# Title: Investigating The Presence of SARS-CoV-2 in Wastewater at Three Wastewater Treatment Plants in Gauteng, South Africa

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### Abstract:

Introduction

The coronavirus disease (COVID-19) pandemic caused by widespread severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, resulted in global alarm due to its high mortality rate and negative socio-economic impacts. Subsequently, SARS-CoV-2 genetic material detection in wastewater raised concerns over potential occupational exposure to wastewater treatment plant (WWTP) personnel and implications for water reuse.

**Objectives** 

- Determination of SARS-CoV-2 RNA in wastewater and aerosols
- Comparison of viral load differences between samples from the different sampling/process points and treatment plants
- Evaluation of the infection potential of SARS-CoV-2 detected in wastewater and its implications for worker health and water reuse
- Determination of viral DNA/RNA pathogens present alongside SARS-CoV-2 in wastewater.

### <u>Methods</u>

Samples (wastewater, contact surface swabs, and bioaerosols) were tested for SARS-CoV-2 RNA using real-time reverse transcriptase PCR, with positive samples subsequently analysed for SARS-CoV-2 RNA infectivity using Vero-E6 cells. Results and Discussion

SARS-CoV-2 RNA was detected in 65% (111/172) of the samples. The percentages of positive detection varied between sample types, namely the influent (74%, 26/35), primary sludge (100%, 26/26), secondary settling tank effluent (71%, 20/28), activated sludge (66%, 19/29), final effluents (57%, 16/28), surface swabs (25%, 4/16) and bioaerosol (0%, 1/10) samples. The detected SARS-CoV-2 RNA concentrations across all three WWTPs ranged between 1.11x  $10^{\circ}$  and 4.19 x  $10^{2}$  gene copy equivalents/ml, with viral RNA decay along treatment stages observed only in two plants. Infectivity tests showed no infection potential. Testing for other viral pathogens is still underway.

#### **Conclusion**

The presence of SARS-CoV-2 RNA in the final treated effluents suggests residual viral RNA persists despite wastewater treatment. However, infectivity studies suggest that SARS-CoV-2 loses viability in wastewater, indicating no direct evidence of health risks to WWTP personnel or during treated wastewater reuse.