**Virus concentration using HA negatively charged filter: “Mg-method”**

(Katayama *et al*., 2002, *Appl. Environ. Microbiol.* **68**:1033-1099)

**Materials**

* HA filter: 0.45 µm pore size amd 90 mm diameter (Millipore HAWP-090-00). Filter holder (Advantec) which holds an HA filter should be covered with aluminum foil and autoclaved prior to use.
* 2.5 M MgCl2: dissolve 101.72 g of magnesium chloride hexahydrate (MgCl2・6H2O) with DI water to obtain a final volume of 200 ml, followed by autoclaving.
* 0.5 mM H2SO4: add 1 ml of 100 mM H2SO4 (pH 1.0) into 200 ml of autoclaved DI water. pH should be 3.0.
* 1.0 mM NaOH: add 2 ml of 100 mM NaOH into 200 ml of autoclaved DI water. pH should be 10.8~11.5.
* A tube to receive and neutralize an eluate: add 50 µl of 100 mM H2SO4 (pH 1.0) and 100 µl of 100X TE buffer into a 50 ml conical tube.
* Centriprep YM-50 centrifugal ultrafiltration device (Millipore)

**Procedures**

1. Transfer the collected water sample into a clean, sterile bottle (or, just adjust volume of water in the sampling bottle) to obtain a desired volume: e.g. 100 ml for raw sewage, 1,000 ml for treated wastewater, etc.
2. Add 2.5 M MgCl2 into the water sample to obtain a final concentration of 25 mM; which means 1/100 volume of 2.5 M MgCl2 should be added into 1 volume of the water sample (e.g. 1 ml of the MgCl2 solution for 100 ml sample, 10 ml for 1,000 ml sample). Mix them thoroughly.
3. Place the autoclaved filter holder on a vacuum bin and pass the water sample (w/ MgCl2) through the filter.
4. Acid rinse: Pass 200 ml of 0.5 mM H2SO4 (pH 3.0) through the filter to remove cations on the filter.
5. Pull out the filter holder from the vacuum bin. Hang the 50 ml conical tube containing 100 mM H2SO4 and 100X TE buffer under the filter holder with tapes and place them on another clean, autoclaved vacuum bin.
6. Alkaline elution: Pass 10 ml of 1.0 mM NaOH to elute viruses on the filter, and the eluate can be received and neutralized in the 50 ml conical tube; this is called “1st concentrate”. It may be refrigerated or frozen, if needed.
7. 2nd concentration: Add the 1st concentrate into a Centriprep YM-50 and centrifuge at 2,500 rpm for 10 mins. Discard the portion that passed through the ultrafiltration membrane. Centrifuge again at 2,500 rpm for 5 mins to obtain a final volume of 600~650 µl; this is called “2nd concentrate”.

**Viral DNA/RNA extraction**

**Procedures**

1. Extract viral DNA/RNA using ZR Viral DNA/RNA Kit (Zymo) according to the manufacturer’s protocol.
2. Use 200 µl aliquots of the “2nd concentrate” for the DNA/RNA extraction.
3. Recover the DNA/RNA from the column with 50 µl pure water (25 µl X twice)