



Collaboration on Sewage Surveillance of SARS CoVID-19 (ColoSSoS)

Monitoring SARS CoVID-19 virus presence and persistence in the Australian sewage networks.

Objective 1. Sampling plan for SARS-CoV-2 sewage surveillance (first published March 2020 – latest review and update September 2020)

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Purpose of sampling

- In areas where there are no known active or recent positive cases of coronavirus (COVID-19), a positive wastewater testing result may provide an early warning and indicate there may be people within that community who have not been diagnosed, but have or have recently had coronavirus (COVID-19).
- Define outbreak peaks, disease persistence and re-emergence
- Increase understanding of the extent of asymptomatic infections

What to sample

- Across each state
 - Sample as per utilities routine program at Sewage Treatment Plant (STP) inlets:
 - 24h composite samples from autosampler, preferably refrigerated (composite sampling is the recommended method to obtain most accurate and precise results): Individual discrete samples taken at regular intervals over 24 h. 1 h regular interval is the common practice of utilities, however if possible reduce the volume of each discrete sample and decrease the sampling interval to 15 minutes to capture a more representative composite sample; or
 - Grab samples (minimum of 1 grab sample per week unless specifically directed by the relevant state health agency)

- Metropolitan areas
 - Additional to the metro STPs, sample selected locations within metro sewage network – will be a function of number of sampling opportunities (e.g. manholes, pump stations), total number of samples that can be stored and processed (at least 1 sample per week).
- **IMPORTANT Note:** Sampling program must include consistent sampling method for any one sampling point.

Metadata

- Clearly record the following mandatory items in the metadata and chain of custody:
 - type of collected samples (composite or grab),
 - if applicable: Configurations of the autosampler (refrigeration temperature and mode of operation – see figure at the end of this document)
 - sampling location and date,
 - time sampled,
 - temperature at laboratory when received,
 - storage facility
 - time to being cooled and stored
 - Flow rate (ML/D) at the sampling period/date
- Desirable data, not-mandatory (these data can be obtained from utility records post-sampling): Usual EPA sewer monitoring parameters, like BOD, TSS, E. coli, pH, weather conditions, chlorine residual for any treated water, and ammonia (as a crude indicator of sewage biological age).

Length of sampling program

- Plan on 10 months, so 40 weeks of sampling

Sampling frequency

- At least once per week, with twice per week being preferable (following DHHS advice in Victoria)

Sampling and storage

- 0.5 L per location as in 2 clean and sterile 250 mL polycarbonate bottles (1L is a preferred volume in 4 clean bottles however due to storage issues the sample volume is reduced to 0.5L with DHHS agreement in Victoria),
- no fixative needed,
- transported on ice from point of sampling to point of storage,
- stored at 4°C

IMPORTANT note: All collection bottles used in grab or composite sampling must be suitable for microbiological sampling. That is, they must be clean and sterile. Under no circumstance should bottles be left in an autosampler for 'repeat sampling' as this can lead to carry-over contamination. The sampler must wear the necessary PPE (mask and laboratory grade gloves) to avoid contaminating the sample. Gloves should be changed as required to avoid cross contamination.

Safety

- Standard PPE for handling raw sewage, including safety glasses, masks and gloves.

Actions

1. Each state to produce a state map showing locations of STPs, hospitals and COVID-19 cases (at publicly-available resolution, typically Local Government Area). Catchment population size and average daily dry weather flow to be provided as metadata for each STP.
2. Each state to produce a metro map depicting same as above plus sewage mains, network sampling points and manholes.
3. Determine how many samples would be taken given proposed sampling plan, and assess practicalities, e.g. storage space.

Types of Composite Samples

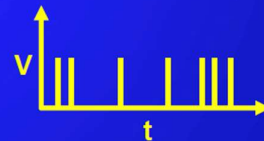
- **Composite Sample is defined by the time interval between aliquots, and the volume of each aliquot (t, V).**

- **Time Proportional (t_c, V_c): Interval time and sample volume are constant**



- **Flow Proportional: Interval time or sample volume may vary**

- **Constant volume (t_v, V_c)**



- **Constant time (t_c, V_v)**

