

# An overview of qualitative and quantitative testing of wastewater for SARS-CoV-2 from inception to date from the SACCESS network partners

Nkosenhle Ndlovu : Medical Scientist

Centre for Vaccines and Immunology, National Institute for Communicable Disease



# Overview

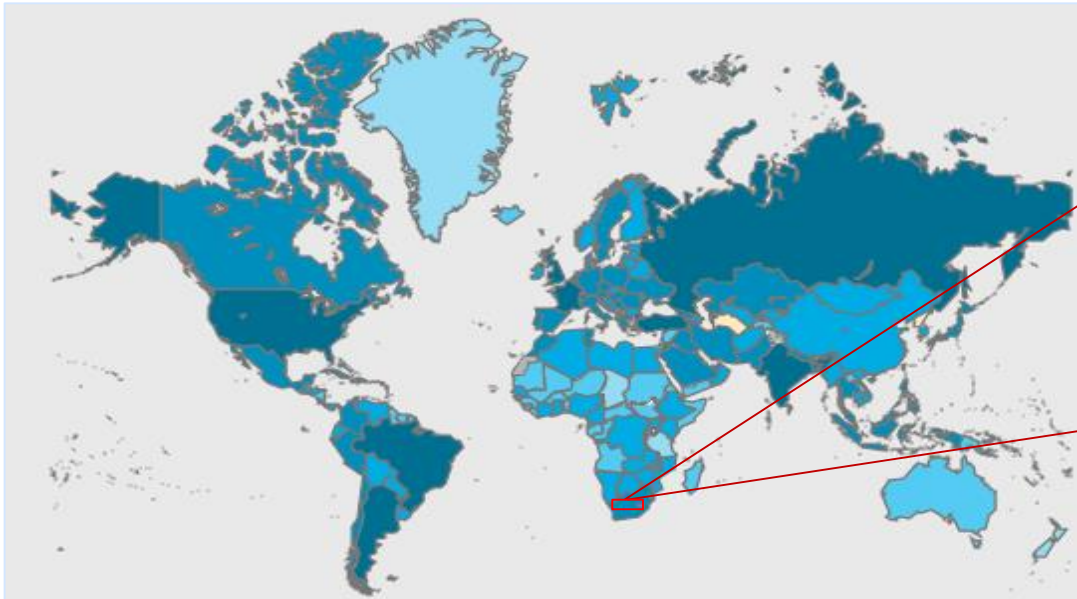
- Background
- Evidence of wastewater as tool for SARS-CoV-2 Surveillance
- The SACCESS network partners
- Roadmap of the SACCESS network
- Coverage of the SACCESS network
- Total number of tests within the SACCESS network
- Processes at NICD
- Qualitative and quantitative testing for SARS-CoV-2 at NICD
- Qualitative results of the SACCESS network
- Quantitative results from NICD
- Acknowledgements

Section A

Section B



# Background



NICD surveillance report as of 24 August 2021

10,346 – New cases

2 708 951 - Total confirmed cases to date

9 953 - Total deaths to date

WHO Dashboard globally, as of 5:56pm CEST, 24 August 2021

434,852 - New cases

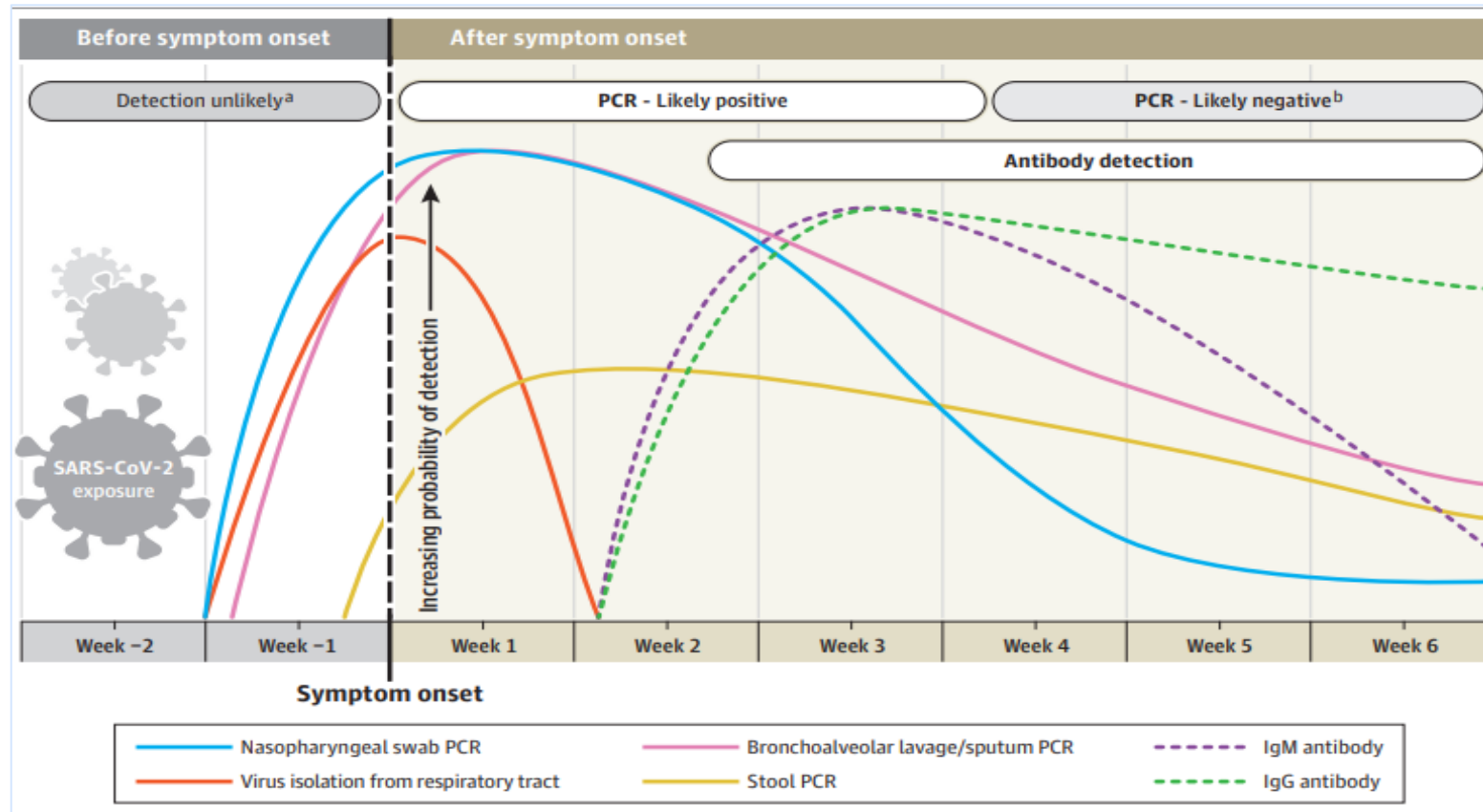
212,357,898 – Total confirmed cases to date

4,439,843 - Total deaths to date

- SARS-CoV- 2 is part of the coronaviridae family
- RNA virus – susceptible to heat
- Contains a lipid envelop



# Background



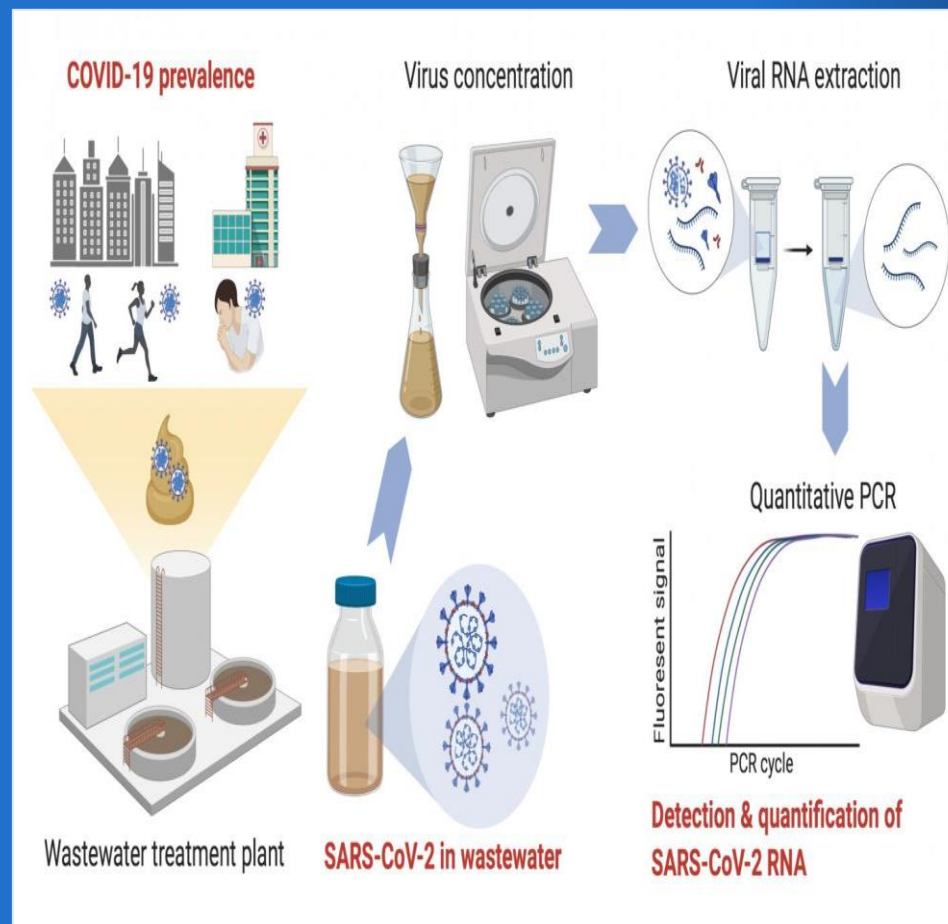
Sethuraman N et al. Interpreting diagnostic tests for SARS-CoV-2. Jama.

# Evidence of wastewater as tool for SARS-CoV-2 Surveillance

**Table 1**  
Overview of selected SARS-CoV2 studies in wastewater conducted in 2020.

Study region	Genetic traces/genes analysed	Results	Reference
Paris (France)	RdRp	Samples in three WWTPs positive between 5th March and 23th April	Wurtzer et al., 2020
Milan and Rome (Italy)	ORF1ab, S	6 out of 12 samples positive in raw wastewater	La Rosa et al., 2020
Netherlands	N1, N2, N3, E	4 out of 7 wastewater samples positive at the beginning of March. 9 out 9 wastewater samples positive in the middle of March	Medema et al., 2020
Milan (Italy) (WWTP and river)	ORF1ab, N, E	First sampling: 3 out of 4 raw wastewater samples positive 2 out of 2 effluent samples negative 2 out of 2 river samples positive	Rimoldi et al., 2020
Brisbane (Australia)	N and confirmation via Sanger and MiSeq Illumina sequencing	2 out of 9 samples positive in raw wastewater	Ahmed et al., 2020
Massachusetts (USA)	N1, N2, N3, E	10 out of 10 raw wastewater samples positive	F. Wu et al., 2020
Israel (different cities and facilities)		2 out of 15 samples positive in March 2020 9 out of 11 samples positive in April 2020	Bar Or et al., 2020
Bozeman, Montana (USA)	N1, N2	7 out of 7 positive in March/April 2020	Nemudryi et al., 2020
Istanbul (Turkey)	RdRp	9 out of 9 sludge samples positive	Kocameci et al., 2020
Valencia (Spain)	N1, N2, N3	35 out of 42 influent samples positive 2 out of 18 secondary treated samples positive 0 out of 12 tertiary effluent samples positive	Randazzo et al., 2020
Yamanashi Prefecture, Japan	N1, N2	None of 5 influent samples positive 1 out of 5 secondary effluent samples positive None out of 3 river water samples positive	Haramoto et al., 2020
Ahmedabad, Gujarat, India	ORF1ab, N and S	2 out of 2 influent samples positive 2 out 2 effluent samples negative	Kumar et al., 2020
Louisiana, USA	N1 and N2	2 out of 15 raw wastewater samples positive Effluent samples all negative	Sherchan et al., 2020
Quito, Ecuador	N1 and N2	3 out of 3 river water samples positive	Guerrero-Latorre et al., 2020

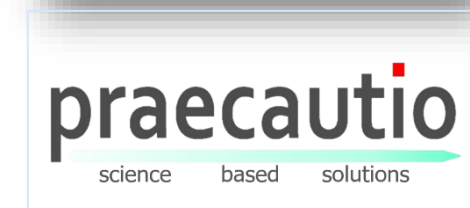
Westhaus S et al. Detection of SARS-CoV-2 in raw and treated wastewater in Germany—suitability for COVID-19 surveillance and potential transmission risks.





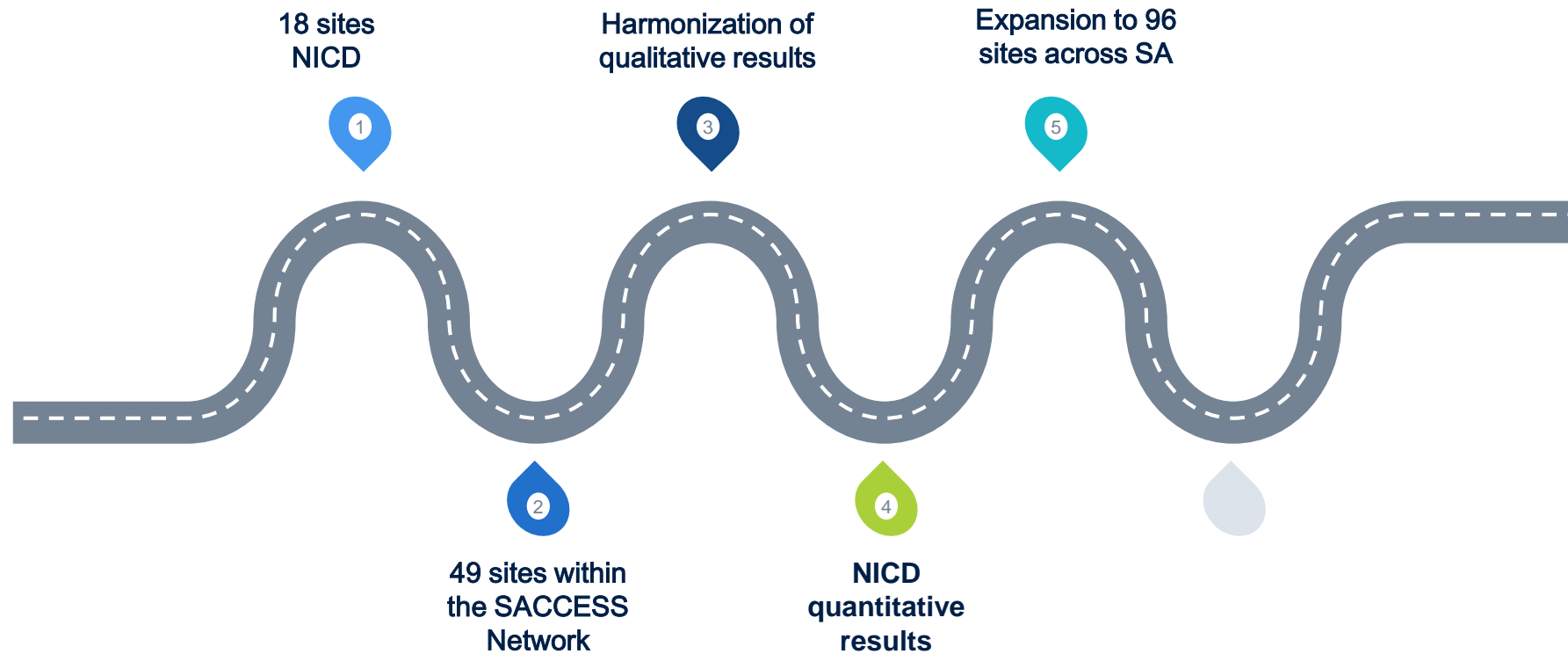
# The SACCESS network partners

Name of Laboratory partner	Method for virus concentration	Method for nucleic acid extraction	RT-PCR assay
NICD	Centricon® Plus-70 centrifugal	QIAamp® viral RNA mini kit	Allplex™ 2019-nCoV Assay
CSIR	PEG	Omega Bio-tek E.Z.N.A total RNA Kit II	2019-nCoV CDC EUA Kit
NIOH	Skim milk flocculation	MagMAX Viral and Pathogen Nucleic Acid Isolation Kit	Thermofisher (TaqPath kit)
Waterlab/UP	Skim milk flocculation	QIAamp® Ultrasens® Virus kit	Allplex™ 2019-nCoV Assay
SAMRC-TB	Ultra centrifugation	ZymoBIOMICS kit	2019-nCoV CDC EUA Kit
SAMRC-BRIP	Centrifugation	RNeasy PowerSoil	2019-nCoV CDC EUA Kit
Lumegen	TFF	Nucleomag RNA/DNA pathogen kit	Thermofisher (TaqPath kit)



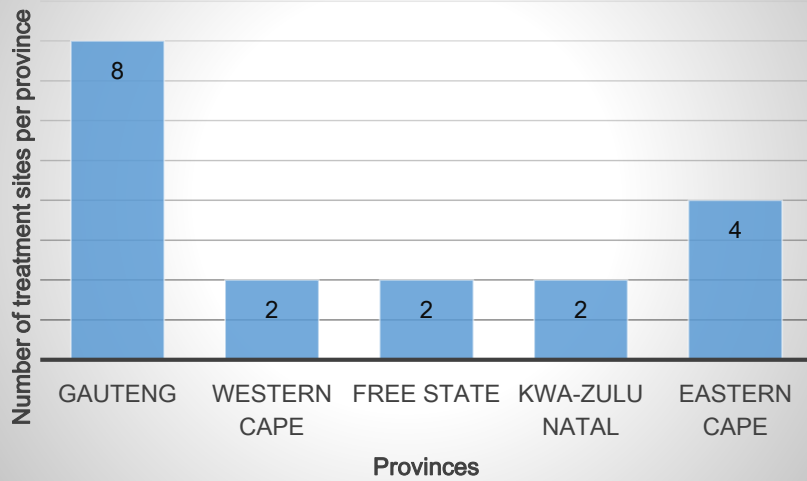


# Roadmap



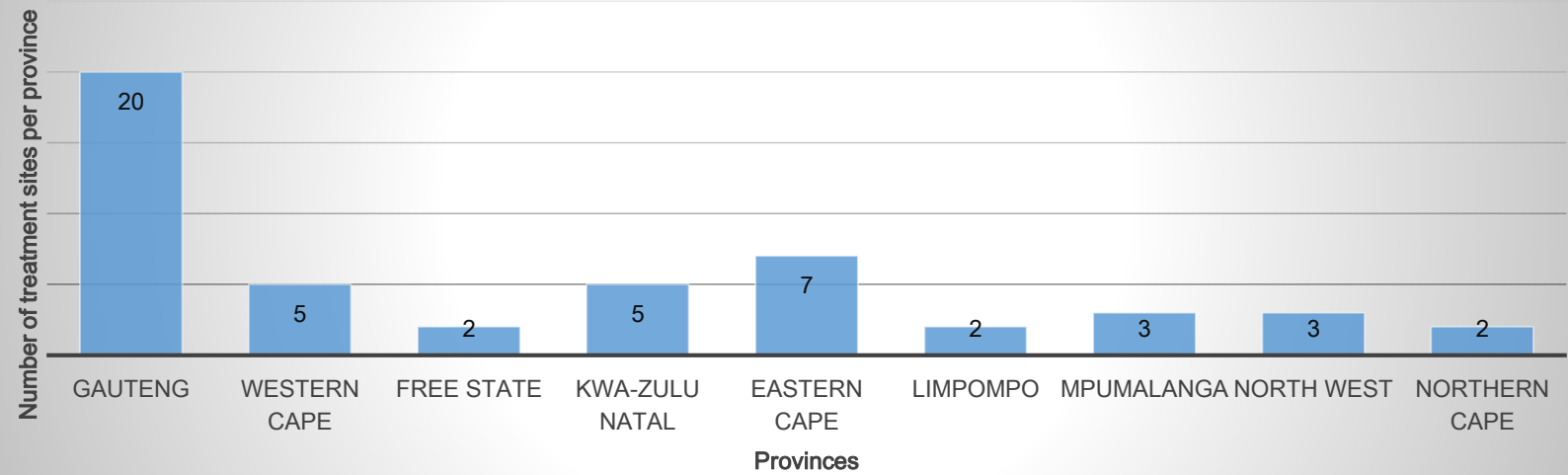


### Coverage in 2020



- 5 provinces
- 18 treatment sites

### Coverage in 2021



- 9 provinces
- 49 treatment sites



# Total number of tests within the SACCESS network for the period 2020 -2021



Testing lab	Total tested	Total positive	% positive
CSIR	41	19	46%
Green hill	27	25	93%
MRC-BRIP	39	36	92%
Water lab	111	46	41%
Lumegen	84	33	39%
NICD	413	355	86%
NIOH	36	22	61%
Praecautio	9	7	78%
SAMRC TB	36	30	83%
<b>Total</b>	<b>796</b>	<b>573</b>	<b>72%</b>



# Processes at NICD

- Samples receipt
- Logging samples on system

## Data analysis

- Counting the number of COVID cases per district
- Calculating relative log genome copies/ml
- Entering results from lab partners
- Results review

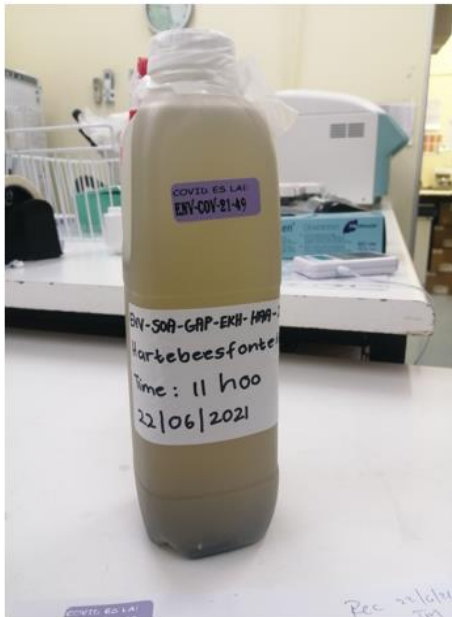
## Wet lab processes

- Specimen concentration
- RT-qPCR

## Reporting

- Qualitative results and quantitative graphs
- SACCESS network
- NDoH
- IMT

# Qualitative and quantitative testing for SARS-CoV-2 at NICD



COVID-ES-LAI ENV-COV-21-49 Rec 22/06/21 TM

Polio & SARS-CoV-2 Environmental Surveillance Form

Site Details	
ID Code	ENV-SOA-GAP-EKH-HAA-21-009
Province	Gauteng
District	Ekurhuleni
Site Name	Hartebeesfontein
Province Code	GAP
District Code	EKH
Site Code	HAA
Compulsory Fields	
Site sample number	007
Sampling method (please tick)	YB6 NO
Time of sample collection (hh:mm)	11:00
Date of sample collection (dd/mm/yyyy)	22/06/2021
Rainfall within last 24 hours (please tick)	YES NO
Atmospheric temperature at time of sample collection (°C)	18 °C
Date sample sent to laboratory (dd/mm/yyyy)	22/06/2021
Details of person collecting the sample	
Name	J. Nel
Telephone	011 999 3955
Signature	[Signature]
Name	J. Nel
Telephone	011 999 3955
Signature	[Signature]

Additional Fields - Please complete if available



Maintained at 4°C before concentration

Centricon Plus-70® centrifugal ultrafilter

# Qualitative and quantitative testing for SARS-CoV-2 at NICD



- Nucleic acid extraction is conducted using the QIamp viral RNA mini kit (Qiagen)
- RT-qPCR - Seegene Allplex 2019-nCoV PCR Kit (Inqaba Biotechnical Industries)
- Exact Diagnostics SARS-CoV-2 Standard (Lasec SA Pty Ltd)



RdRp gene, E gene, and N-gene



E gene, N gene, ORF1ab gene, RdRP gene, Spike gene

# Qualitative and quantitative testing for SARS-CoV-2 at NICD



1:4 = 2400 cp/rxn

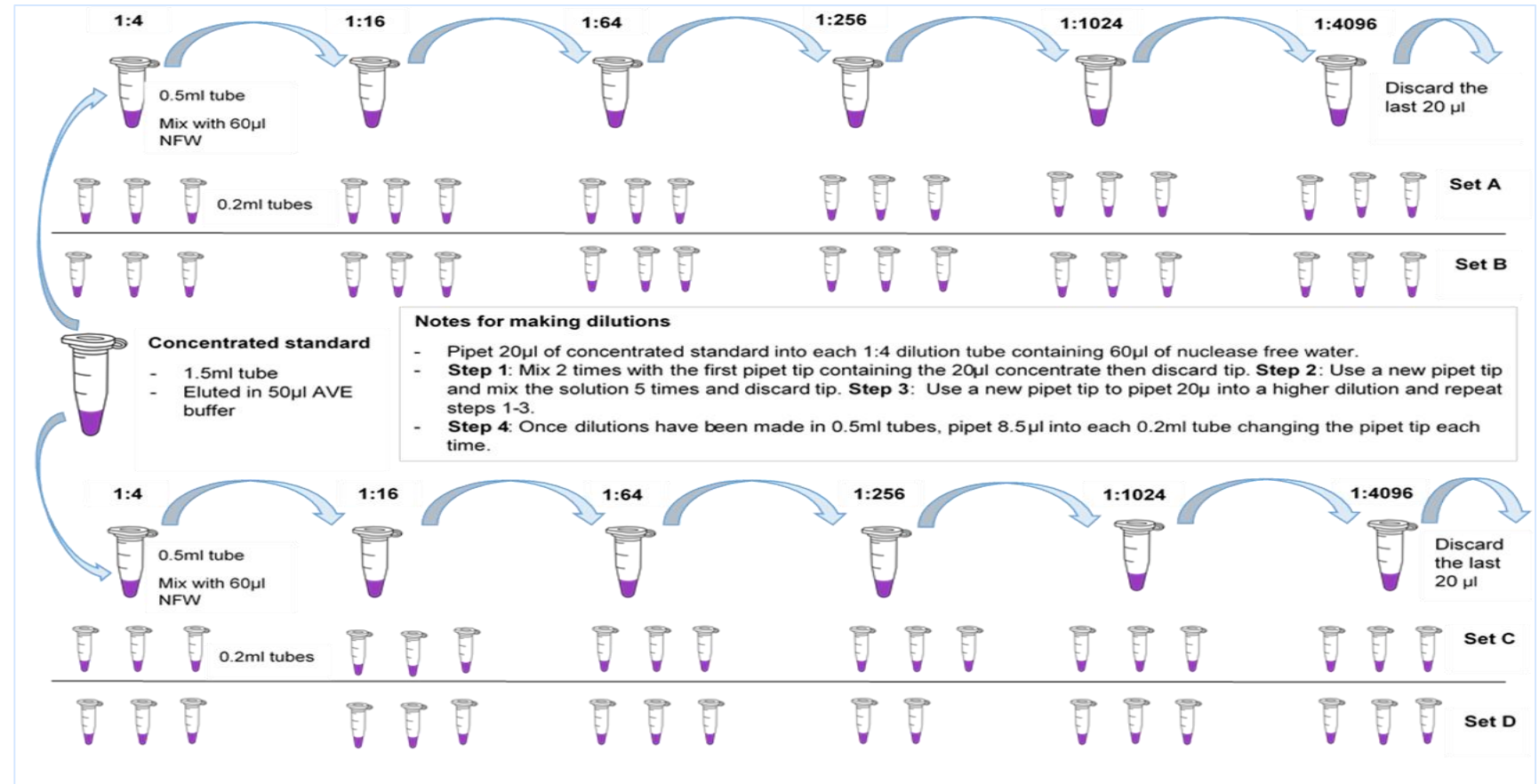
1:16 = 600 cp/rxn

1:64 = 150 cp/rxn

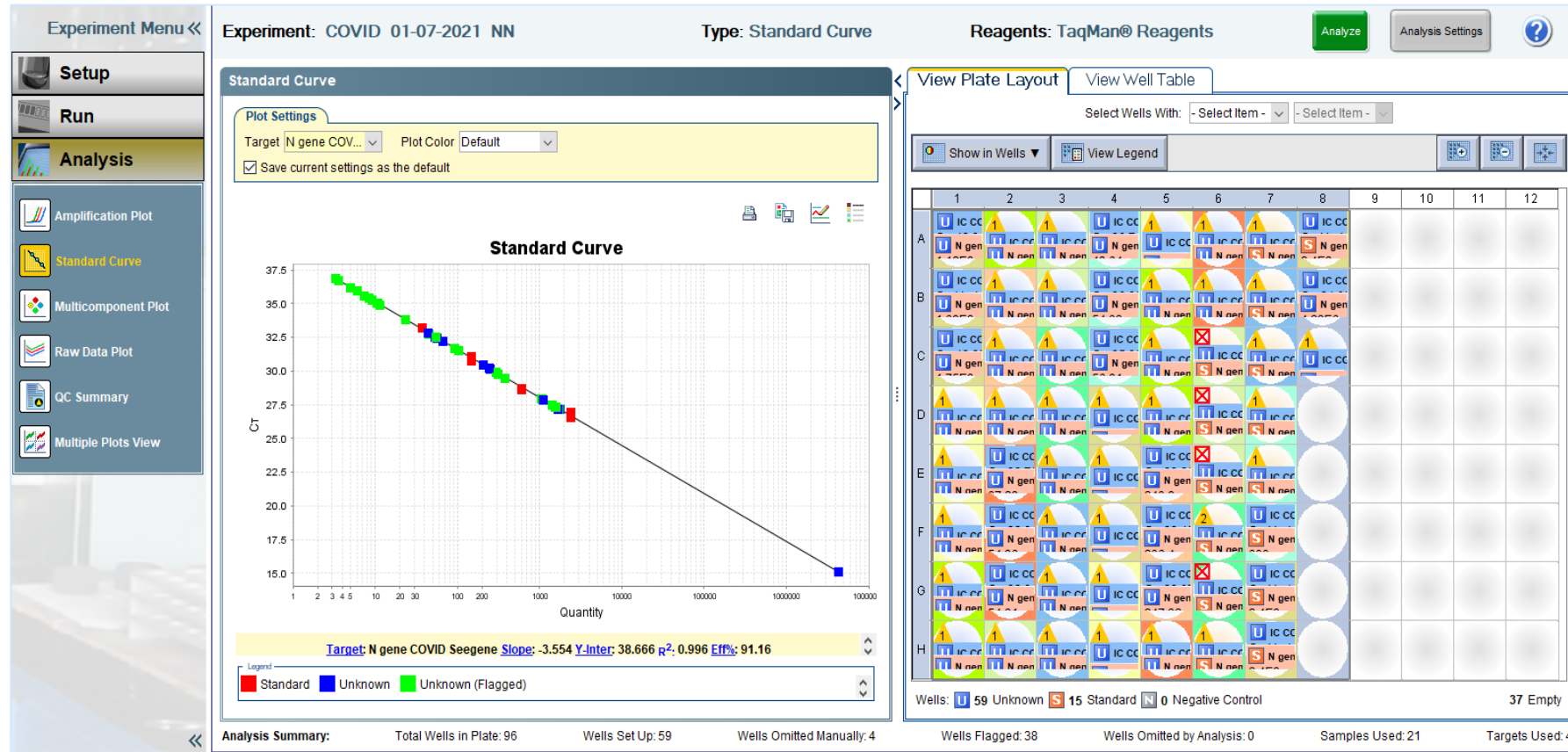
1:256 = 37.5 cp/rxn

1:1024 = 9.375 cp/rxn

1:4096 = 2.34 cp/rxn



# Qualitative and quantitative testing for SARS-CoV-2 at NICD





# The SACCESS SARS-CoV-2 qualitative dashboard

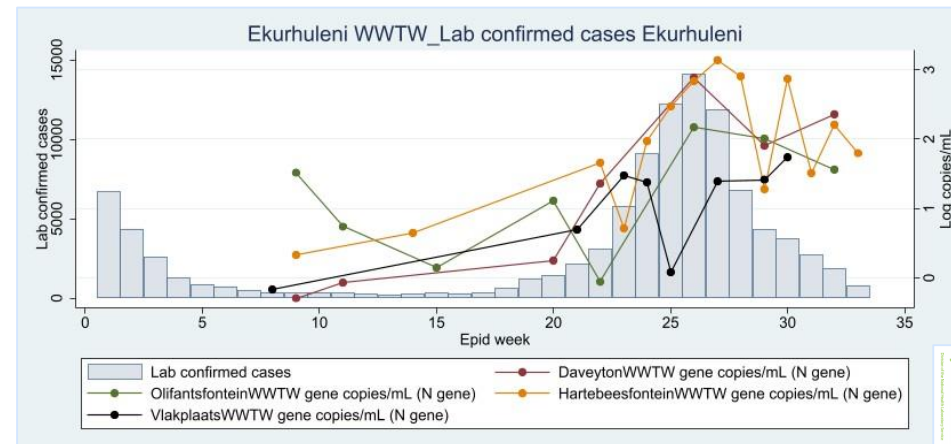
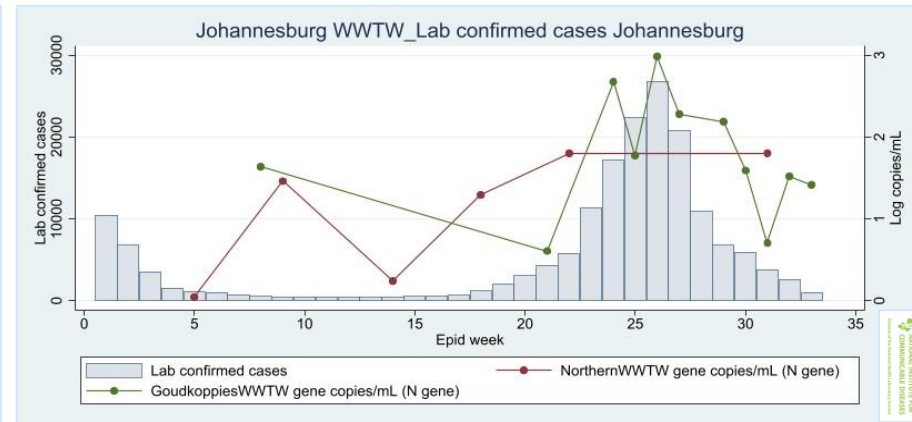
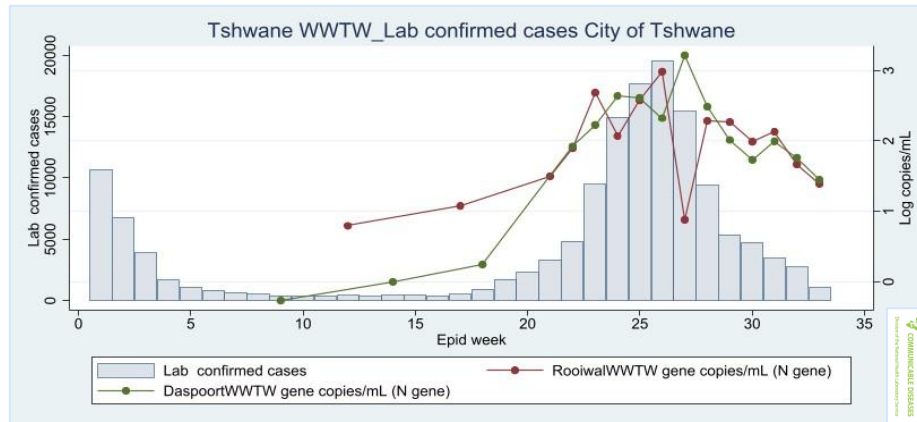
<p>NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES Division of the National Health Laboratory Service</p>	<b>Legend</b>		
	1	2	3
	Not schedule	Pending	Negative
		<b>Interpretation</b>	
Positive:		at least one gene target must be positive	
Negative:		none of the gene targets must be positive except for the internal control	
Invalid:		internal control as well as all gene targets negative on 2 attempts	
	<b>Legend</b>		
	1	2	3
	Not schedule	Pending	Negative
		<b>Interpretation</b>	
Positive:		at least one gene target must be positive	
Negative:		none of the gene targets must be positive except for the internal control	
Invalid:		internal control as well as all gene targets negative on 2 attempts	
Site Name	District Code	Laboratory	Epidemiological Week 2021
Province - Gauteng			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Rooiwal Wastewater Treatment Works	TSH	NICD	
Daspoort Wastewater Treatment Works	TSH	NICD	
Babelegi	TSH	NIOH	
Baviaanspoort	TSH	NIOH	
Sunderland Ridge	TSH	NIOH	
Sandspruit	TSH	Waterlab/UP	
Temba	TSH	Waterlab/UP	
Rayton	TSH	MRC-TB	
Refilwe	TSH	MRC-TB	
Rietgat	TSH	MRC-TB	
Daveyton WasteWater Treatment Works	EKH	CSIR	
Olifantsfontein WasteWater Treatment Works	EKH	CSIR	
Hartebeestfontein	EKH	Waterlab/UP	
Dekema	EKH	Waterlab/UP	
Hartbeesfontein WasteWater Treatment Works	EKH	NICD	
Vlakplaats WasteWater Treatment Works	EKH	NICD	
Olifantsfontein WasteWater Treatment Works	EKH	NICD	
Daveyton WasteWater treatment Works	EKH	NICD	
Northern Wastewater Treatment Works	JBM	NICD	
GOUDKOPPIES Wastewater Treatment Works	JBM	NICD	
Olifantsvlei	JBM	Waterlab/UP	
Flip Human	WR	Waterlab/UP	
S gene, N gene, E gene, RdRp gene and ORF1ab gene	+	-	-
Internal control	+/-	+	-
	POS	NEG	Invalid

Site Name	District Code	Laboratory	Epidemiological Week 2021
Province - Western Cape			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Borcheds Quarry Wastewater Treatment Works	CAT	NICD	
Zandvleit Wastewater Treatment Works	CAT	NICD	
Bellville	CAT	MRC-BRIP	
Cape Flats	CAT	MRC-BRIP	
Stellenbosch	CAT	MRC-BRIP	
Province - Free State			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Bloemspruit Wastewater Treatment Works	MAN	NICD	
Sterkwater Wastewater Treatment Works	MAN	NICD	
Province - Kwazulu-Natal			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Northern Wastewater	ETH	NICD	
CENTRAL WASTEWATER TREATMENT WORKS	ETH	NICD	
Howick	MGU	Greenhill	
Davill	MGU	Greenhill	
Lynnfield	MGU	Greenhill	
Province - Eastern Cape			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Kwanobuhle Waterwaste	NMM	NICD	
Brickfield Pretreatment	NMM	NICD	
East Bank waterwaste	BCM	NICD	
Mdantsane waterwaste	BCM	NICD	
Potsdam	BCM	Praecautio	
Central	BCM	Praecautio	
Zwelitsha	BCM	Praecautio	
Mpumalanga			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Kanyamazane	MBO	Waterlab/UP	
Kingstone vale waste water treatment plant	MBO	Lumegen	
Riverview waste water treatment plant	EHL	Lumegen	
North West			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Klerksdorp mains waste water treatment plant	MAT	Lumegen	
Potchefstroom waste water treatment plant	JBMA	Lumegen	
Rustenburg -Water and Sanitation Services	BCJ	Lumegen	

# NICD SARS-CoV-2 quantitative results



## Gauteng wastewater treatment plants

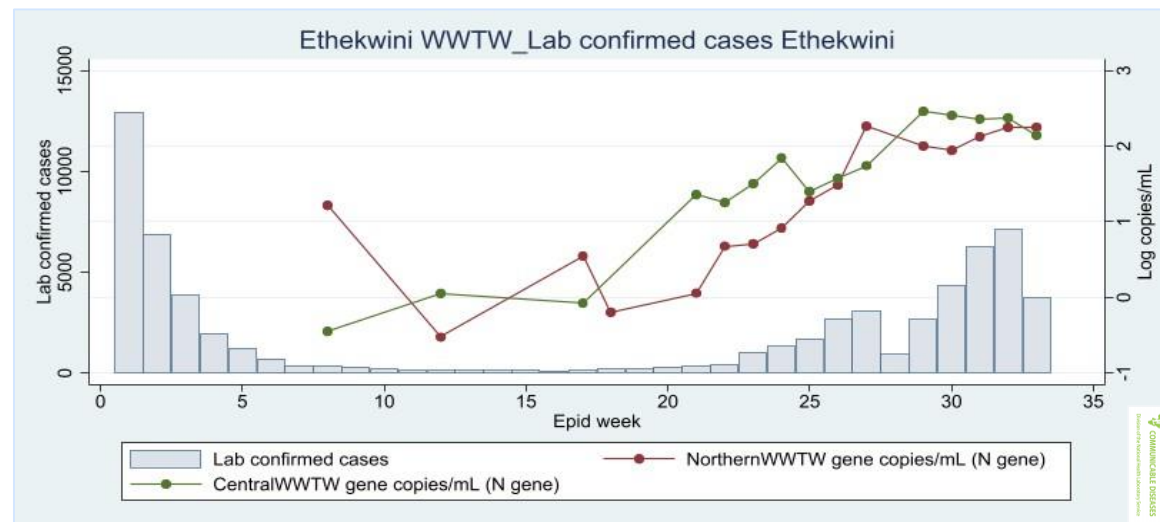






# NICD SARS-CoV-2 quantitative results

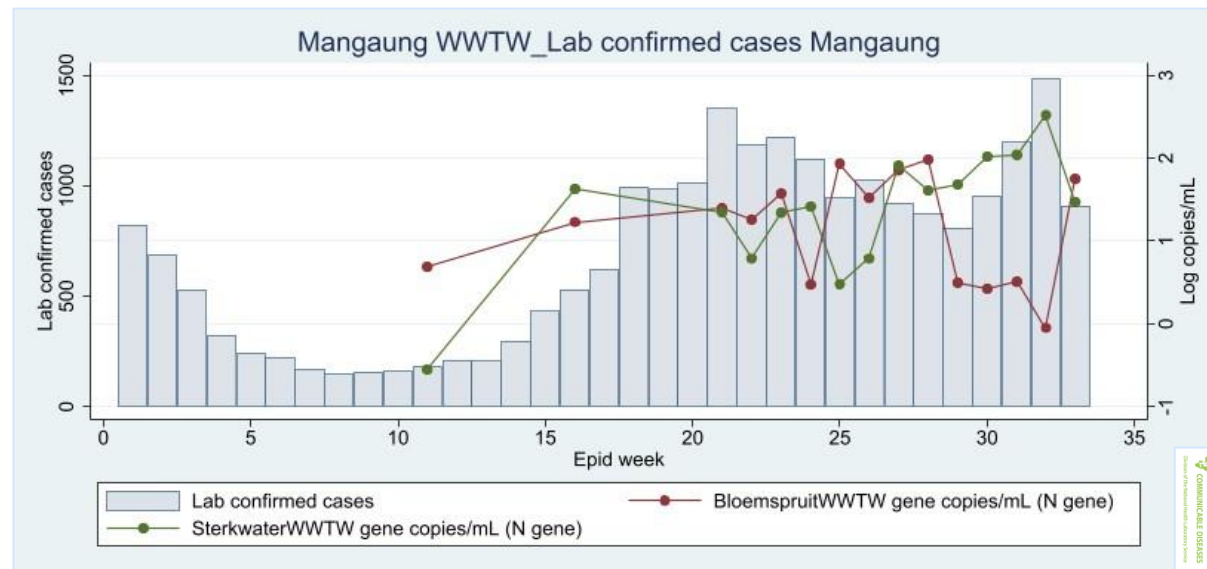
## KwaZulu-Natal wastewater treatment plants





# NICD SARS-CoV-2 quantitative results

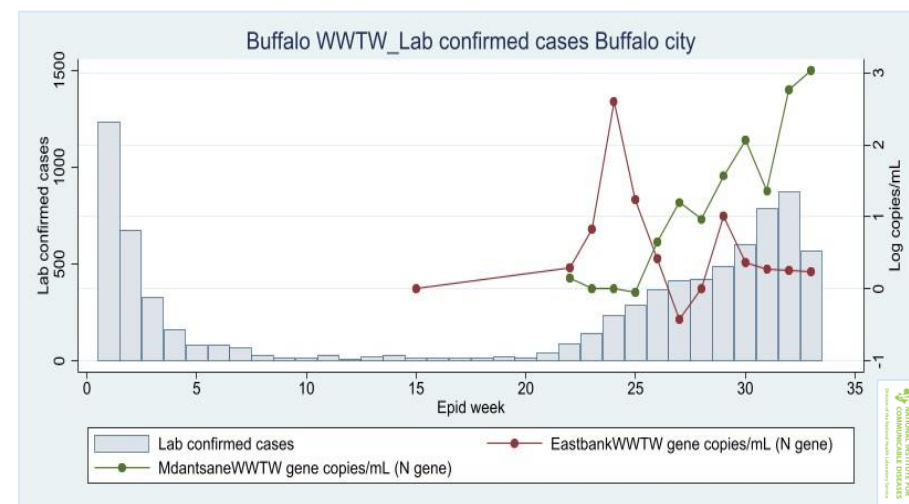
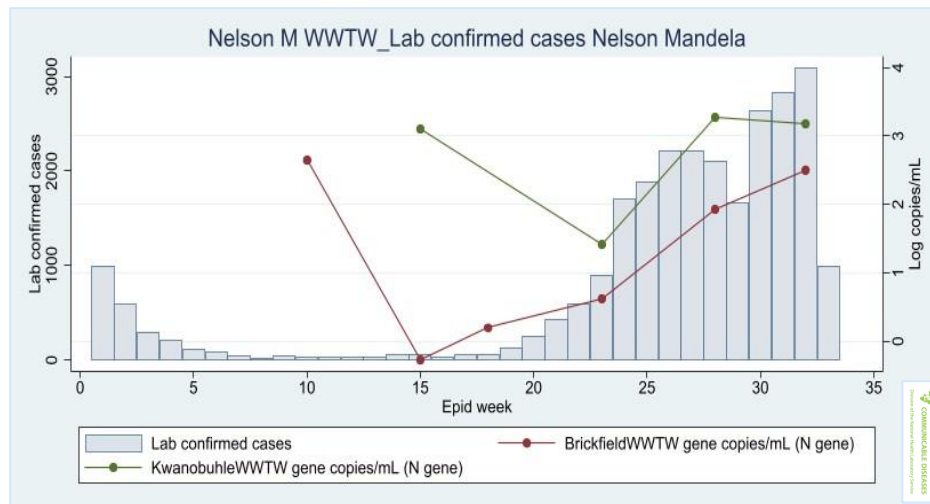
## Free State wastewater treatment plants





# NICD SARS-CoV-2 quantitative results

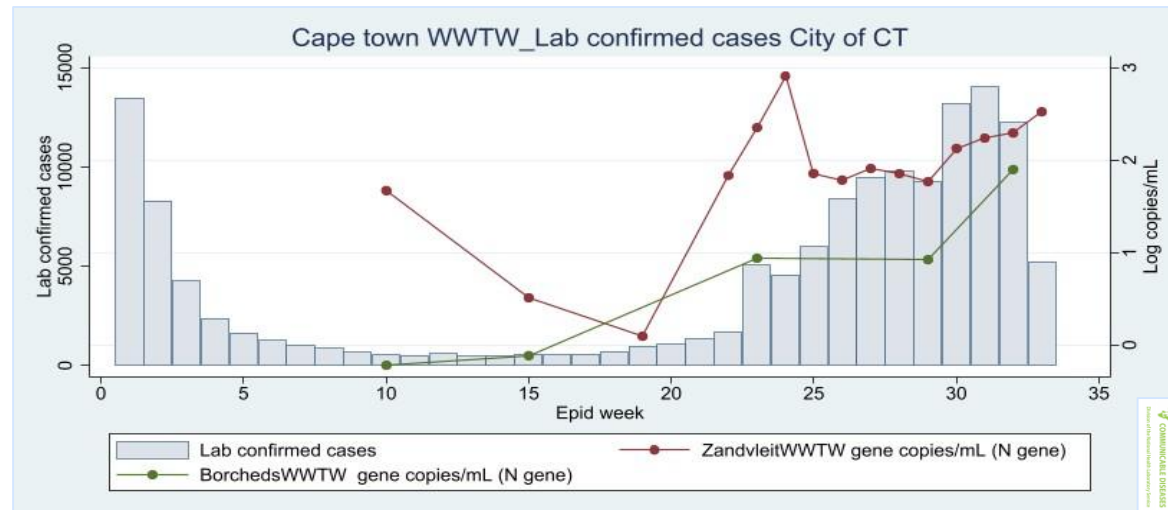
## Eastern Cape wastewater treatment plants





# NICD SARS-CoV-2 quantitative results

Western Cape wastewater treatment plants





# Acknowledgements

Water Research Commission  
NICD- Centre for Vaccines  
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