Evaluation of wastewater-based epidemiology of COVID-19 approaches in Singapore's 'closed-system' scenario

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Evaluation of wastewater-based epidemiology of COVID-19 approaches in Singapore's 'closed-system' scenario: A long-term country-wide assessment

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Population of over 5.8 million
Density of 7,810 people per km²
Land area : 728 km²

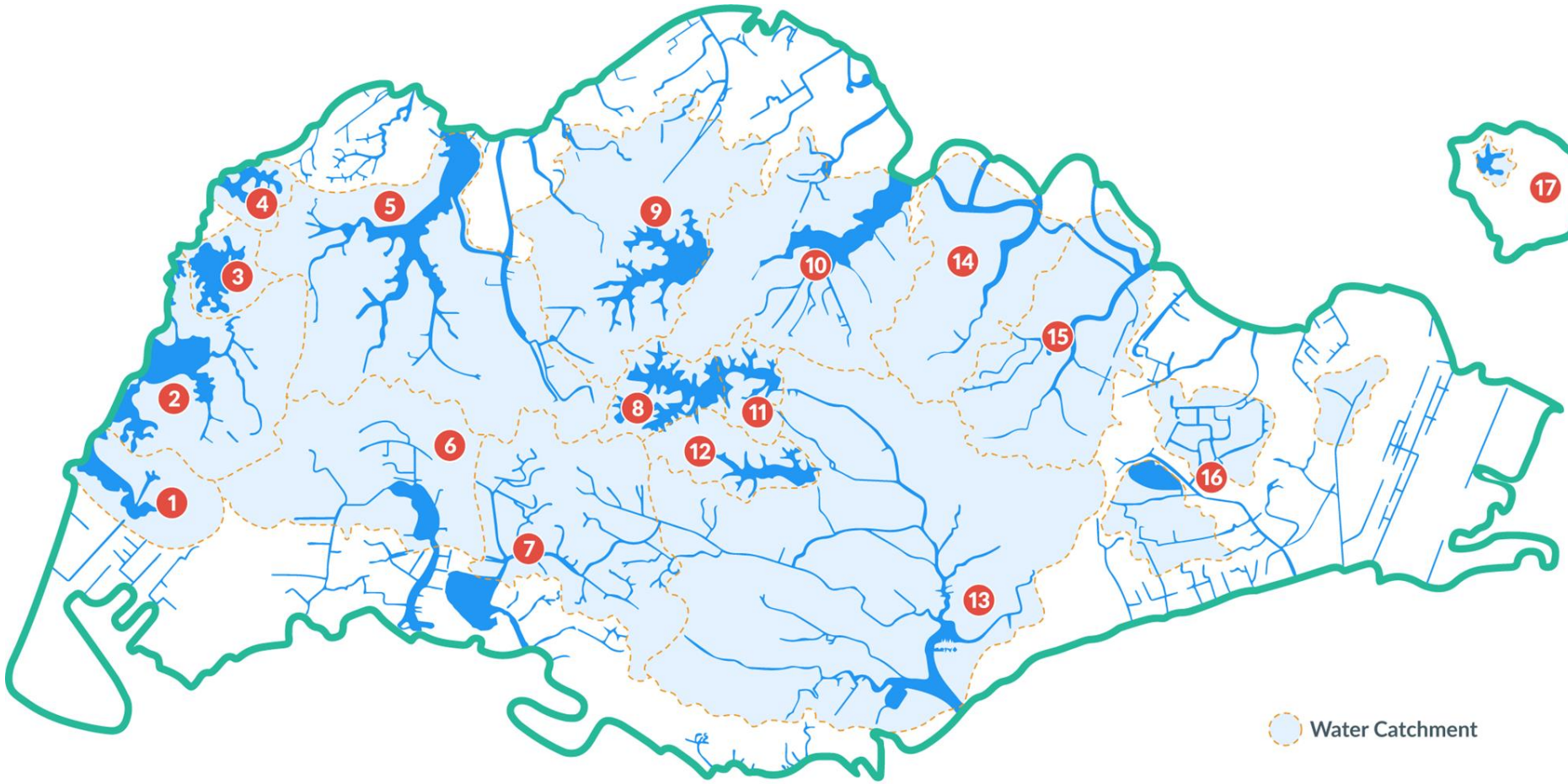
During the height of the pandemic, Singapore was essentially closed to the outside world.

Singapore's water system is closely monitored, precise/accurate data are readily available.

All water reclamation facilities and multiple points within the sewer collection system were monitored.

Epidemiological data/testing available for the entire country.

GOAL: Model and normalize SARS-CoV-2 virus to anthropogenic markers (population predictions).



IMPORTED WATER

WATER FROM LOCAL CATCHMENT

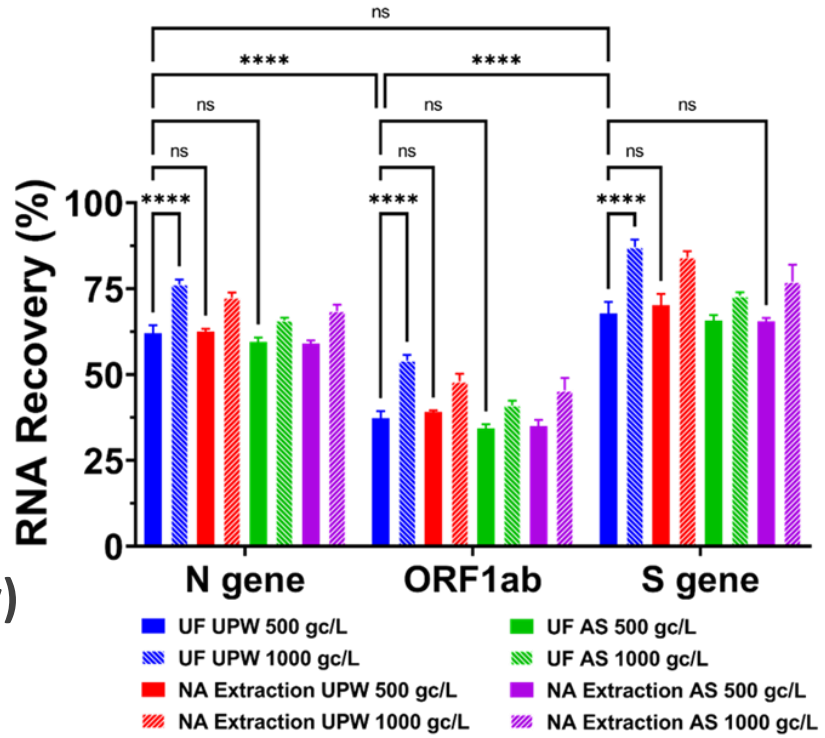
NEWATER

DESALINATED WATER

1. Tengeh Reservoir
2. Poyan Reservoir
3. Murai Reservoir
4. Sarimbun Reservoir
5. Kranji Reservoir
6. Jurong Lake
7. Pandan Reservoir
8. Upper Peirce Reservoir
9. Upper Seletar Reservoir
10. Lower Seletar Reservoir
11. Lower Peirce Reservoir
12. MacRitchie Reservoir
13. Marina Reservoir
14. Punggol Reservoir
15. Serangoon Reservoir
16. Bedok Reservoir
17. Tekong Reservoir

Entire Country Monitoring of all WWTPs

- 85-week monitoring, twice a week (weekend & weekday)
- Heat Inactivation upon sample receipt (BSL2)
- Ultrafiltration followed by vacuum concentration
- Automated extraction of nucleic acid
- 4-plex RT-qPCR with 3 SARS-CoV-2 gene targets (Proprietary)
- Treatment process efficacy also evaluated



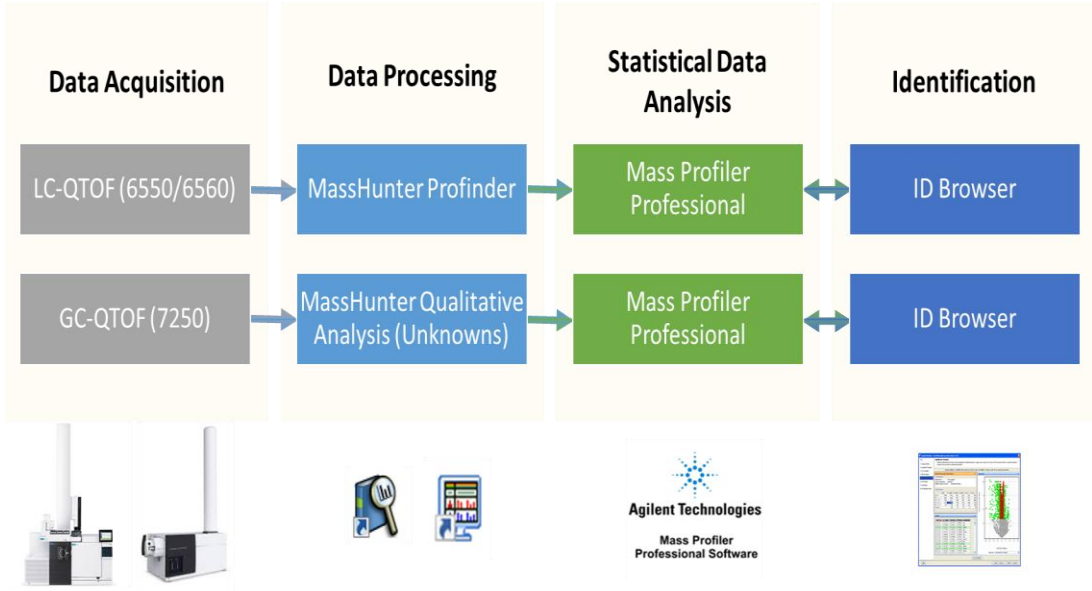
Heat Inactivation (60 °C for 90 min) → Centrifugation (large debris) – 250 mL tube → Ultrafiltration (10 kDa) → Vacuum Concentration and Reconstitution → Nucleic Acid Extraction (Automated/Magnetic Beads – MS2 phage control) → RT-qPCR

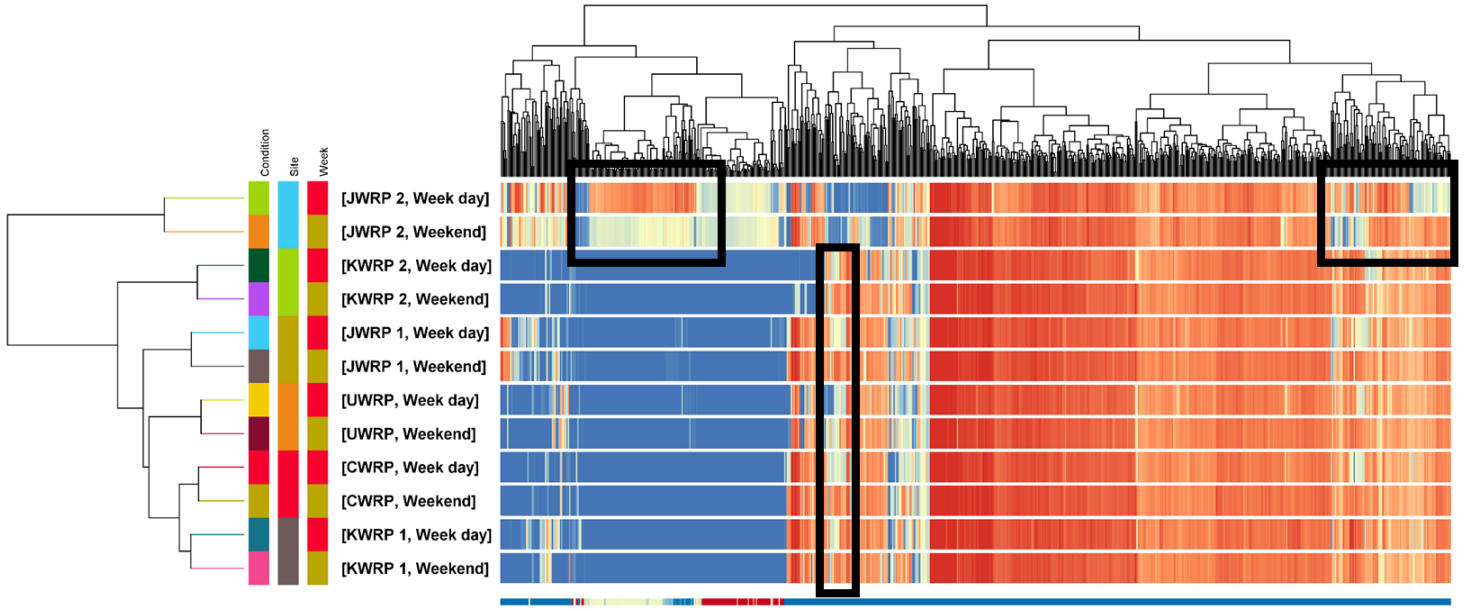


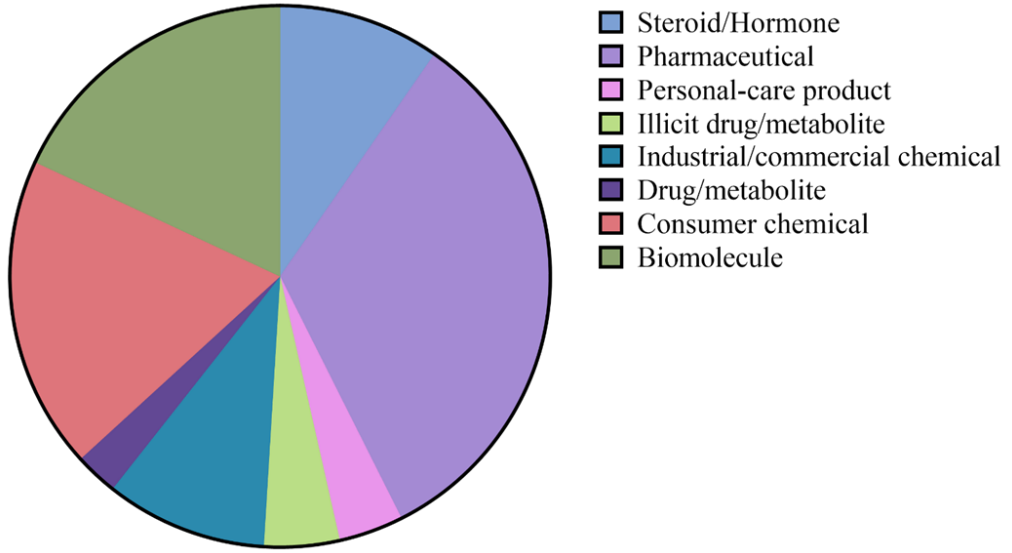



Chemical markers in wastewater

- Untargeted high-resolution mass spectrometry (LC/GC-HRMS)
  - ❖ >7,500 compounds detected



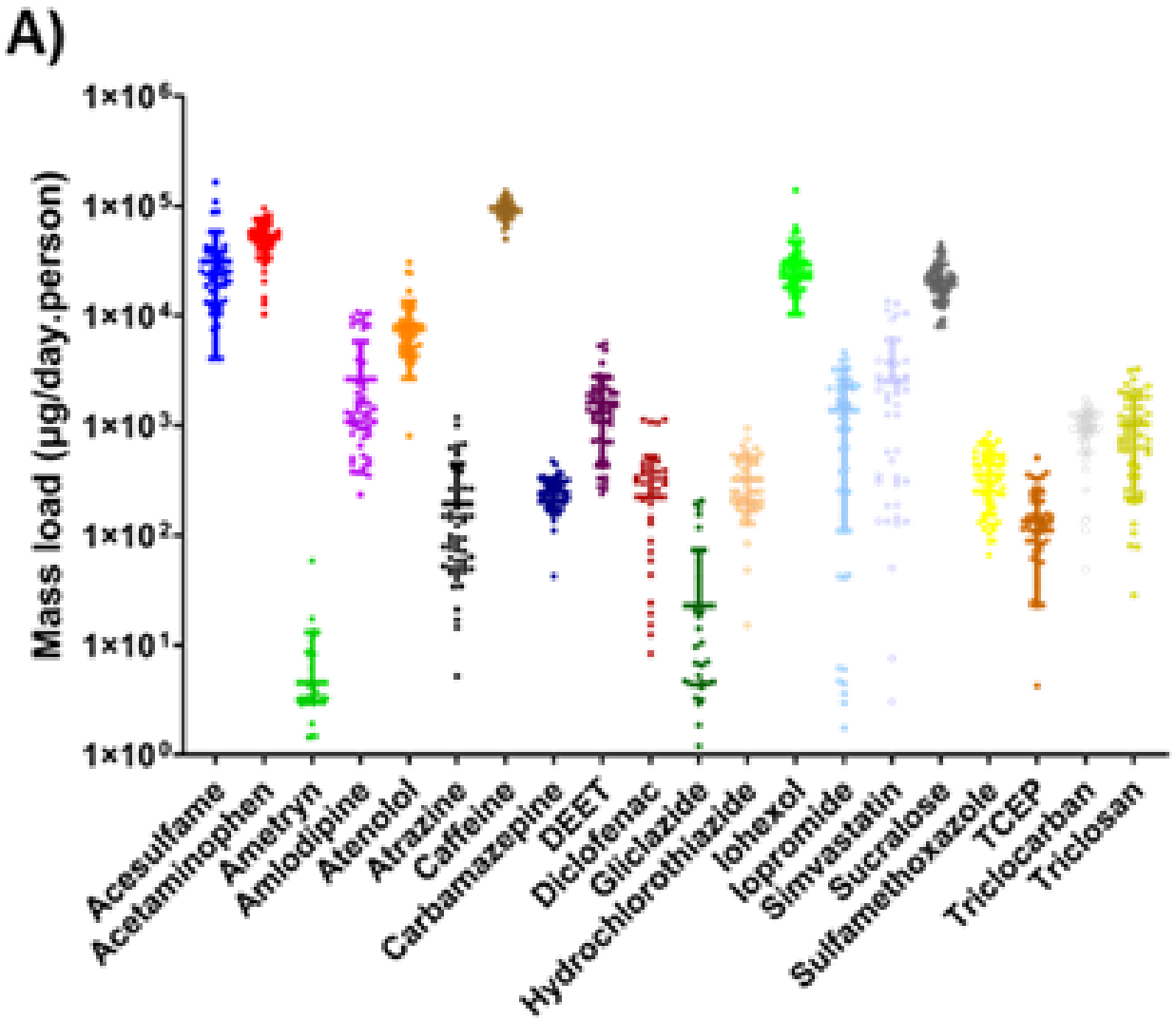




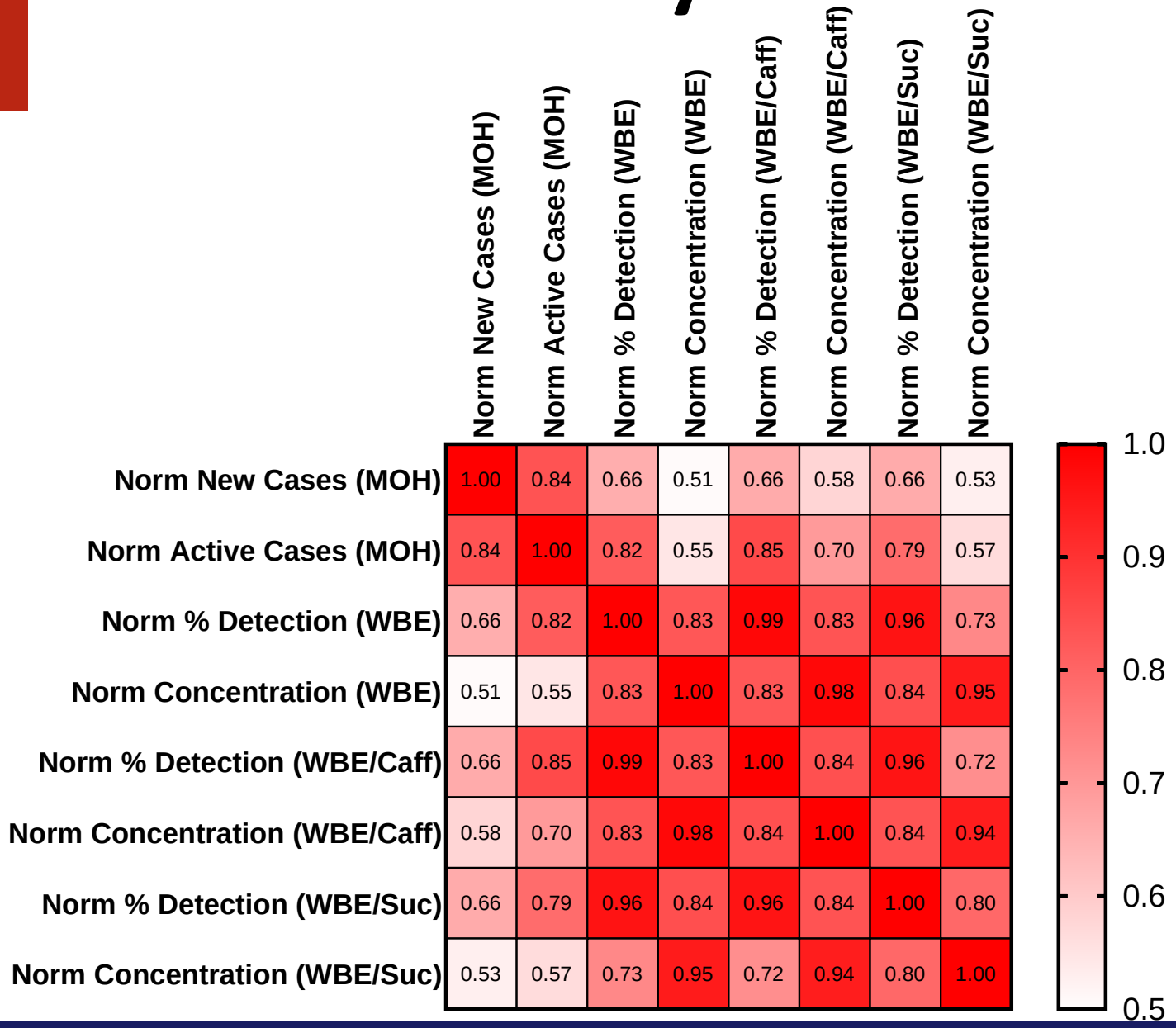
Validation of markers in wastewater (LC-MS/MS)

	Concentration (ηg/L)			
	Mean	SEM	Min	Max
Acesulfame	31162	3722	7507	164695
Acetaminophen	51507	2534	10261	95539
Ametryn	5	1	< LOQ	59
Amlodipine	2639	434	233	10735
Atenolol	8084	741	800	30460
Atrazine	193	35	< LOQ	1189
* Caffeine	92485	2279	50291	136274
Carbamazepine	246	11	42	473
DEET	1627	165	241	5611
Diclofenac	223	41	< LOQ	1125
Gliclazide	23	7	< LOQ	205
Hydrochlorothiazide	323	27	< LOQ	940
Iohexol	29317	2611	11098	139063
Iopromide	1385	175	< LOQ	4633
Simvastatin	2505	489	< LOQ	13529
Sucralose	21282	1139	8033	45749
Sulfamethoxazole	359	28	67	847
TCEP	135	15	< LOQ	506
Triclocarban	954	53	49	1759
Triclosan	1028	113	28	3269

* Selected to be marker for WBE normalization



WBE Data Analysis

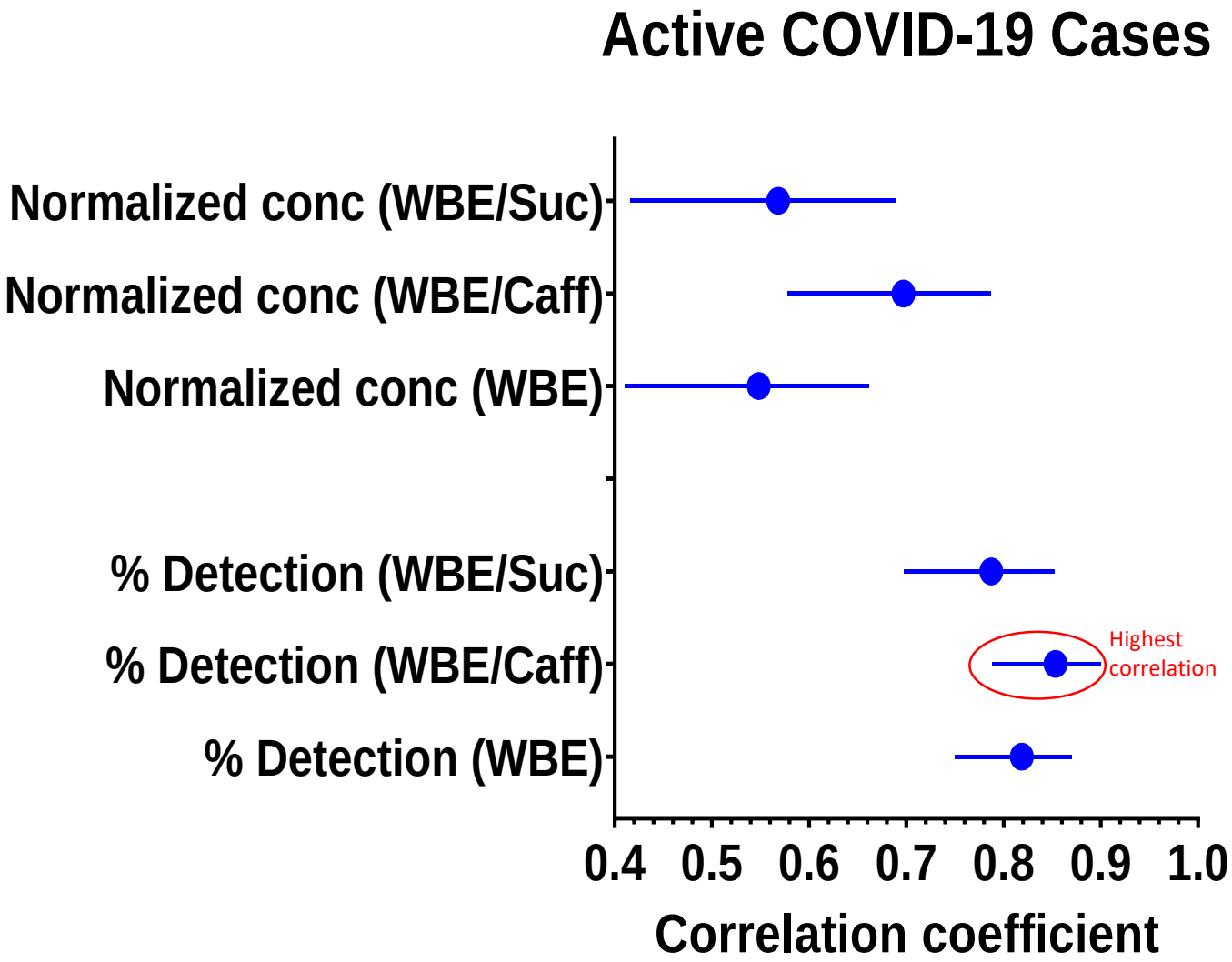


$$\boldsymbol{Norm\ Conc\ (WBE)} = \sum_{genes}\left(w_{wrp}\ x\ \left(\frac{Conc\ x\ Flow}{Caff}\right)\right)\Big/_{Max}$$

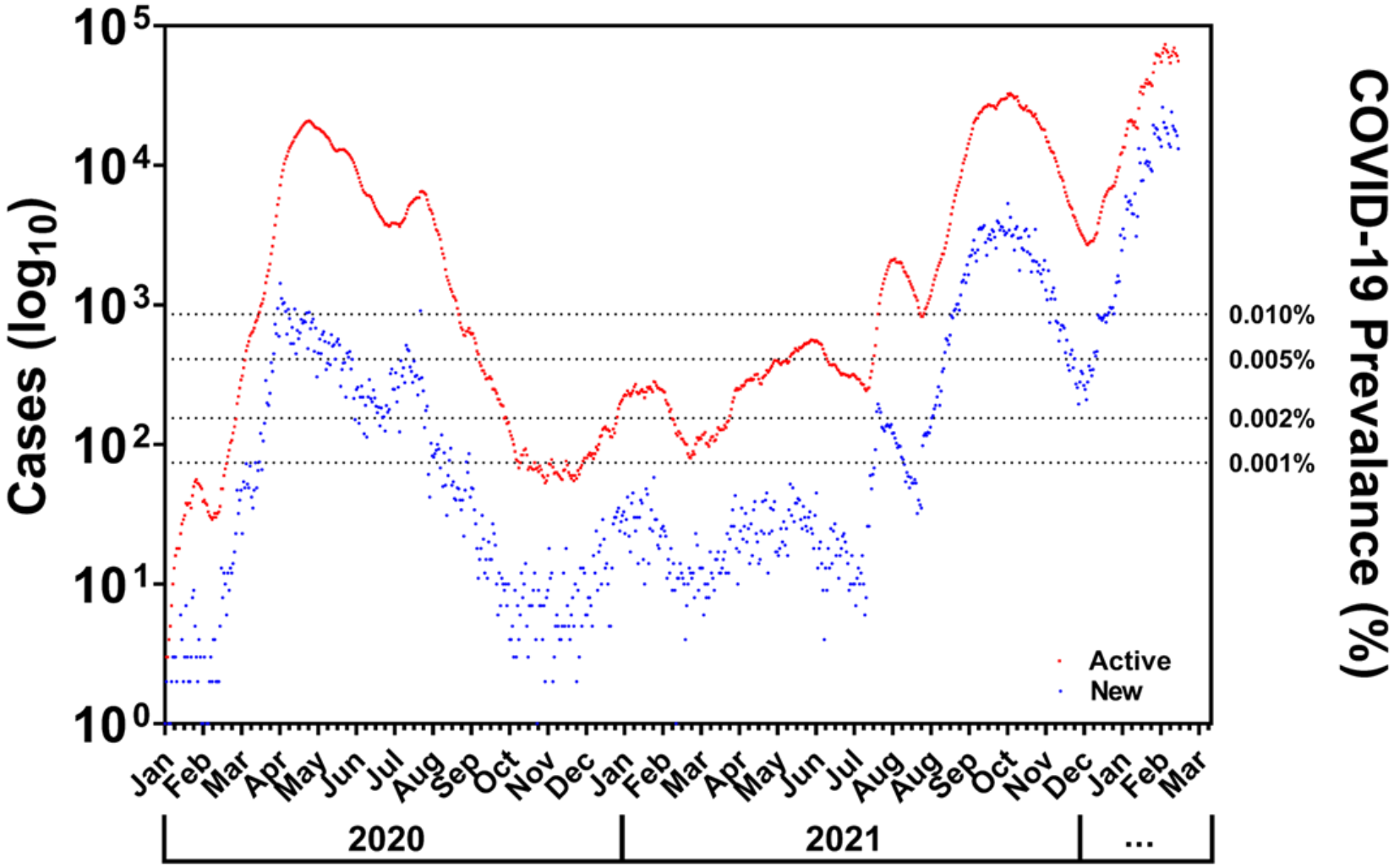
W_{wrp} = WRP % Population
Conc = gene concentration (gc/L)
Flow = WRP flow (L)
Caff = Caffeine concentration (µg/L)
Suc = Sucralose concentration (ug/L)

WBE Data Analysis

- In a low prevalence situation, chemical marker normalization is reduced when compared to flow-based analysis.
  - ❖ Flow normalized data – **0.819**
  - ❖ Flow/caffeine normalized data – **0.854**



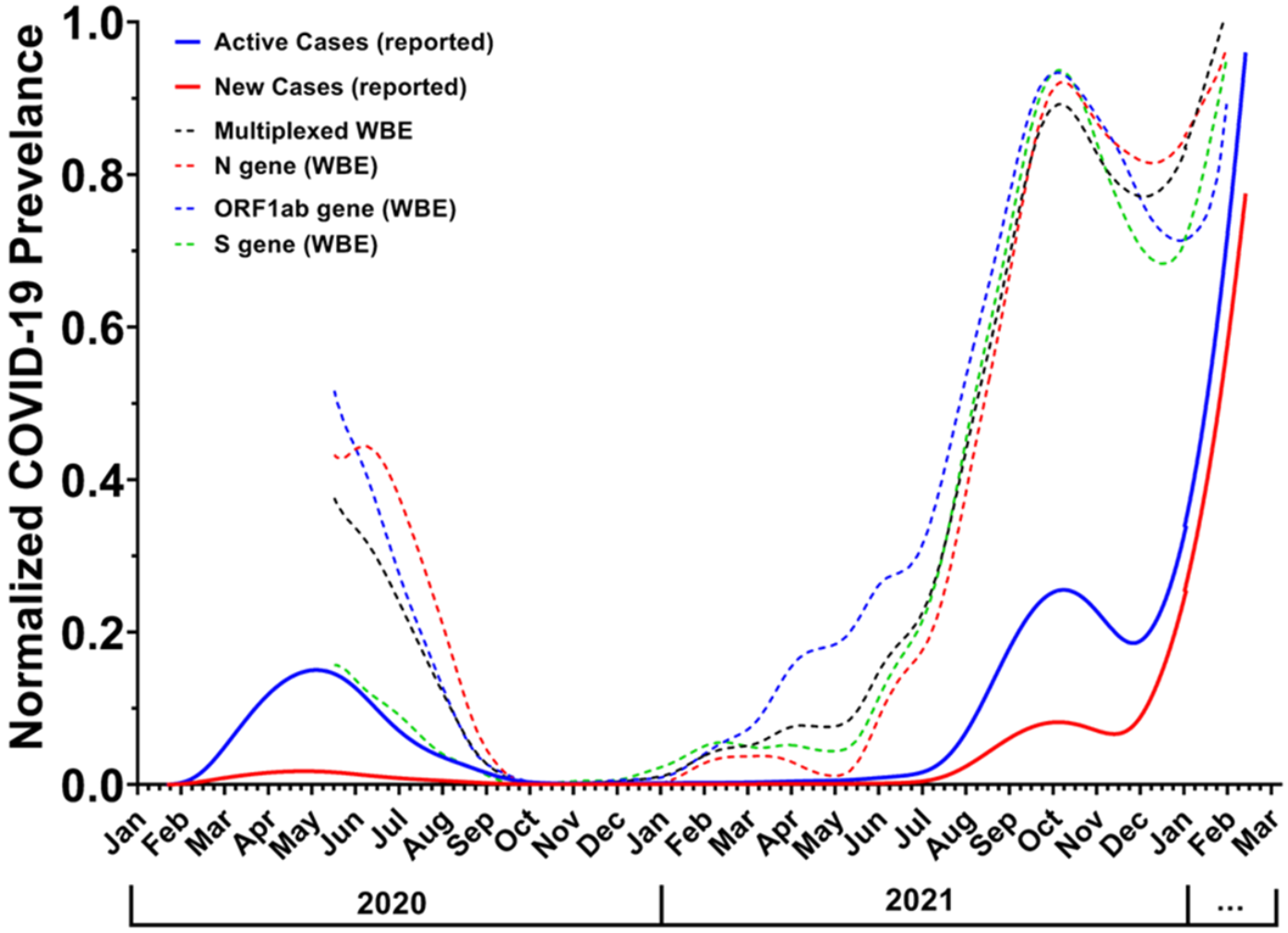
WBE of SARS-CoV-2 in Singapore

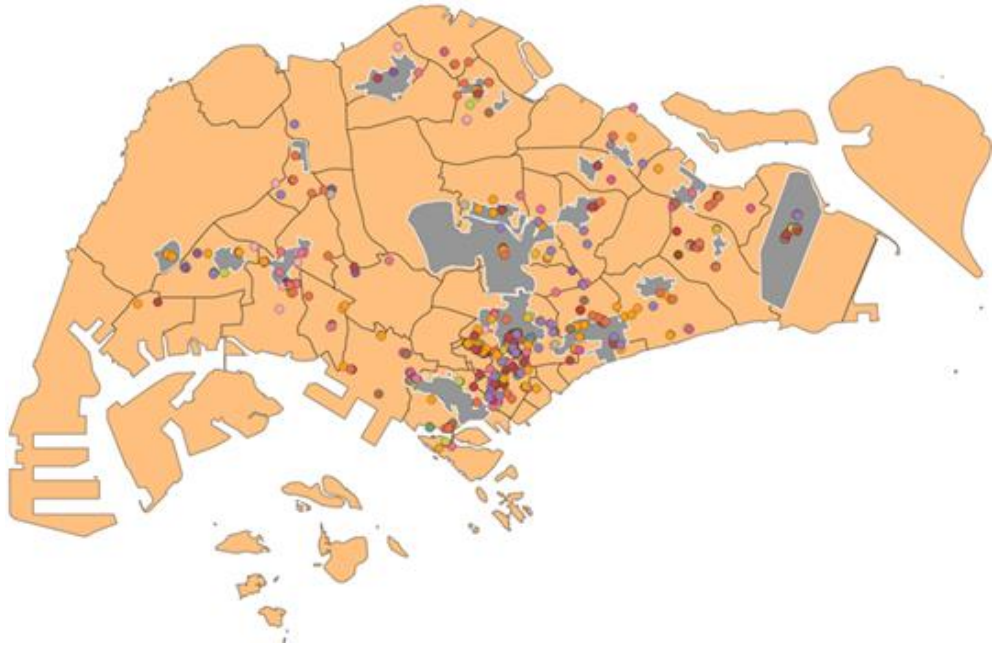


- **Epidemiology data**
  - ❖ 3 waves: initial; Delta; (rising) Omicron
- **Sewages from WWTPs**
  - ❖ 416/1009 positive
  - ❖ Differential sensitivity of 3 genes during different waves
    - S gene was consistently negative during January 2022 despite high signals in the other two genes
  - ❖ Important to use multiple gene targets

Detection limit
~0.001%
(~1 case in 100,000)

WBE of SARS-CoV-2 in Singapore

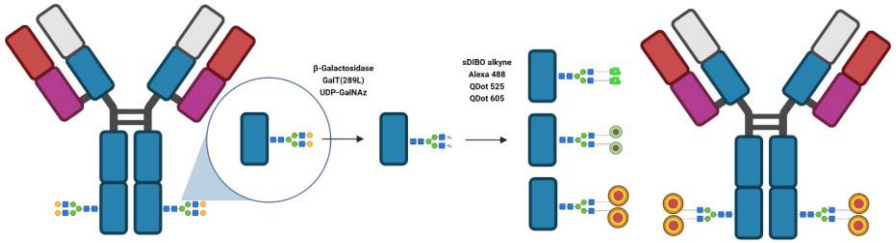


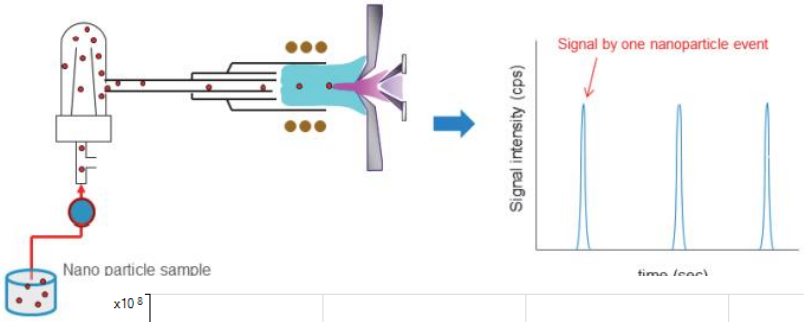


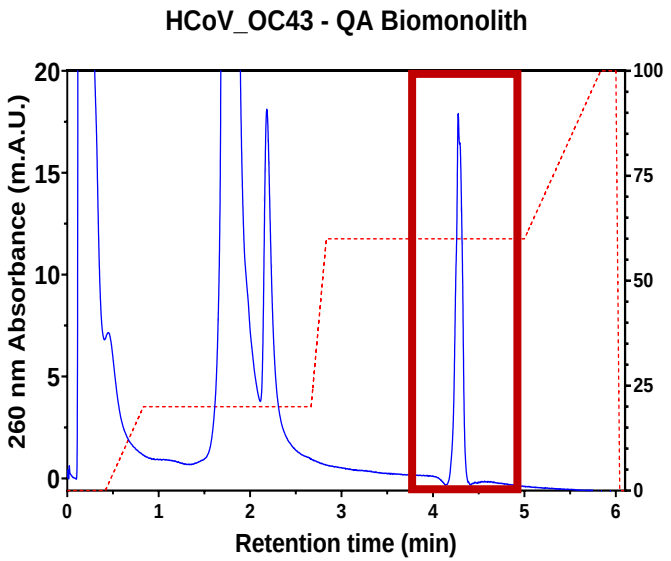
Sampling boundaries for community level/point-of-source WBE analysis and visited places by active COVID-19 cases, different colors represent a different week in the 28 weeks monitoring period.

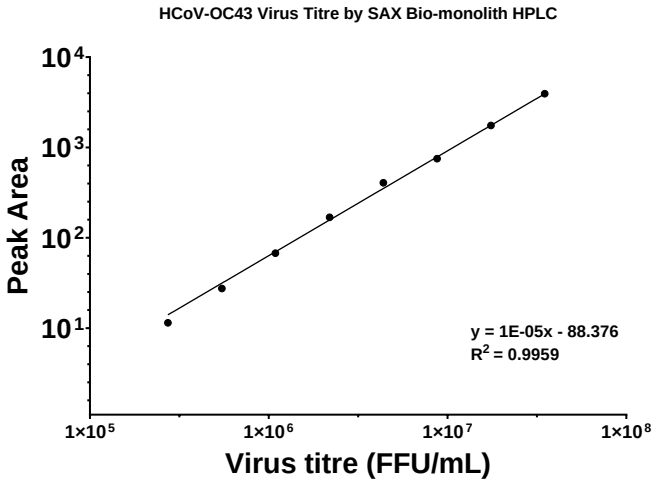
Anion Exchange & ICP-MS Techniques:

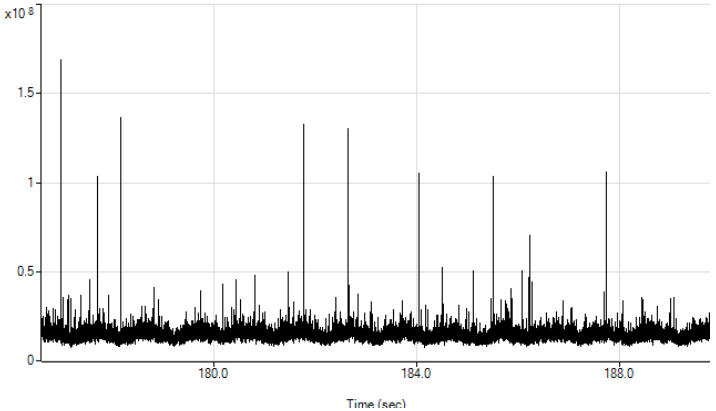
- Rapid analysis of virus particles in under 10 minutes from sample to results
- Strong anion-exchange Bio-monolith HPLC column
- System: Agilent Bio-Inert 1260 Infinity II HPLC
- Use of metal tagged Abs for specificity and lower detection limits

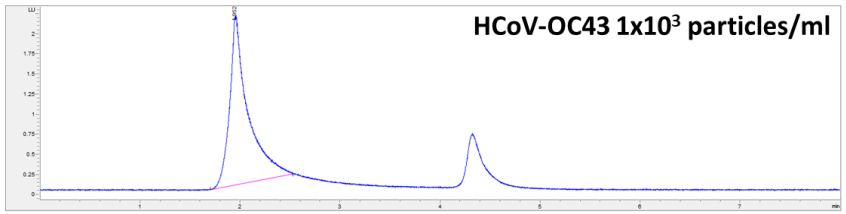




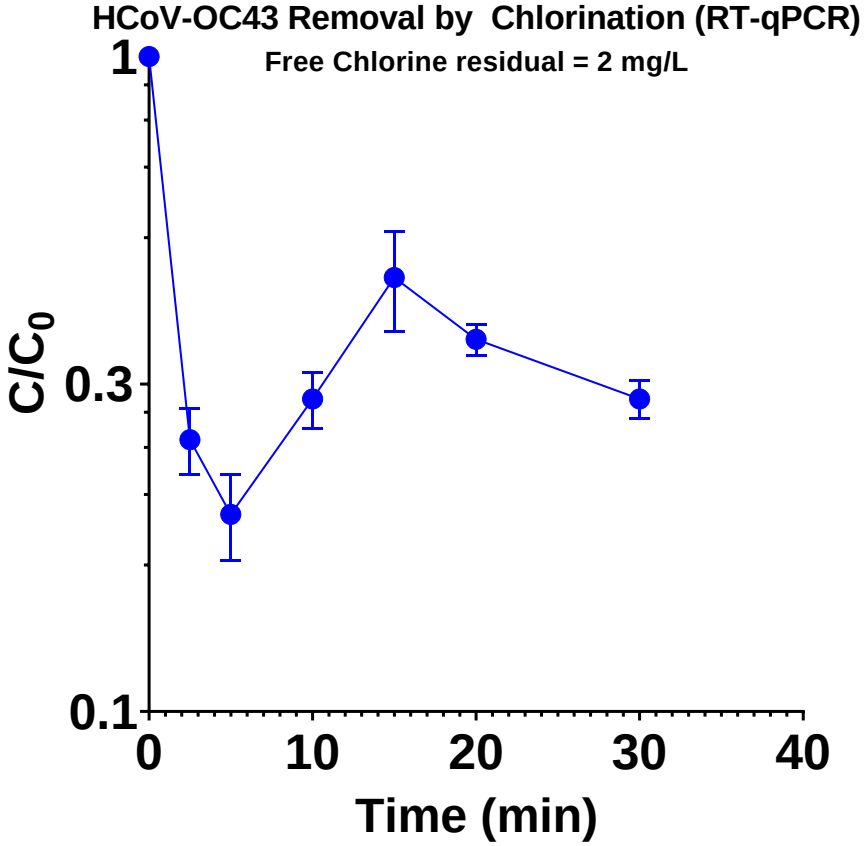


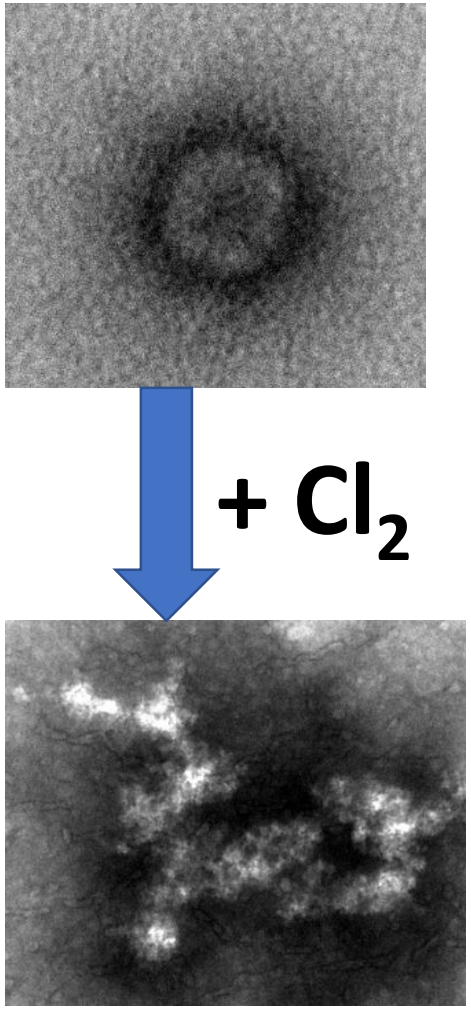


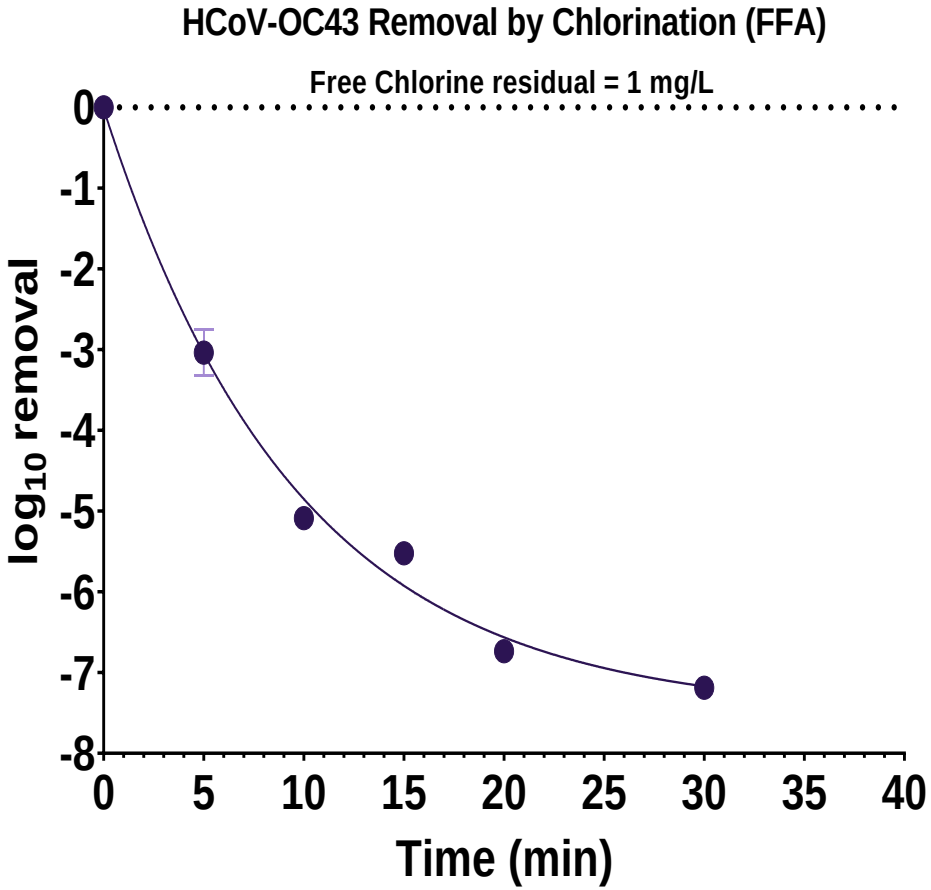




Water/wastewater treatment Evaluations

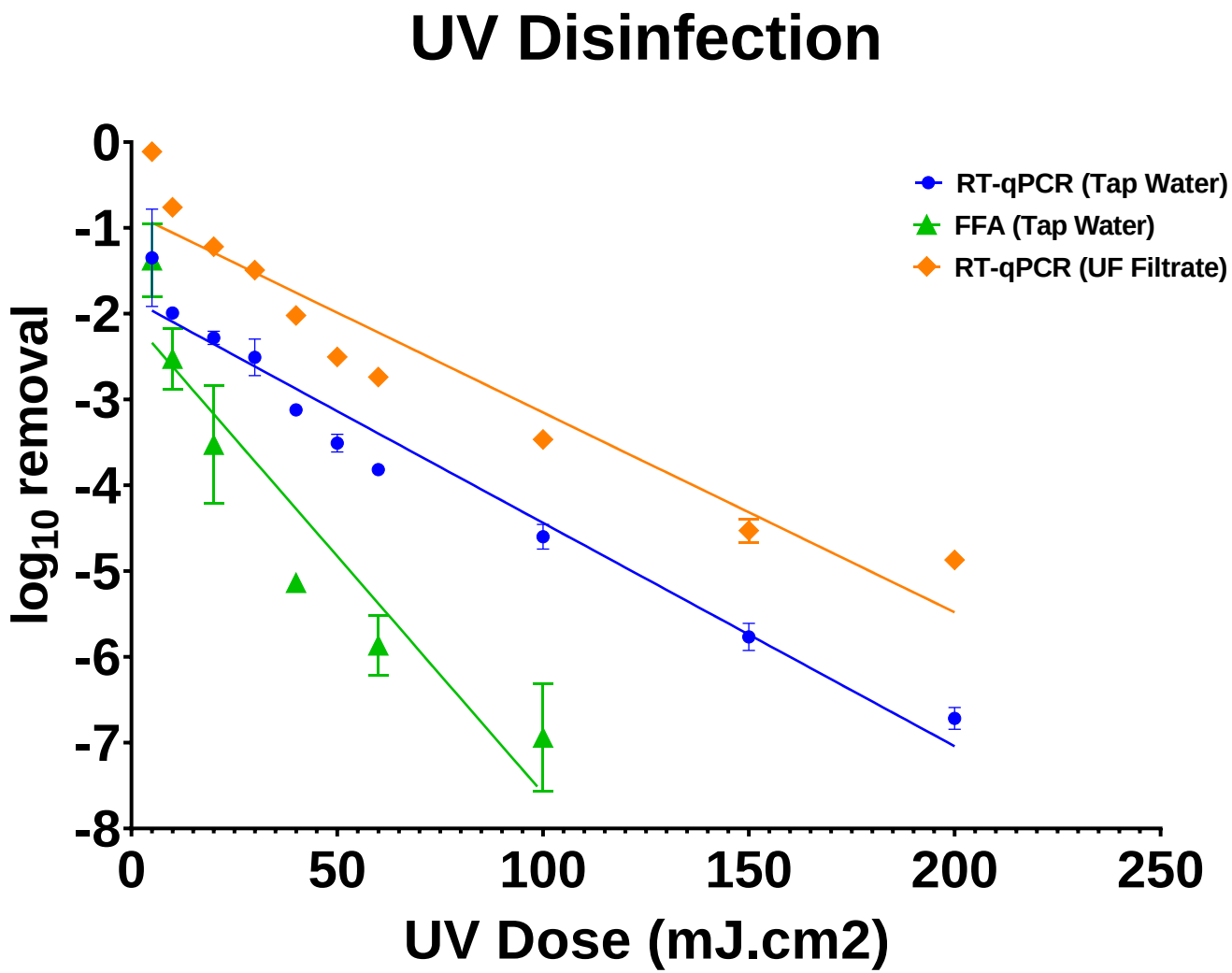






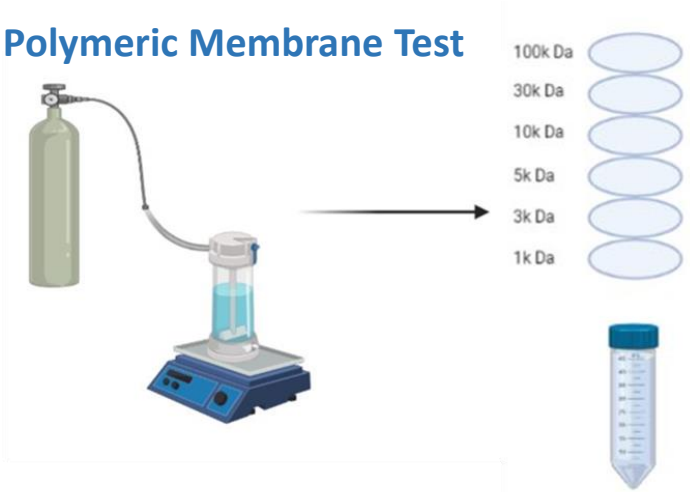
Water/wastewater treatment Evaluations

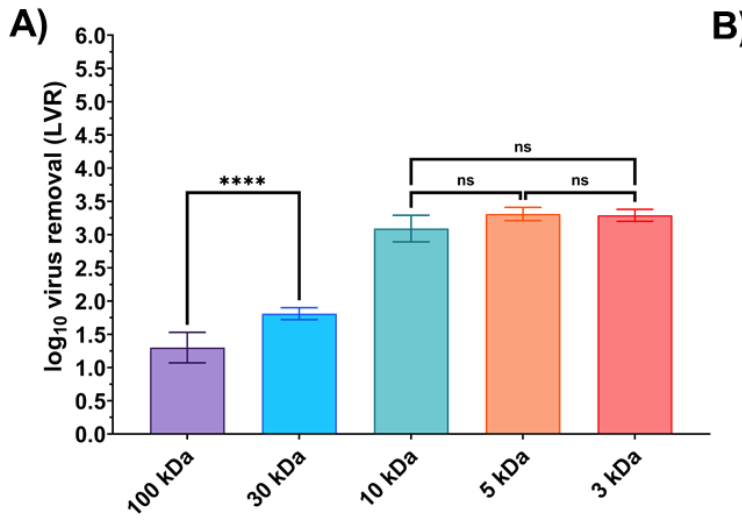
- RT-qPCR and FFA assays offers different inactivation curves
  - ❖ 4-log dose (35 mJ.cm^2 vs. 83 mJ.cm^2)
- Increased dose needed for UF filtrate samples (Higher UV254 nm absorbance); 136 mJ.cm^2

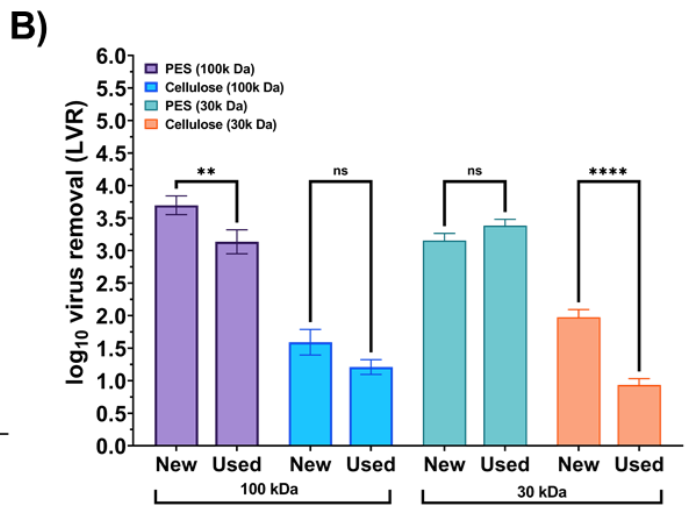


Filtration Membrane Evaluations

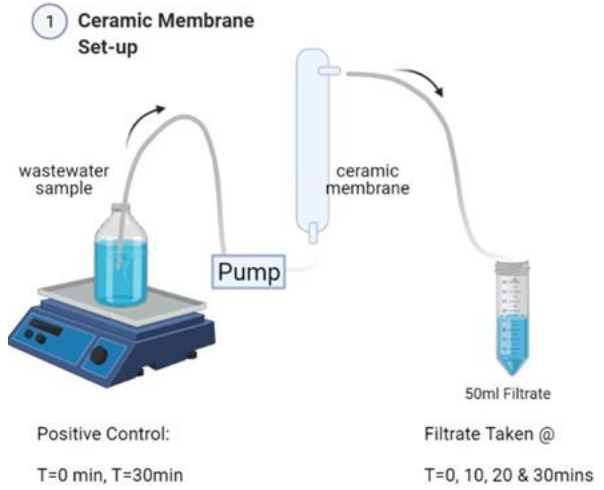
Polymeric Membrane Test

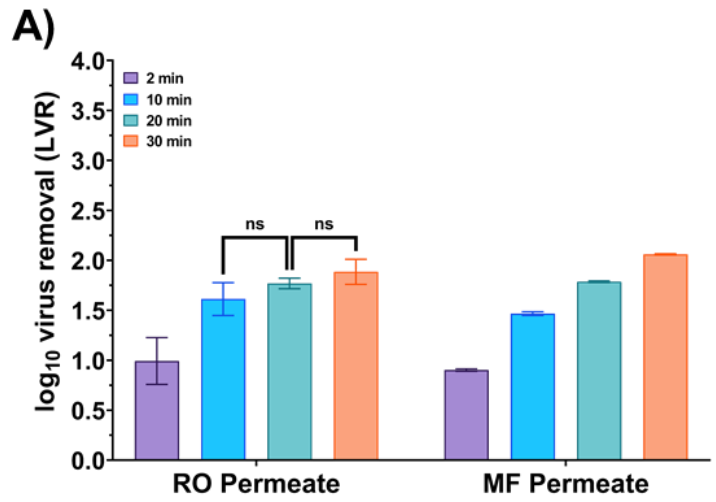


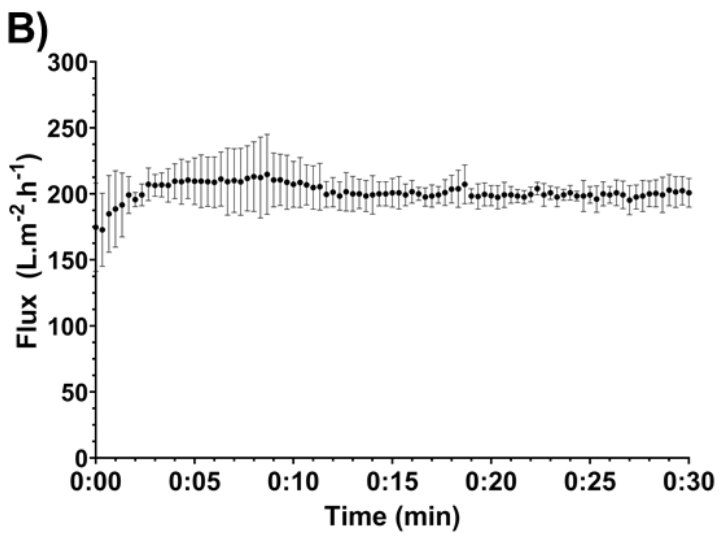




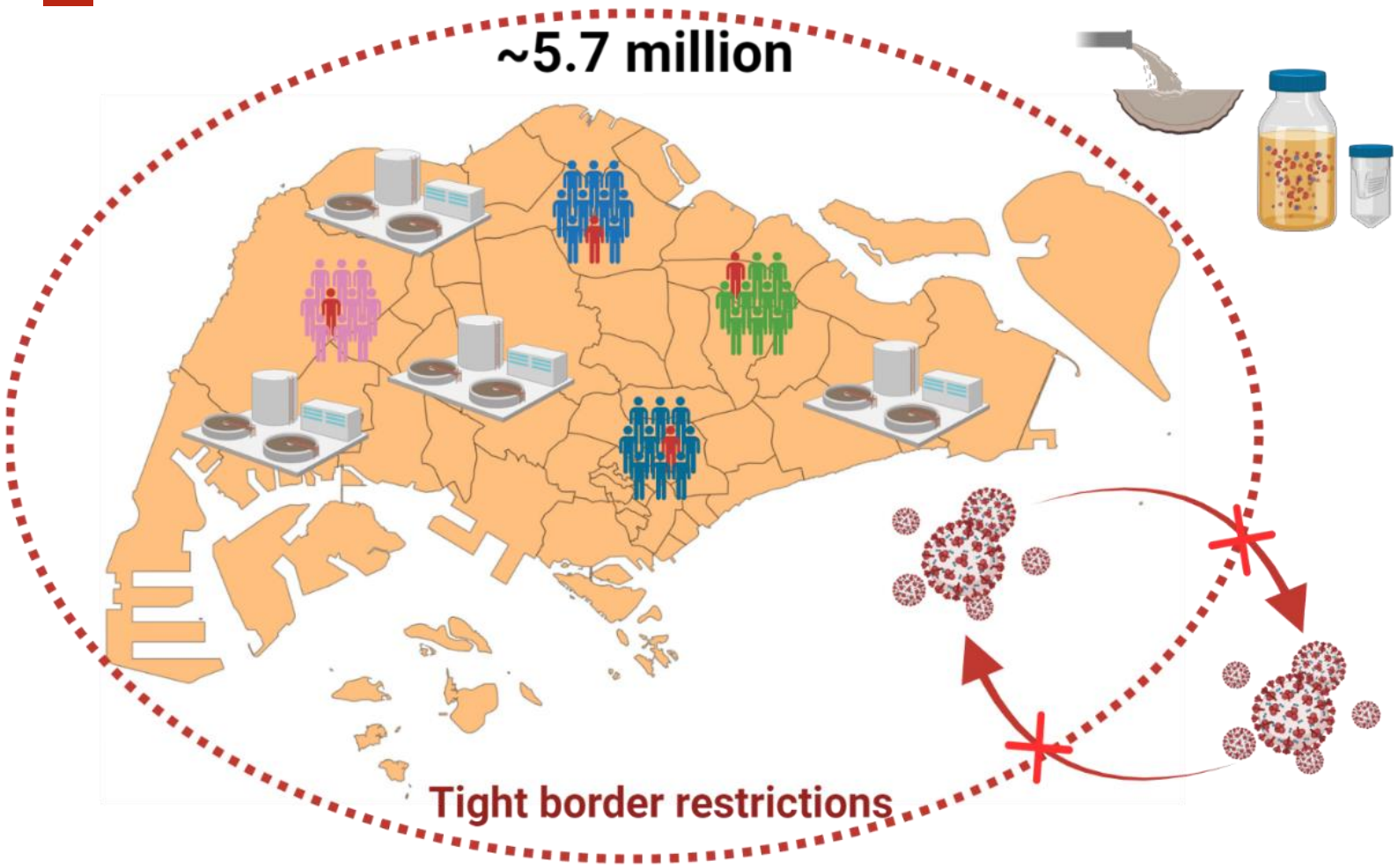
Ceramic Membrane Test

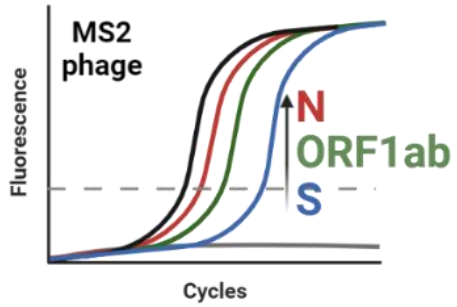


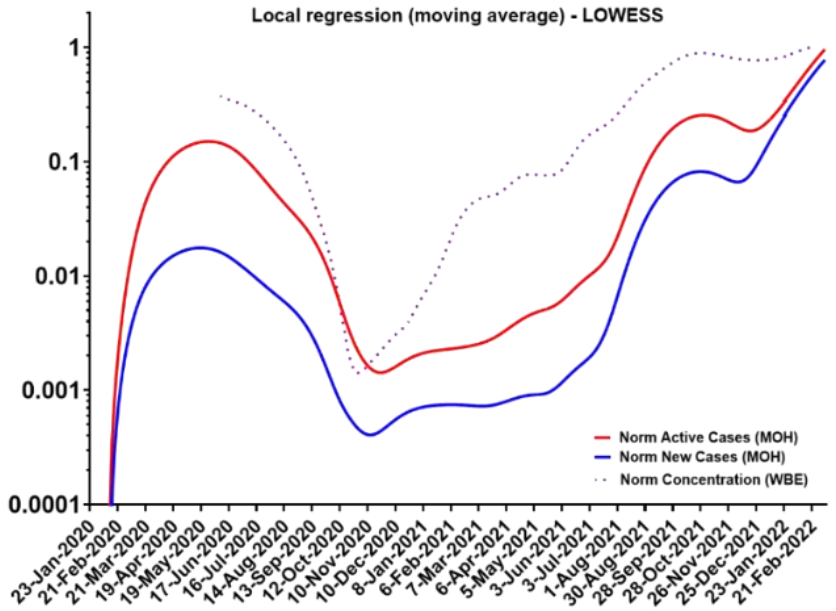




Conclusions of WBE in Singapore







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