

METHODOLOGIES 101 – a guide to wastewater testing methods explored in RSA



The South African science community has made remarkable progress in detecting coronavirus RNA in wastewater. It has taken several research initiatives, individuals, academics and practitioners to get to this point in the collective fight against Covid-19. Ultimately, the biggest development has been the establishment of the South African Collaboration Covid-19 Environmental Surveillance System (SACCESS) network. Another key element has been the standardisation of methodology and sampling methods.

The compendium explores the different methodologies various institutions applied in the detection of SARS-CoV-2 RNA in wastewater.



The following methods were explored by the **National Institute for Communicable Diseases (NICD)**:

- The preferred concentration method was ultrafiltration using the Centricon® Plus-70 centrifugal ultrafilter device
- The PEG/dextran two-phase separation method was used in the absence of centricon ultrafilters

The **Water Research Commission (WRC)** used three methods (in one instance two methods at a single testing site)

- The PEG 8000/NaCl precipitation method and Skimmed Milk flocculation were used at the same testing sites and therefore could be directly compared.
- The Aluminium Hydroxide Adsorption-Precipitation method was used together with Skimmed Milk flocculation and were both found to be faster and cheaper than PEG 8000/NaCl

Recommendation: Although various methods can be used interchangeably between laboratories, because of inherent variability it is recommended that the same method be applied to the same site when monitoring trends over time

Observation: The commercial multiplex Seegene assay and QuantiFast Pathogen Kit in-house N1 and N3 assays all detected SARS-CoV-2. The Seegene kit is more sensitive than the Quantifast N1 assay, and has the advantage of amplifying 3 targets in one reaction, and is more likely to be more consistent across laboratories



The following methods were explored by the **Institute of Water and Wastewater Technology, Durban University of Technology**

- PEG 600/NaCl. The method described by Kocamemi et al. (2020) was slightly modified and used in this study
- The PH of each solution had to be adjusted to 7.0 – 7.2 with this method as characteristics of wastewater from the four domestic wastewater treatment plants varied
- Another method explored is Centricon Plus 70 10k

Lesson learned – Characteristic of each sample must be analysed and considered because of the varying characteristics of wastewater between and within the plants

[Click here to download the Compendium](#)

[Click here for more information on the COVID surveillance programme](#)



Tangential Flow Filtration (TFF) method was used by Lumegen Laboratory

- They found that the added benefit of the method was its preservation effect around viral particles to a greater degree than other methods

Observation: The application of TFF is widely used in the areas of biotechnology, mainly for the purification of cells, virus and bacterial proteins



In the Western Cape the standard curve method was applied by **South African Medical Research Council**

- An important yet unexpected outcome was that this study brought together professionals and experts spanning skill sets, disciplines and sectors including public health, microbiology, town planning and wastewater treatment facilities, and formed the basis for a comprehensive weekly follow-up study that includes a larger number of wastewater treatment plants being sampled over consecutive days with the overall agenda of advancing equity and health.

RESEARCH PARTNERS

