Nucleic acid extraction from viral concentrates using QIAmp Viral RNA Mini Kit

The aim of the protocol is to extract nucleic acids (NA) from irrigation water viral concentrates.

Equipment:

Centrifuge	☐
QIACUBE + tubes+ adaptors (optional)	☐
Micropipettes + filter tips	☐
Sterile graduated disposable micropipettes	☐
Sterile plastic tubes of 1.5 mL	☐
Plastic supports	☐

Reagents (see appendix):

QIAmp Viral RNA Mini Kit (22906) - QIAGEN.	☐
Ethanol (96-100%)	☐

Extraction protocol:

A nucleic acid extraction is performed using **QIAmp Viral RNA Mini Kit** (QIAGEN 22906) following the manufacturer's instructions. The total volume of viral concentrate used is **140 µl**.

1. Pipet 560 µl of prepared Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge tube.

2. Add 140 µl of viral concentrate to the 560 µl of Buffer AVL-Carrier. Mix the tube 15 seconds..

3. Incubate at room temperature (15-25ºC) for 10 min.

4. Spin the tubes to remove drops from the inside of the lid.

5. Add 560 µl of ethanol (96%-100%) to the sample. Mix by vortexing during 15 seconds. Spin the tubes to remove drops from the inside of the lid.

6. Carefully apply 630µl of the solution from the step 5 to a QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. Centrifuge at 6000xg for 1 min. Place the QIAamp spin column into a clean 2mL collection tube and discard the tube containing the filtrate.

7. Repeat the step 6 until all sample has passed through the spin column.

8. Add 500 µl of buffer AW1. Centrifuge at 6000xg for 1 min. Keep the spin column, and discard the collection tube and the filtrate.

9. Add 500 µl of buffer AW2. Centrifuge at 20.000xg for 3 min. Keep the spin column and discard the collection tube and the filtrate.

10. Repeat step number 9 but centrifuge for 1 min.

11. Place the QIAamp Mini Spin Column into a clean 1.5mL centrifuge tube (collection tube). Open the column and add 60 µl of AVE. Incubate the Spin Column for 2 min and centrifuge at 6000xg for 1 min.

12. Store the eluted NA at -80ºC for further molecular analysis.