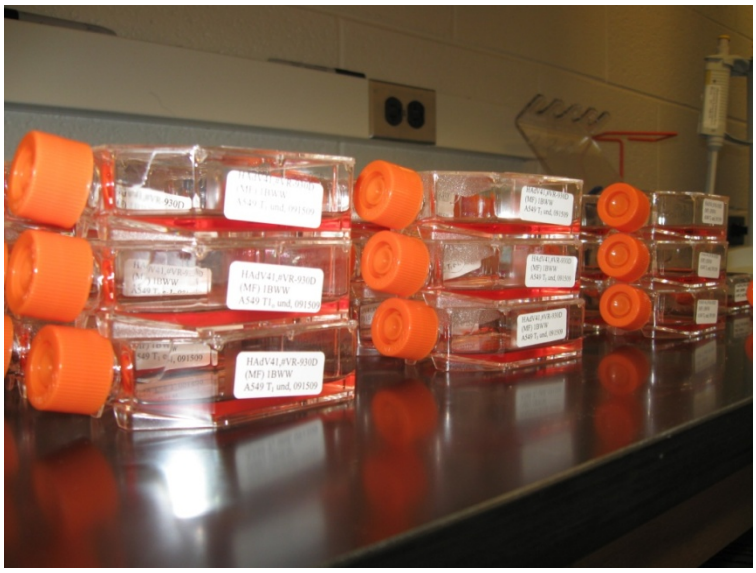
The Rose WQEMM laboratory has various cell lines available to propagate and detect enteric human viruses. Cell lines allow culturable viruses to infect the cell culture and produce plaques. The presence of plaques or "cytopathic effect" (aka CPE), demonstrates the virus is viable and infectious. For example, we are using A549 cells to detect and enumerate human adenoviruses from municipal wastewater using the MPN approach. This cell culture approach is also being used to determine inactivation of human viruses in point-of-use disinfection experiments.

Our lab also uses virus cell culture in combination with ICC-PCR, PCR, QPCR, and Q(RT)PCR.

Approaches used by the Rose WQEMM Laboratory

- A549 cell line for adenovirus strains
- BGM cell line for enteric viruses

Cell culture flasks (25 cm^2) labeled and ready for virus inoculation
(photo taken by Dr. Angela D. Coulliette, Michigan State University)



Coliphage

Coliphage is a type of bacteriophage that infects *Escherichia coli* and can be detected wherever fecal contamination occurs. Concentrations of coliphage virus can be identified using a double agar layer method, appropriate hosts, media, and incubation. Results are reported as plague forming units/volume sample. Coliphage is a good indicator of enteroviruses because they exhibit similar seasonal variation, propensity for removal and resistance to environmental stress, and they do not regrow in the environment. This indicator is commonly used by our laboratory as part of the toolbox for characterization of surface waters in the Great Lakes.

Approaches used by the Rose WQEMM Laboratory

- USEPA Method 1601, Double agar layer (DAL)
- USEPA Method 1602, Single agar layer (SAL)