NJHealth New Jersey Department of Health Public Health & Environmental Laboratories	Public Health and Environmental Laboratories 3 Schwarzkopf Drive, Ewing, NJ 08628 POLICIES AND PROCEDURES				
CLIA: 31D0881184 31D2272677 (BTRL)	CLIS: 0002435 32800 (BTRL)	CAP: 9552101 (BTRL Only)	FDA: 34-A-00001	NJDEP: 11036	US EPA: NJ01351
Service Area: PHLS Program: All Unit: Other	Title: **Wastewater - [redacted] Virus Concentration and Kingfisher Extraction**				
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1. **Purpose:** This procedure describes a standardized laboratory protocol for using Nanotrap Microbiome A particles and [redacted] enhancement reagent 1 to concentrate viral particles from wastewater sources using the [redacted] technology. Microbe capture from 10 mL samples has been optimized and is compatible with MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. The extracted nucleic acids will be used for downstream processes, such as polymerase chain reaction (PCR) for the detection of pathogens, such as SARS-CoV-2, as well as other viral pathogenic organisms of interest. The extracted nucleic acids can also be used for Whole genome sequencing.

2. **Definitions:**
   2.1 ER1: [redacted] Enhancement Reagent 1
   2.2 PPE: Personal Protective Equipment

3. **Equipment and Supplies:**

Name	Description
KingFisher™ Apex with 96 DW Head	Thermo Fisher Scientific; Cat# 5400930
KingFisher Apex 24 Combi head	Thermo Fisher Scientific; Cat# 24079940
KF Apex 96 KF heating block	Thermo Fisher Scientific; Cat# 24075920
KF Apex 24 DW heating block	Thermo Fisher Scientific; Cat# 24075940
KingFisher 24 Deep-well Plate, Barcoded	Thermo Fisher Scientific; Cat#95040470B
KingFisher 24 Deep-Well Tip Comb & Plate, Barcoded	Thermo Fisher Scientific; Cat#97002610B
KingFisher 96 Deep-well Plate, Barcoded	Thermo Fisher Scientific; Cat# 95040450B
KingFisher 96 Plate (200 µL), Barcoded	Thermo Fisher Scientific; Cat# 97002540B
Reference 2 Variable Volume Pipette	Eppendorf; Cat# 4924000100

4. **Reagents and Media:**

Reagent Name	Reagent Description
Ethanol VWR; 1006-012 Molecular grade water	VWR; 45001-044

[redacted] Microbiome A Particles [redacted]	[redacted]
[redacted] Enhancement Reagent 1 (ER1)	[redacted]
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit	Thermo Fisher Scientific™; Cat# A42357

5. Specimen Handling and Storage Requirements

5.1 Precipitate can form in ER1 if stored below room temperature. Allow ER1 to return to room temperature to dissolve the precipitate (can invert 2-3 times to help resuspend, do not heat).

5.2 Samples will be collected from wastewater treatment in a way that provides a representative sample from the treatment plant (24-hour composite sample, if possible); a minimum of 200 mL is required. Specimens can be stored at 2-8°C overnight prior to transporting and up to 1-3 days after reaching PHEL. Samples should be stored on ice, to maintain sample integrity, while transporting to the Public Health Environmental Laboratories. If the samples are not going to be processed within 3 days of receipt at PHEL, they should be frozen at -20°C until processed. Frozen samples should be thawed slowly, in 2°C refrigerator over several days.

6. Biosafety:

6.1 PPE - Wear lab coats, gloves, and safety glasses.

6.2 Follow BSL-2 laboratory procedures.

6.3 Ensure that chemicals, spent containers, and unused contents are disposed of in accordance with governmental safety standards.

7. Equipment Calibration and Maintenance Procedure

7.1 Kingfisher Flex system maintenance performed as needed, typically annual service is recommended under service contract per manufacturer's instructions

7.2 Any time maintenance is performed, either scheduled or due to equipment failure/troubleshooting, print the manufacturer provided documentation and place in the instrument maintenance binder located next to each instrument

8. QC Requirements and Frequency of QC

8.1 Description of Daily QC

8.1.1 Extraction control (BCoV)

8.1.1.1 This should only be performed during method development and evaluation or if a run returns aberrant results, not on every instrument run.

8.1.1.2 BCoV tests should be negative for viral targets and positive for the BCoV control.

8.1.1.3 If the BCoV does not amplify targets as expected, but the target amplifies, the test is acceptable.

8.1.1.4 If the BCoV does not amplify targets as expected, and the target also does not amplify, the test is unacceptable and all samples must be reextracted.

8.1.2 Combined Flu-SC2-BCoV or PMMoV positive control.

8.1.2.1 The combined positive control should be positive for all viral targets during dPCR.

8.1.3 No Template Control (NTC)

8.1.3.1 Each PCR plate must contain a negative NTC to detect possible contamination

8.1.3.2 Add 10µL of nuclease-free water to the NTC RT-PCR reaction instead of extracted RNA. Add water to the NTC well prior to leaving the assay preparation area.

8.1.3.3 NTC must be negative for all targets. If NTC has any amplification greater than 2 gene copies on any target, repeat the PCR.

8.1.4 Negative Control

8.1.4.1 Negative Control must be negative for all targets. If NTC has any amplification greater than 2 gene copies on any target, repeat the PCR.

9. Documentation for QC/Calibration/Maintenance

9.1.1 Each daily run is recorded by printing a plate map for the PCR plate and a master mix sheet, on which will be recorded information regarding control performance, the PCR instrument used and lot numbers of all PCR reagents (Appendix FluSC2 Plate Map).

9.2 Corrective Action for Out of Range QC

9.2.1 Failure of any of the controls to meet expected values outlined below in the 'expected results' section requires the run to be repeated.

9.2.2 If quality controls fail upon repeat testing, further investigation should be initiated to determine the source of the error.

10. Procedure – [redacted] Microbiome A MagMAX KingFisher Apex Procedure – Part 1

10.1 *Prepare "Sample Plates 1" and "Sample Plates 2"*

10.1.1 Invert environmental water sample 5 times to mix. After inverting, place on a flat surface for 45 seconds. Decontaminate outside of bottle with bleach.

10.1.2 Add 4,875 µL of environmental water sample to one well (one well per sample) of a new KingFisher 24 Well Deep Well Plate.

10.1.3 Add another 4,875 µL of environmental water sample to the same well location on a second KingFisher 24 Well Deep Well Plate. For example, if you loaded a sample into well A1 of the first plate, load the second volume of that sample into well A1 of the second plate.

10.1.4 Add 50 µL of [redacted] Enhancement Reagent 1 (ER1) Solution to each sample on the two KingFisher 24 Well Deep Well sample plates (100 µL total).

10.1.5 Add 75 µL of [redacted] Microbiome A Particles to each sample on the two KingFisher 24 Well Deep Well sample plates (150 µL total).

10.2 *Prepare "Lysis Plate"*

10.2.1 Add 500 µL of MagMAX Microbiome Lysis Solution to a new (the third) KingFisher 24 Well Deep Well Plate matching the number and location of the "Sample Plate'' wells.

10.3 *Prepare "Tip Plate"*

10.3.1 Insert a new tip comb into a new KingFisher 24 Well Deep Well Plate.

10.4 *Run NT Script (Request file at* [redacted] *)*

10.4.1 Run **WW_Concentration**

10.4.2 Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

10.5 Once the protocol is completed, the "Lysis Plate" will contain lysate that is ready to proceed to Part 2 (*caution* sample may be hot).

11. Procedure – [redacted] Microbiome A MagMAX Kingfisher Apex

11.1 *Prepare MagMAX Bead Binding Plate*

11.1.1 To a new KingFisher 96 Deep Well Plate, add 400 µL of the cleared lysate (NT lysate) from each well of the lysis plate used in "Part 1 step 5" of the protocol.

11.1.2 Add 530 µL of MagMAX Binding Solution to each well in which lysate was added.

11.1.3 Add 10 µL of MagMAX Proteinase K to each well in which lysate was added.

11.1.4 Add 20 µL of MagMAX DNA/RNA Binding Beads to each well in which lysate was added. The total final volume should be 960 µL in each sample-containing well of this plate.

11.2 *Prepare Wash Plate 1*

11.2.1 Add 1 mL of MagMAX Wash Buffer to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher 96 Deep Well Plate- MagMAX Bead Binding Plate wells.

11.3 *Prepare Wash Plate 2*

11.3.1 Add 1 mL of 80% EtOH to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well PlateMagMAX Bead Binding Plate wells.

11.4 *Prepare Elution Plate*

11.4.1 A dd 100 µL of MagMAX Elution buffer to a new KingFisher 96- 200 µL plate matching the number and location of the MagMAX Bead Binding Plate wells

11.5 *Prepare "Tip Plate"*

11.5.1 Insert the KingFisher 96 Deep Well Comb into a new KingFisher 96 Deep Well Plate

11.6 *Run Extraction Script (Request file at [redacted])*

11.6.1 Run **WW_Extraction**

11.6.2 Follow the on-screen instructions loading the previously prepared plates at the appropriate time

11.7 *Storage of extracted materials*

11.7.1 Plate containing extracted total nucleic acids should be stored at -80℃ (freezer in L315) until digital PCR testing.

12. Procedure – Limitations

12.1 A false negative result may occur if specimen is improperly collected, transported or handled. False negatives may also occur if amplification inhibitors are present in the specimen or if inadequate number of organisms are present in the specimen.

13. References

13.1 [redacted]