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# Collection from Wastewater Treatment Plant, Transportation, and Storage of Raw Wastewater

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This method was developed at the FDA's Center for Food Safety and Applied Nutrition for GenomeTrakr's pandemic response project, monitoring SARS-CoV-2 variants in wastewater. Protocols developed for this project cover wastewater collection, concentration, RNA extraction, RT-qPCR detection, library prep, genome sequencing, quality control checks, and data submission to NCBI. This method describes the collection and transport of raw wastewater samples from a wastewater treatment plant.

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Samples can either be collected as "grab" samples or "composite" samples. Grab samples are either a single discrete sample or individual samples collected and pooled over a period not greater than 15 minutes; grab samples are intended to be representative of the wastewater at a single point in time. Composite samples are collected over a greater time frame, generally by an automatic sampler; composite samples are either based to capture variations in flow (flow-paced) or time (time-paced). Methods for collecting both types of samples are provided (Grab Sampling in Section 1 and Composite Sampling in Section 2).

### Specialized Equipment

1. Insulated cooler (Pelican CAT # 20282166, or equivalent)
2. Wet ice (preferred) or blue ice
3. Swing arm pole sampler (NASCO CAT # B01366, or equivalent) for grab samples
4. Hype-Wipes (Fisher Scientific CAT # 14-412-56, or equivalent)
5. Smart Button Temperature Loggers (ACR Data Solutions CAT # 01-0185, or equivalent)
6. Automated sampling system (Teledyne ISCO, or equivalent)
  - ISCO 6712 Full size portable sampler (CAT # 686710070)
  - ISCO 12 bottle configuration kit (CAT # 686700008)
  - Model 934 Nickel Cadmium Battery ( CAT # 601684040)
  - Model 961 battery charger (CAT # 603004059)
  - Polypropylene bottles with caps, 100 round, 1 L (CAT # 606700016)

Collection of raw wastewater from a WWTP can pose physical and biological hazards. The health and safety of collection personnel must never be at risk during sampling. Adhere to all site-specific Health and Safety Plans for the WWTP. At a minimum, personal protective equipment (PPE) should consist of gloves and eye protection.

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Prior to initiating routine collections from a wastewater treatment plant (WWTP), an appropriate sampling location should be identified. Key factors in selection of a sampling location include:

- Readily accessible location for collection of raw influent. Most WWTP have access immediately after bar or rotary screeners.
- Flow and mixing to ensure collection of a representative sample. Sampling from the center channel at 2/3 depth where it is generally most turbulent is recommended. Avoid sampling from edges, corners, and deadheaded channels.
- Ability to access the same location each sampling occasion without impeding normal WWTP operations.

#### Grab Sample Collection

- 1 When possible, new sample containers should be used. If not feasible, sample containers should be clean and autoclave sterilized prior to collection.
- 2 Ensure sample container(s) are labeled with indelible marker or waterproof labeling.
- 3 Grab samples can be collected using a peristaltic pump (section 3.1) or a dip pole (section 3.2). Ensure sample is collected from a consistent location with adequate mixing to be representative of the wastewater.

An automated sampler (e.g., ISCO) can be used as the peristaltic pump, if available.

- 3.1 When using a peristaltic pump, place end of suction line into appropriate collection area (as described above in "before start" section).
  - Start pump.
  - Purge collection tubing by directing the tubing outlet to flow into the influent waste stream until approximately a liter flushes the tubing.
  - Shut pump off.
  - Move collection tubing outlet to the sample container and turn on pump to deliver intended sample volume.
  - Shut pump off and cap the bottle tightly.
  - Run pump in reverse direction to empty the collection tubing, paying close

attention to facilitate all the influent making its way out of the tubing.

- 3.2 When using a dip pole, ensure the collection bottle on the pole is clean and securely fastened to the pole.
- Dip the collection bottle into the appropriate collection area (as described above in "before start" section) to rinse at least three times with the influent waste stream.
  - Collect sample.
  - Transfer the collected wastewater into the sample container.

Do not collect sample directly in sample container.



- 4 Ensure adequate headspace (at least 3-4 cm) exists in the sample container to allow for complete mixing prior to analysis.



#### Composite Sample Collection

- 5 When possible, new sample containers should be used. If not feasible, sample containers should be clean and autoclave sterilized prior to collection.
- 6 Ensure sample container(s) are labeled with indelible marker or waterproof labeling.
- 7 Ensure suction line of the ISCO is placed downstream in the headworks, preferably after screener and de-gritting. Generally, the weighted suction strainer should but placed in an area of high hydrological turbulence and not touching the bottom or sides of influent channel (as described above in "before start" section).
- 8 Follow manufacturer's instructions to calibrate the collection volume of the ISCO to ensure adequate headspace (3-4 cm) will exist in the sample bottle(s) to allow for complete mixing prior to analysis in the laboratory.

Calibration should not need to be done on each sampling occasion.



- 9 Fill base of ISCO with ice and secure collection bottles in proper orientation. Add ice to remaining space around the collection bottles to ensure sample(s) remain cool from collection to transport, as necessary. If available, include temperature recording devices (Smart Buttons) to monitor sample temperature.



- 10 Composite samples can be collected as flow-paced or time-paced. Follow manufacturer's instructions for proper programming of the ISCO and then initiate collection.



If using the WWTP's automated sampler, be aware that they are generally not set up for aseptic sampling. It is strongly recommended to discuss sampling requirements with facility management prior to initiation of any sampling.

- 11 After appropriate time, return to WWTP to collect filled sample bottles.

If sample bottle(s) are overfilled, do not pour out sample. Allow transfer to a larger vessel for adequate mixing at the laboratory. Re-calibrate the ISCO for collection volume prior to subsequent collections.

## Sample Transportation

- 12 Ensure wastewater sample bottles are tightly sealed and decontaminate the surface with a bleach-based disinfectant (i.e., Hype-Wipes).
- 13 Place wastewater samples in secondary containment (polyethylene bag) and then place on wet ice in an insulated container (i.e., cooler). When possible, transport bottles in a rack to ensure they remain upright.
- 14 Ensure adequate ice is present to secure the bottles in the transport container and maintain sample temperature below  $\delta$  8 °C for the duration of transportation.



If samples are to be shipped by commercial carrier, adhere to appropriate packaging and labeling requirements of IATA regulations for biological substances.

## Sample Storage

- 15 If samples cannot be processed immediately, samples should be stored at  $\delta$  2-8 °C for up to 7 days.
- 16 If samples need to be stored longer than 7 days, frozen storage at  $\delta$  -70 °C or  $\delta$  -20 °C is recommended.