Attachment 1: Criteria for rapidly selecting laboratories for this type of urgent and quality critical work

Traps for beginners: why we need to get the choice of labs right…

Type 1 errors leading to the virus being apparently detected in sewage samples when in fact it's absent, or being detected at higher than its true concentration.

1. Sample contamination. PCR reactions are highly sensitive and notorious for detecting contaminants from traces of their target genetic material from environmental and laboratory contamination. For instance, the PCR reactions themselves generate a lot of the material that is sought by the reaction and so it is very easy to accidentally detect that material - particularly during incident or high pressure situations.

2. Non-target amplification due to too broad a specificity. There may be other genetic sequences that are detected by the assay that makes them appear to be positive for the virus. Genetically similar viruses or other similar genetic sequences could be present in wastewater resulting in the apparent detection of the COVID-19 virus at locations that are in fact free of the virus.

3. Non-patient introduction of COVID-19 viral RNA. In locations such as pathology, research and hospital labs, there may be COVID-19 virus material generated from diagnostic tests, or used as control material, that might be improperly disposed of and might be present in sewage.

Type 2 errors leading to the virus being apparently absent in sewage samples when in fact it's present, or being apparently detected at lower than its true concentration.

4. Inhibition. Wastewater is diverse and variable and many substances in wastewater will inhibit PCR reactions and stop them working at all or reduce their sensitivity.

5. Missing some viruses due to too narrow a specificity. The COIVD-19 virus continues to mutate - there are multiple clades in circulation and primers will only detect a sub-set of those in circulation. The more specific the primers the less variants of the virus will be detected.

6. Poor extraction. A particular problem given the diverse nature of wastewater is extracting viruses and it's possible that extraction efficiencies will be highly variable between wastewater samples.

7. Interfering substances. A range of substances in wastewater might interfere with virus persistence and might break down the viruses, e.g. if the wastewater flow is particularly warm or has extreme pH or elevated detergent present.

Communication issues

8. Poor communication. Major dramas could arise from poor communication of the results – sewer workers walking off the job, public fear and concern about sewer outfalls, misunderstanding of results leading to over- or underreaction by third party decision-makers. Results need to be rapidly, but carefully and intelligently, communicated. We need all testing to be done in that context and not just done and released publicly without due care.

Implications

To manage issues such as the above requires carefully designed and proven protocols, the use of numerous positive and negative controls, including trip controls and laboratory controls.

Furthermore, to enable comparison of results, there needs to be come proficiency testing between labs, and at least some standardisation. For instance, whilst there can be good reasons to use different extraction and analysis methods, at least some samples should be analysed using common, standard extraction and analysis methods, to enable comparison. Likewise, some common reference materials and controls should be used.

This is important because poor quality results have major potential negative implications. Not finding the virus where it is present could lead to a false sense of security or backing off on controls. Finding the virus where it is not could lead to major local control impacts and undue concern. These could be major consequences in both cases.

Recommendations for labs given the above:

- There is no point every lab in the country doing the testing. Better to focus on, and support, a small number of labs and, if we need more, to then have those labs train the others and share protocols and reference standards.
- The labs need to be able to rapidly do this work so need to have:
    - Solid experience with **molecular viral analysis** from **wastewater**
    - **Scale** to handle multiple samples – potentially hundreds per week.
    - **Be trusted by utilities and health agencies** – confidentiality issues are extremely sensitive on this one – we can't have results being 'leaked' or published prior to utility and health agency agreement and knowledge.
    - **Be engaged under contract** by utilities already to enable rapid testing and avoid delays with forming new contracts (at least at first).
    - **Reliability** and QA/QC – NATA accredited for their normal work.
    - Safety issues covered
    - Be open during this period
    - Not be flooded with more pressing priorities
    - Sensitivity is critical – so pathology labs might not have the required sensitivity for testing sewage so probably not an option – the labs need to be environmental/sewage molecular testing labs.
- This means the major water utility labs and their existing viral analysis labs, and their experienced and trusted collaborating virology labs that have experience with wastewater sampling, are the first port of call. There isn't time for a formal request or tender. Most of the testing will be directly funded by utilities and/or health agencies, or if it's funded via WRA from grant support we'd still use the existing utility and health agency contracts and labs to keep things consistent.
- In future additional labs can be set up and protocols shared but that is not a fast process and they'd need to be set up. So that would be once we get routine testing underway. This 'tech transfer' might include regional labs and international labs.
- To help with scale-up, if we can secure a decent length of funding we can look at up-staffing or funding could be used to support routine duties, or routine duties could be outsourced to less sophisticated labs, to free up the more advanced labs' staff and equipment to support this surveillance – if required.
- *Action*: To get going as fast as possible and avoid delays in contracting etc., conduct the work with labs that are experienced and trusted by the utilities and that already have contracts in place at first and then build up from there to draw in other labs and expertise as time and scale permit and require.

Laboratories that meet ColoSSoS Laboratory Criteria include:

WA – Path West
SA – SA Water Research Laboratory; Flinders University; UniSA (Barbara Drigo)
VIC – ALS
NSW – Sydney Water Laboratories