Collection Protocols for Metagenomics

Sewage sample collection and handling:

From sewage treatment plant:

1 to 2 L of unprocessed, non-filtered and untreated sewage to be collected over 24 hours from each site in clean and sterile containers.

If continuous sampling for 24 hours is not feasible, collecting 1-2 L can done over a course of 1 hour by collecting a subset of the volume periodically over the 1-hour.

The raw sewage can be stored frozen at −80 °C until transportation to the lab if needed. If the sewage samples are to be processes immediately, please see sample processing below.

All sewage samples are to be transported untreated with any chemicals or DNA stabilizers and remained frozen upon arrival until DNA extractions.

From open sewage:

Collect 1 to 2 L of sewage directly from the open sewage system at various points to ensure a representative sample of the system, in clean, sterile containers.

If possible, aim to sample at different times to capture diurnal variations, ensuring a comprehensive representation of the microbial community.

If immediate processing is not possible, store the raw sewage at −80°C. If the samples are to be processed immediately, proceed to sample processing.

Photograph the location if possible.

Sample processing and preparation:

Label each sample with unique name, the date, time, and exact location of collection. If possible photograph the sewage bottle.

Provide as much metadata as possible, e.g., exact location, arrival conditions, colouration, and which sewage plant. No human or personal data were collected or linked to the biological samples.

If the samples are frozen, defrost 500 mL from each sample slowly over 2 days at 4 °C in order to maintain the community stable as much as possible and avoid drastic or sudden changes to the bacterial communities.
If an outbreak needs to be investigated immediately defrost the samples at RT.

Once thawed, centrifuge the 500 mL sewage to end up with two sewage pellets (each pellet is accumulated from spinning down 250 mL – this will depend on which centrifuge is available in the lab). Centrifugation is done on the raw thawed sewage for 10 minutes at 10,000 x g to collect sewage pellets from each sample. E.g., if the centrifuge allows tubes of 50 mL, split the 250 mL sewage into 5 50mL tubes then centrifuge them 10 min at 10,000 x g then collect all those pellets as one pellet from the 250 mL.

Keep the supernatant in separate containers, freeze it down at −80°C until needed for viral detection and analyses.

All sewage pellets to be stored at −80 °C until DNA extractions.

Proceed to DNA extraction similar to the faecal metagenomic DNA extraction and sequencing protocols.

Faecal sample collection and handling:

Collect faecal samples using a clean, sterile scoop or spatula. If collecting from a toilet, ensure collecting directly from the faeces as much as possible to avoid diluting the sample with extraneous materials.

Place the collected faecal sample into a sterile, leak-proof container. Each container should only hold one sample to prevent cross-contamination.

If collecting from pit latrines or similar facilities, take samples from different locations within the pit to ensure a representative sample.

Aim to collect approximately 2 grams of faecal material in a sterile tube.

faecal samples for DNA extraction are to be transported cooled down (on ice or frozen gel packs) until a freezer is available (24-48 hours chilled down are acceptable) without any buffer added, natural faeces.
If RNAlater is available (or similar nucleic acid buffers and shields), please submerge the faeces in RNAlater, then the samples can be transported at room temperature. Faeces in RNAlater can be frozen when freezer is available.

Clearly label each sample with the date, time, and specific location of collection.

Proceed to DNA extraction.