



The Environmental Biotechnology Lab at BGU  
Avram and Stella Goldstein-Goren, Department of Biotechnology Engineering

# Wastewater Based Epidemiology: SARS-CoV-2 variants of concern detection and quantification in wastewater



Prof. Ariel Kushmaro



# The Environmental Biotechnology Lab at BGU

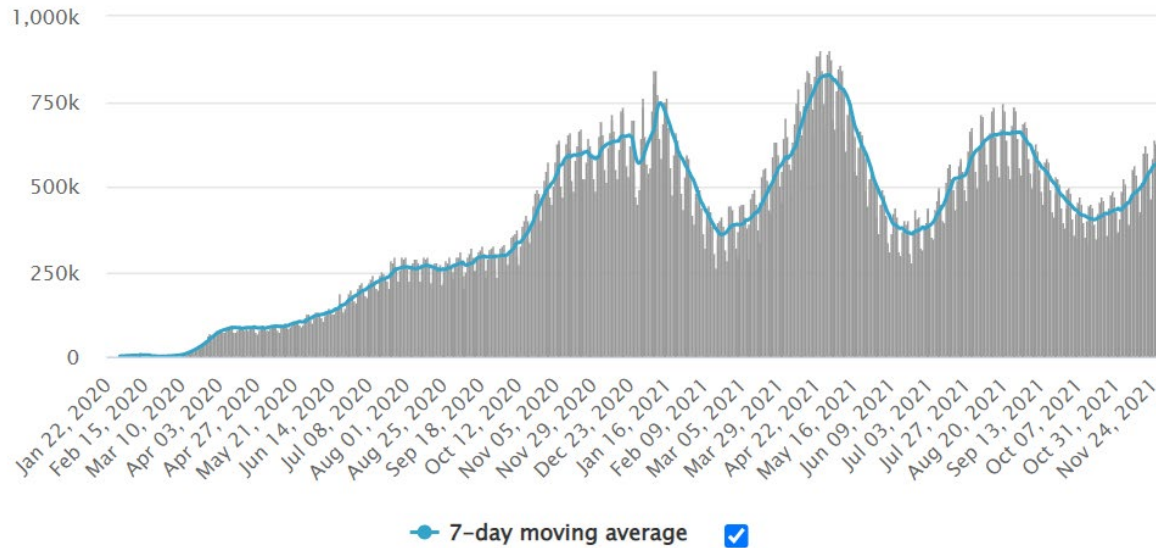
Avram and Stella Goldstein-Goren, Department of Biotechnology Engineering



# SARS-CoV-2 Pandemic

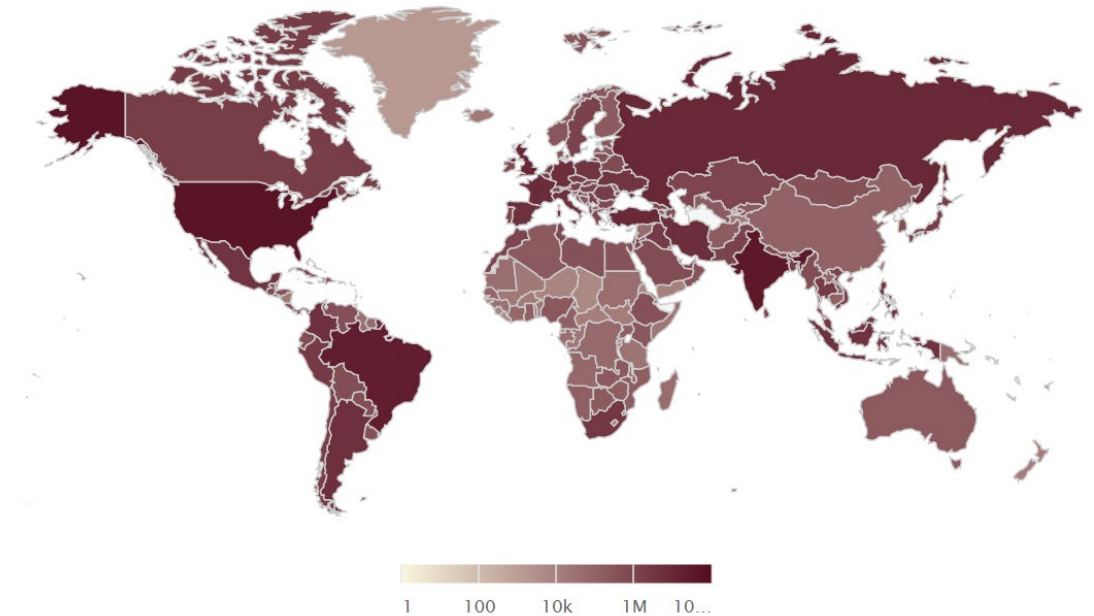
## Daily New Cases

Cases per Day  
Data as of 0:00 GMT+0

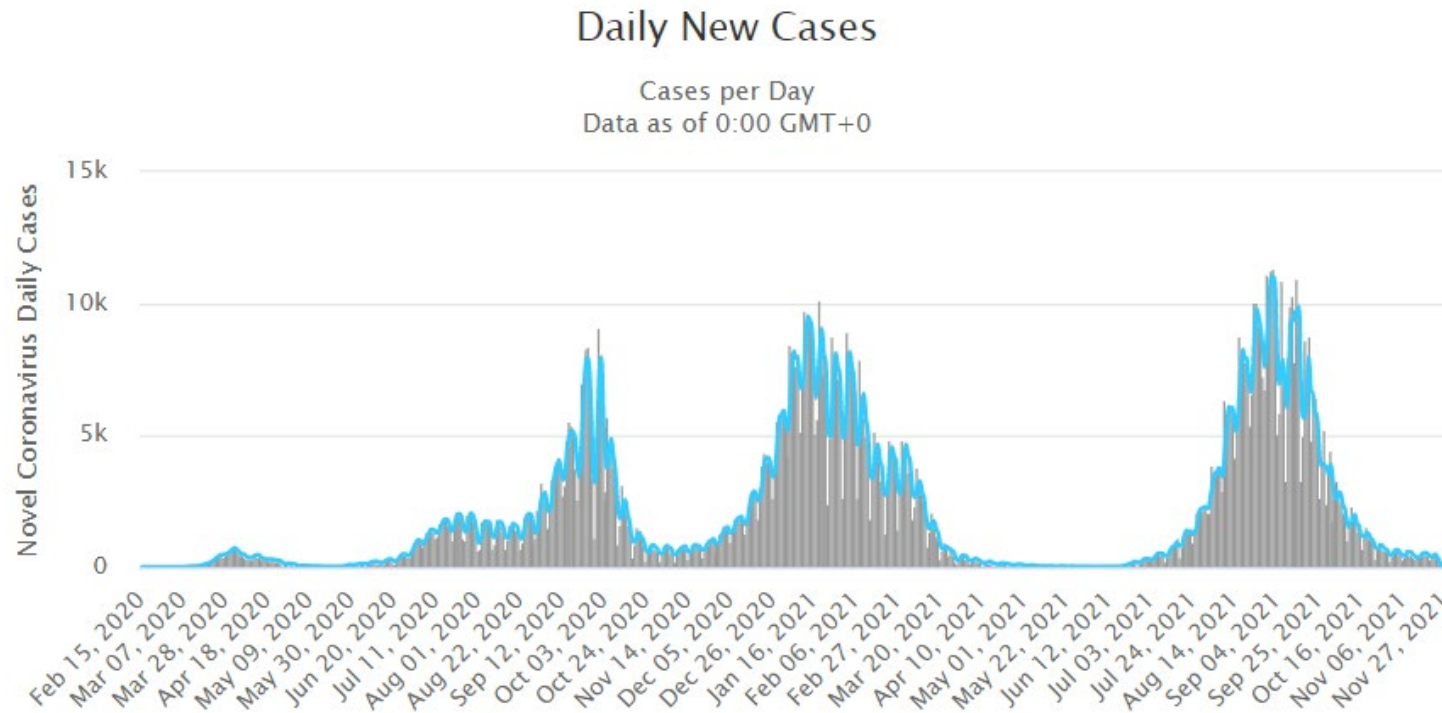


## Total number of cases

Per country over time



# SARS-CoV-2 Pandemic in Israel



Israel

Coronavirus Cases:

**1,342,976**

Deaths:

**8,195**

Recovered:

**1,329,263**

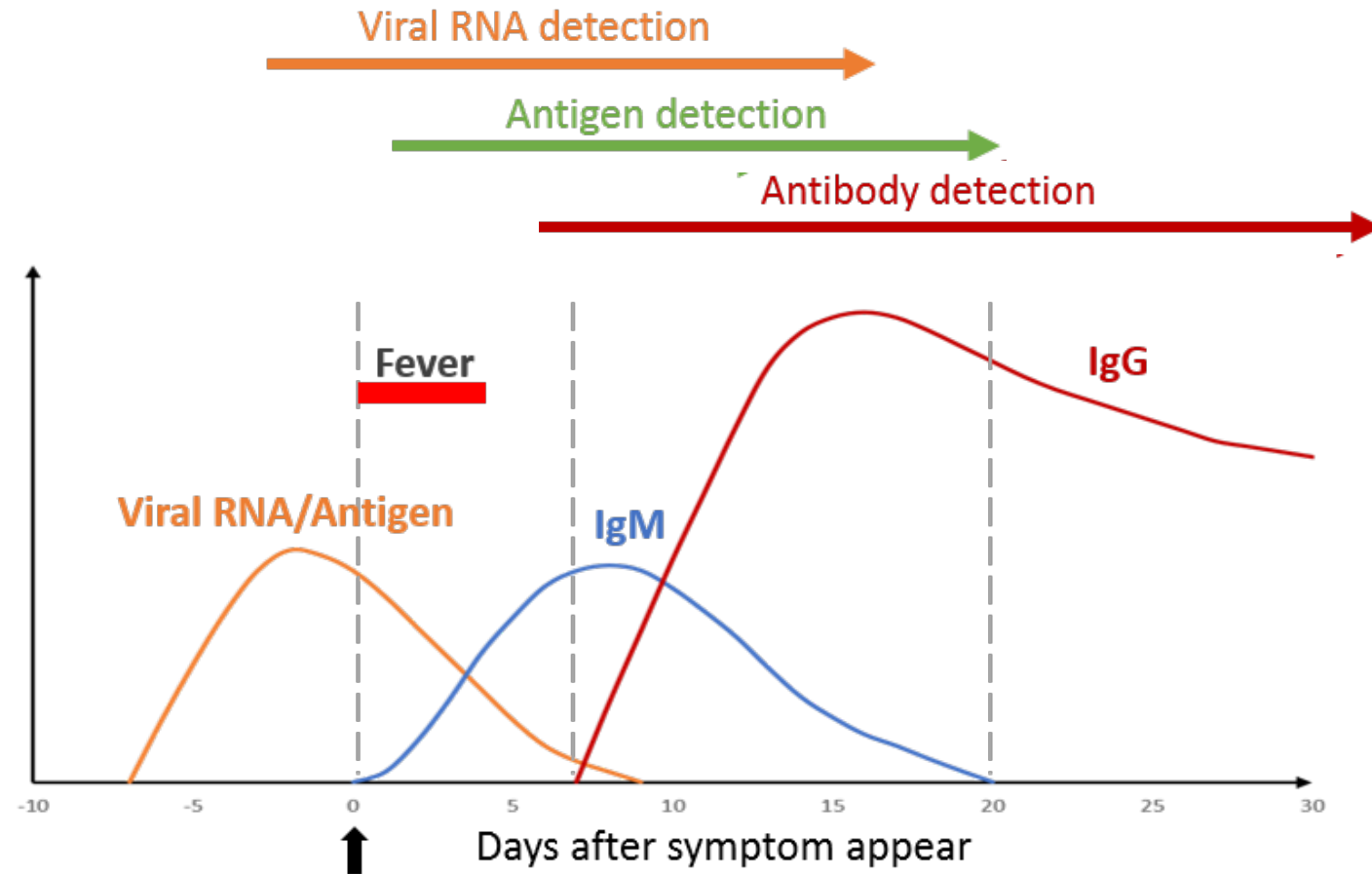
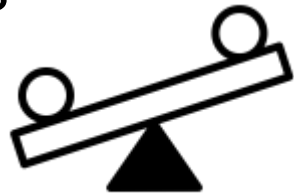


Break the chain of transmission:



# The problem

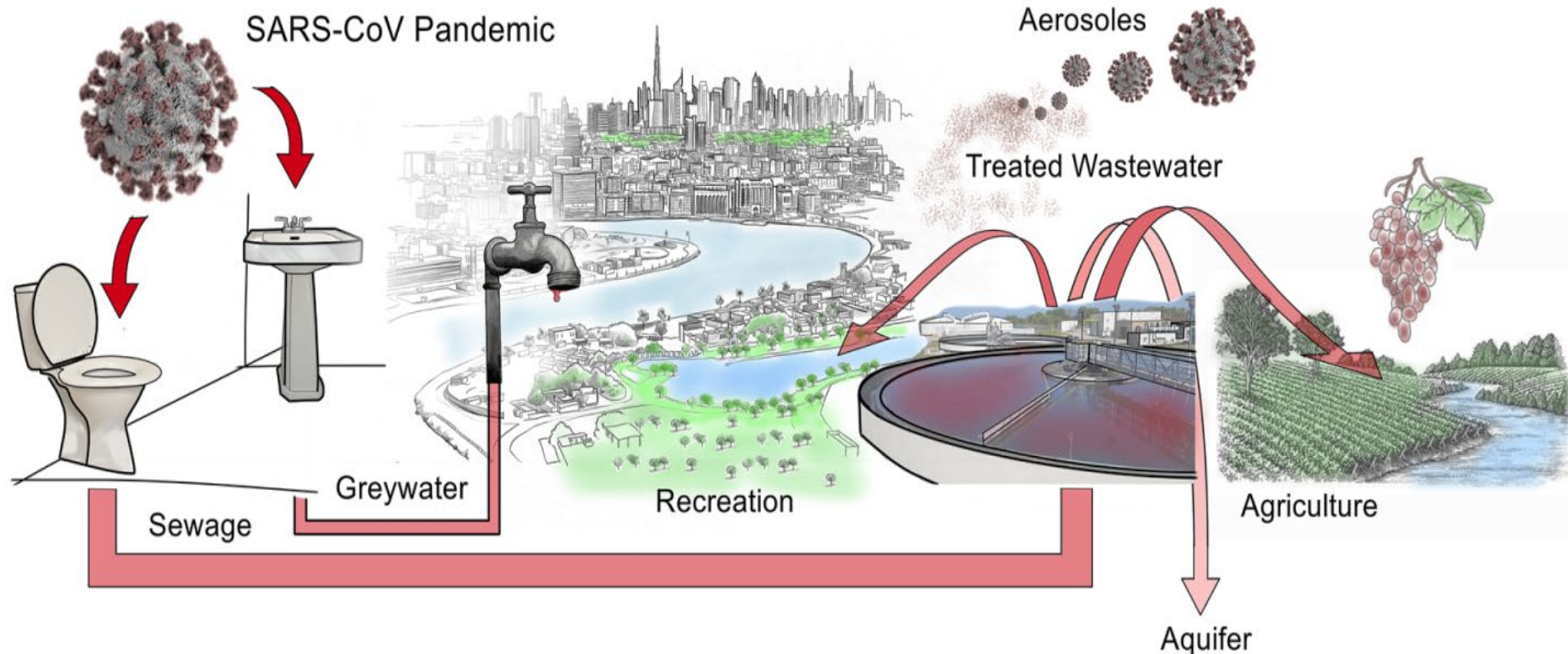
- ❖ Main detection methodology is through RT-qPCR
- ❖ Early detection is essential for pandemic containment
- ❖ Asymptomatic; False Negative; Volunteer bias



# Wastewater Based Epidemiology

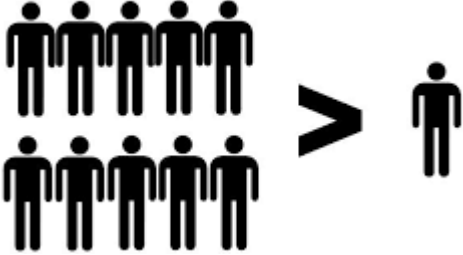
## Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic

Anne Bogler<sup>1,2,3</sup>, Aaron Packman<sup>2</sup>, Alex Furman<sup>3</sup>, Amit Gross<sup>1</sup>, Ariel Kushmaro<sup>4,5</sup>, Avner Ronen<sup>1,4</sup>, Christophe Dagot<sup>7</sup>, Colin Hill<sup>8</sup>, Dalit Vaizel-Ohayon<sup>9</sup>, Eberhard Morgenroth<sup>10,11</sup>, Enrico Bertuzzo<sup>12</sup>, George Wells<sup>2</sup>, Hadas Raanan Kiperwas<sup>9</sup>, Harald Horn<sup>13</sup>, Ido Negev<sup>9</sup>, Ines Zucker<sup>14,15</sup>, Itay Bar-Or<sup>16</sup>, Jacob Moran-Gilad<sup>17</sup>, Jose Luis Balcazar<sup>18,19</sup>, Kyle Bibby<sup>20</sup>, Menachem Elimelech<sup>21</sup>, Noam Weisbrod<sup>1</sup>, Oded Nir<sup>1</sup>, Oded Sued<sup>9</sup>, Osnat Gillor<sup>1</sup>, Pedro J. Alvarez<sup>22</sup>, Sandra Cramer<sup>23</sup>, Shai Arnon<sup>1</sup>, Sharon Walker<sup>24</sup>, Sima Yaron<sup>25,26</sup>, Thanh H. Nguyen<sup>27</sup>, Yakir Berchenko<sup>28</sup>, Yunxia Hu<sup>29</sup>, Zeev Ronen<sup>1</sup> and Edo Bar-Zeev<sup>1,3,3</sup>





# Normalization for SARS-CoV-2 detection



Flow rate

Population size

Secretion rate

Wastewater source

SARS-CoV-2 signal strength

$$\text{Normalized Viral Load (NVL)} = \frac{\text{RNA copy number} \left(\frac{\#}{L}\right) * \text{Commulative sampling flow (L)}}{\text{Population size}/1000}$$





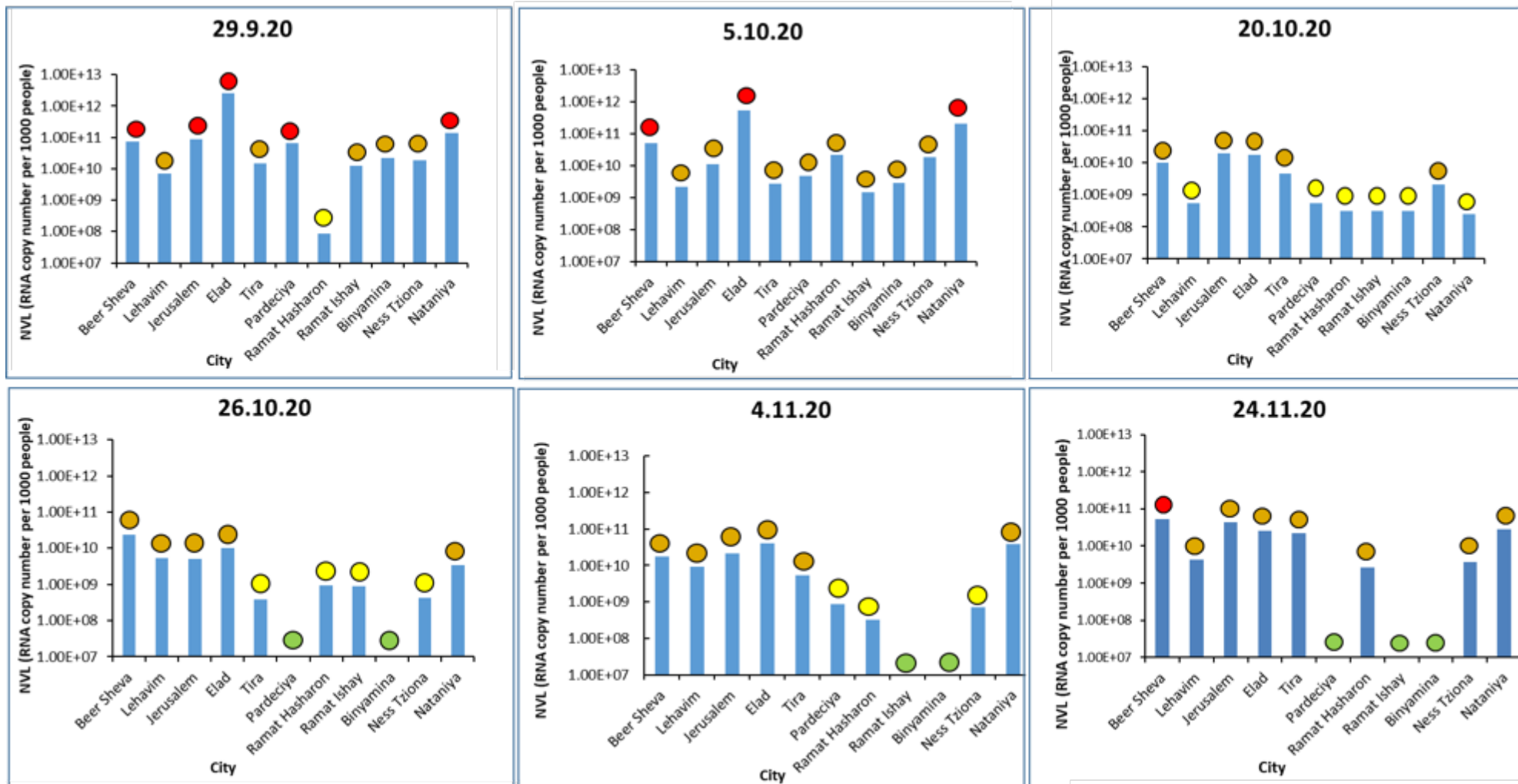
# 'Traffic' scale for SARS-CoV-2 detection

>5\*10<sup>10</sup>

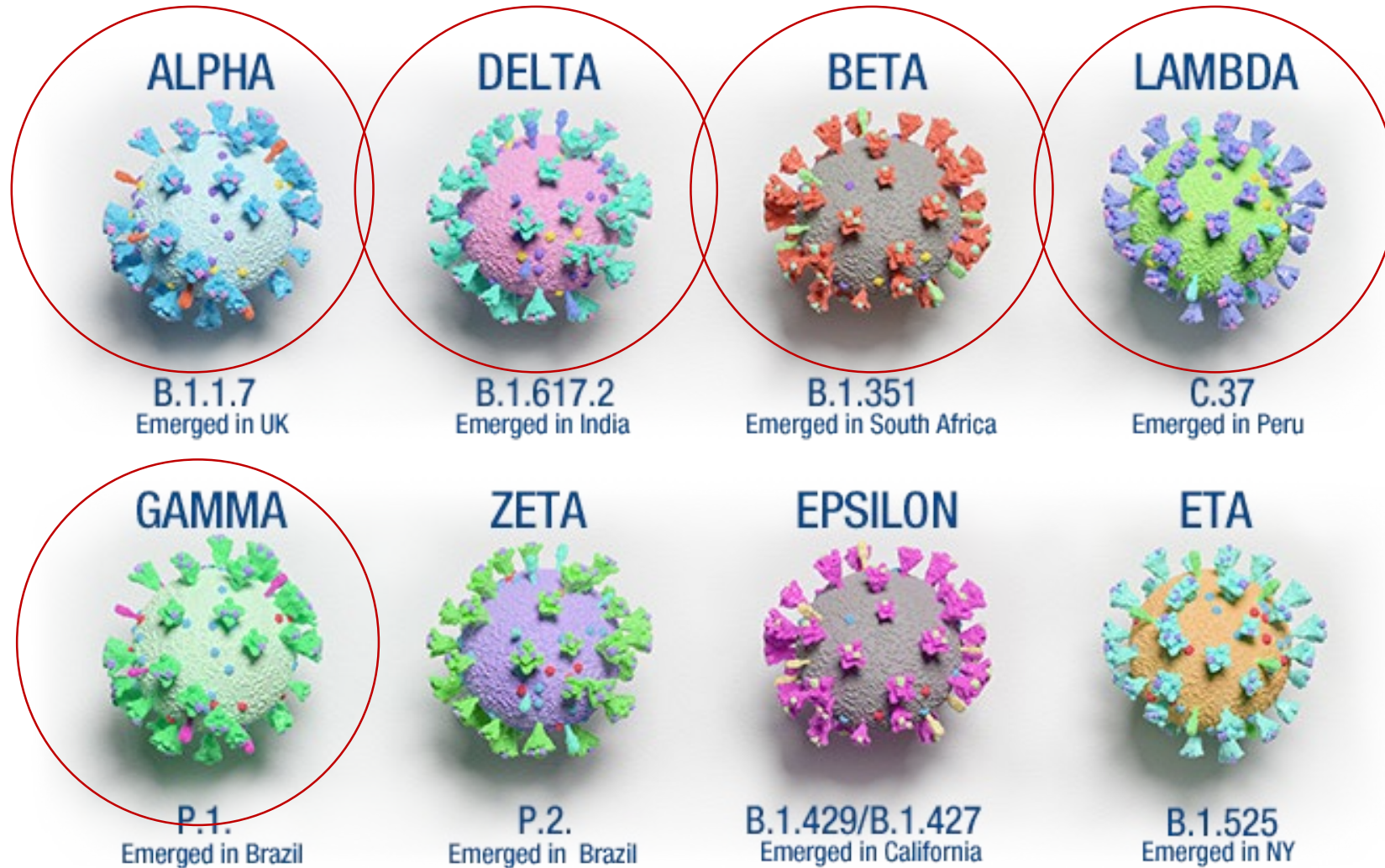
-1\*10<sup>9</sup>  
5\*10<sup>10</sup>

<1\*10<sup>9</sup>

Not Detected



# Variants of Concern vs. Variants of Interest



# Variants of Concern vs. Variants of Interest



Water Research

Volume 207, 1 December 2021, 117808



RT-qPCR assays for SARS-CoV-2 variants of concern in wastewater reveals compromised vaccination-induced immunity

Karin Yaniv <sup>a, 1</sup>, Eden Ozer <sup>b, 1</sup>, Yair Lewis <sup>c</sup>, Ariel Kushmaro <sup>a, d, e</sup>  



Environmental Research

Volume 201, October 2021, 111653

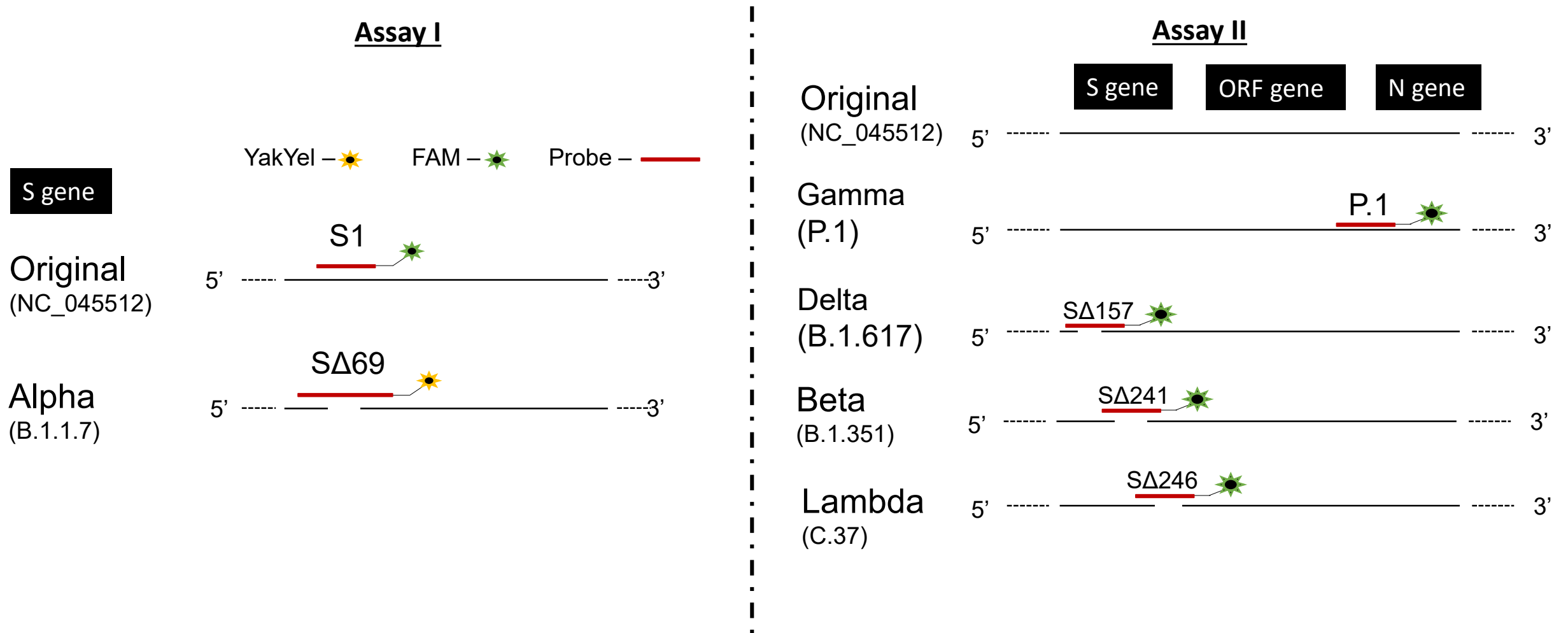


Direct RT-qPCR assay for SARS-CoV-2 variants of concern (Alpha, B.1.1.7 and Beta, B.1.351) detection and quantification in wastewater

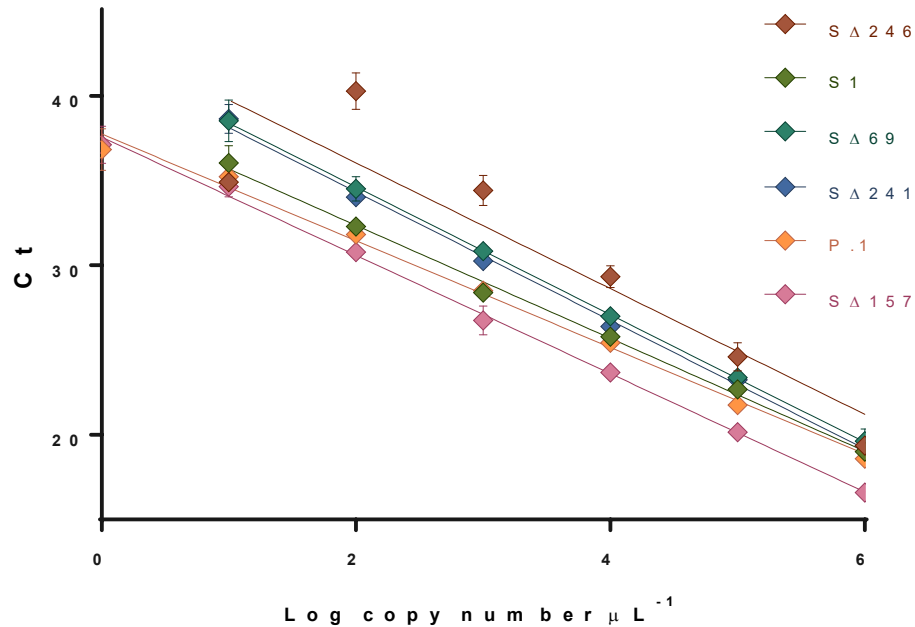
Karin Yaniv <sup>a, 1</sup>, Eden Ozer <sup>b, 1</sup>, Marilou Shagan <sup>a</sup>, Satish Lakkakula <sup>a</sup>, Noam Plotkin <sup>a</sup>, Nikhil Suresh Bhandarkar <sup>a</sup>, Ariel Kushmaro <sup>a, c</sup>  



# SARS-CoV-2 Mutations detection

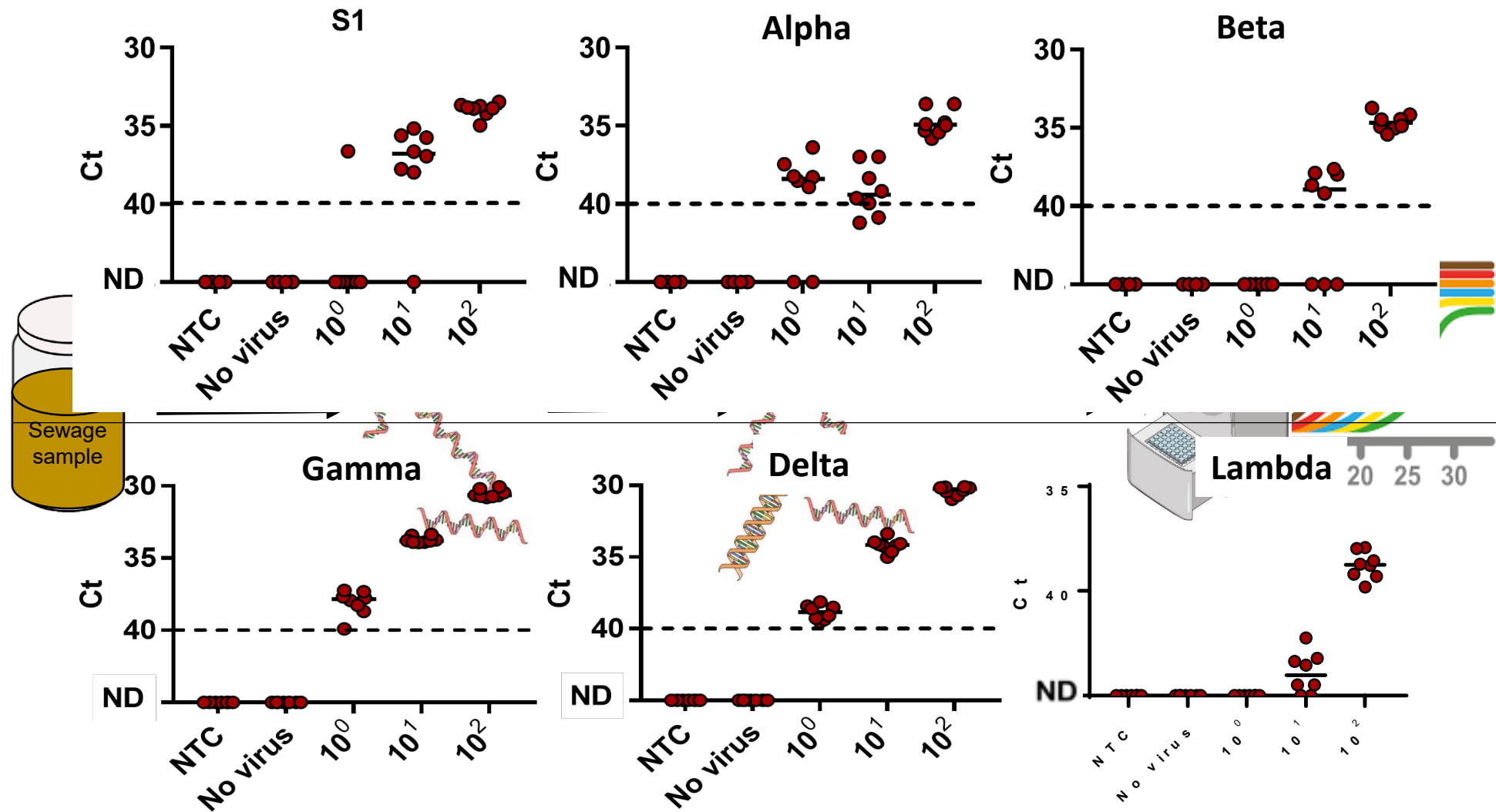


# SARS-CoV-2 Mutations detection



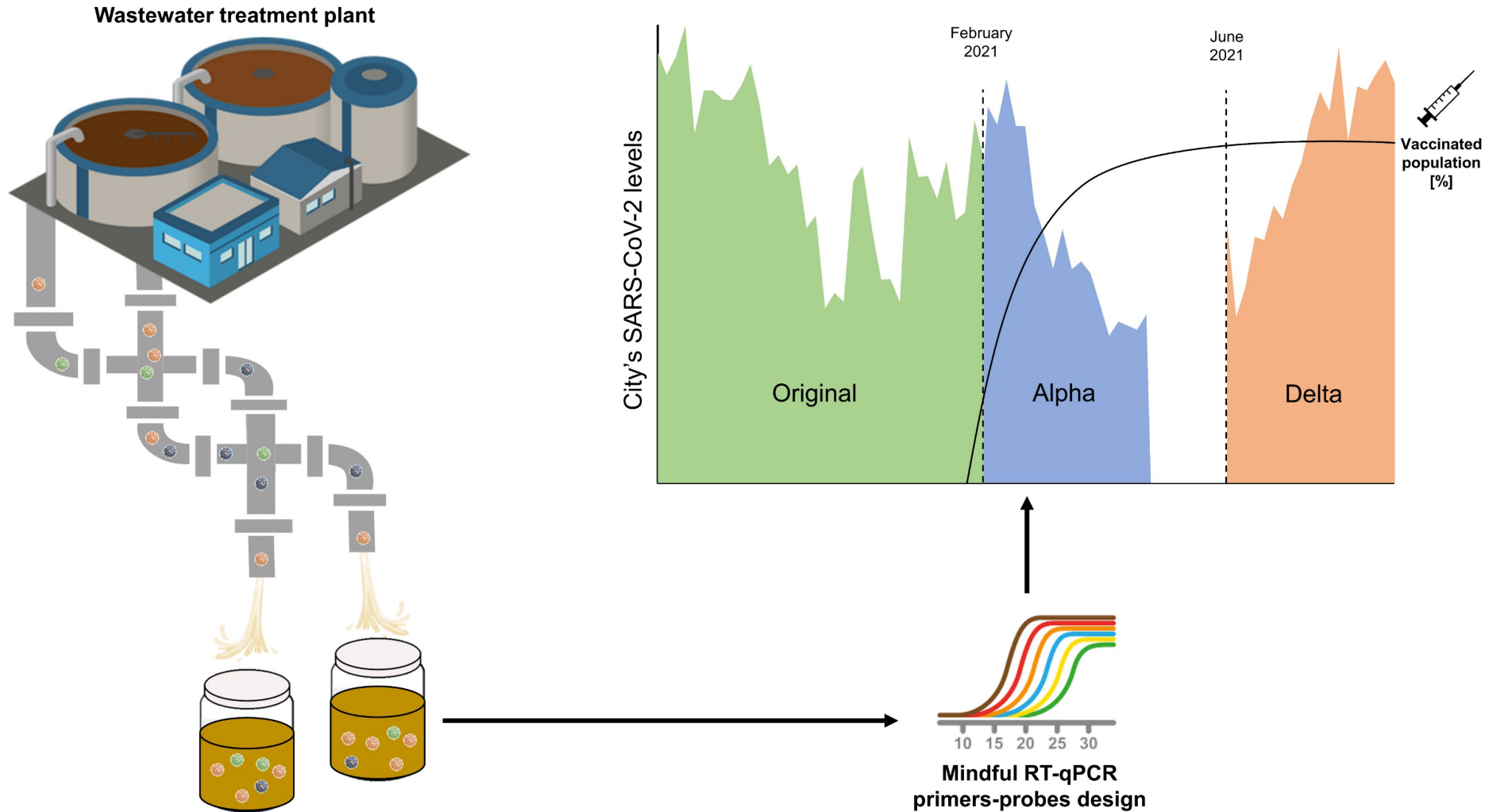
Set	Limit of detection (LOD) [Copy number per $\mu\text{L}$ ]	Linear regression $R^2$	Y intercept
S1	10	0.9977	$39.14 \pm 0.323$
S $\Delta$ 69	10	0.9998	$42.07 \pm 0.1132$
S $\Delta$ 241	10	0.9978	$41.75 \pm 0.3516$
P.1	1	0.9942	$37.75 \pm 0.3865$
S $\Delta$ 157	1	0.9979	$37.57 \pm 0.2561$

# SARS-CoV-2 Mutations detection

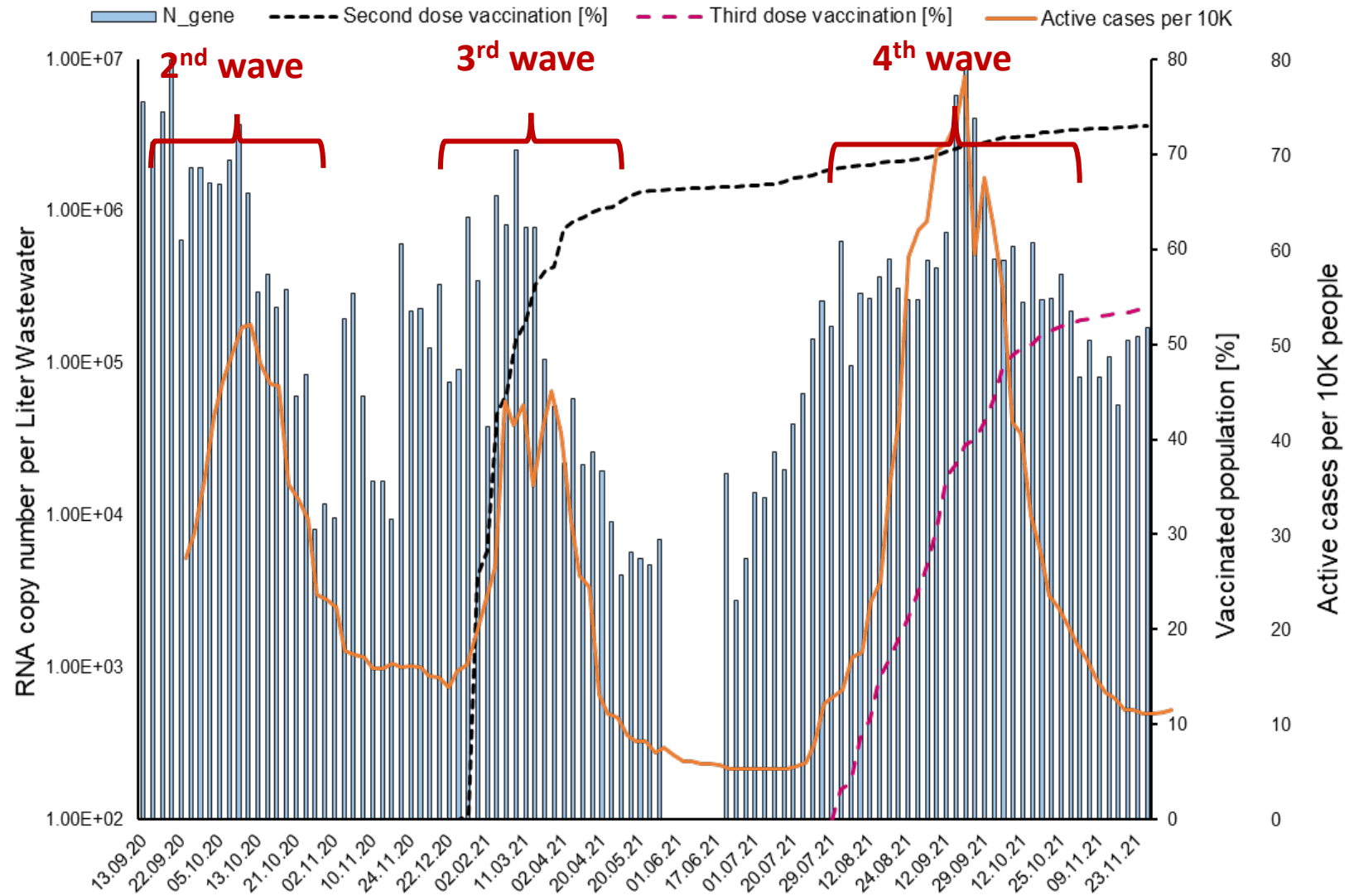




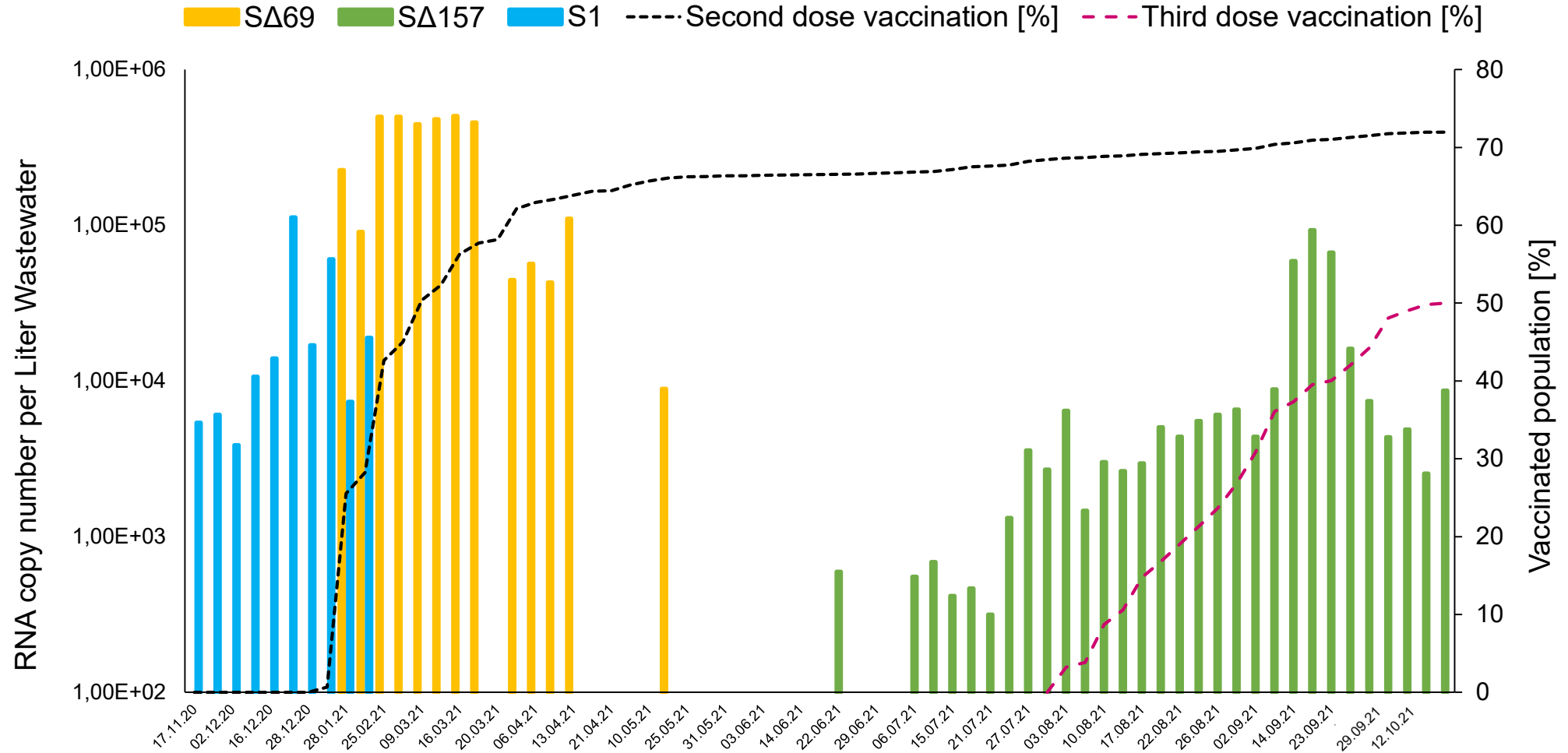
# SARS-CoV-2 Mutations detection in Beer Sheva



# SARS-CoV-2 Mutations detection in Beer Sheva



# SARS-CoV-2 Mutations detection in Beer Sheva





# Conclusion

- ❖ **Successful development of direct RT-qPCR assays for Alpha, Beta, Gamma, Delta and Lambda variants of concern.**
- ❖ **Variants differentiation and quantification was achieved using the developed assays.**
- ❖ **The describe developments are crucial for early warning and epidemiological population surveillance.**
- ❖ **The response time for developing specific assays for future variants of interest/concern is relatively short. The presented detection approach is beneficial.**

# In the media



Jerusalem Post > Israel News

## Israeli tech to hunt for early evidence of coronavirus in sewer systems

Imagine if the world knew that there would be a coronavirus outbreak two weeks before it started spreading, how much better-prepared countries could be in dealing with the pandemic.

By [\\_DOSHUA ROBBIN MARKS](#) THE MEDIA LINE OCTOBER 27, 2020 08:04



## Down the tubes: How Israel's poop may help prevent second coronavirus wave

Scientists say analyzing sewage from across the nation can help pinpoint infection areas and nip new cases in the bud

By [NATHAN JEFFAY](#)

4 May 2020, 2:00 pm

**Ben-Gurion University Researchers To Monitor Sewage For COVID-19 Outbreaks In 14 Israeli Cities**  
Ben-Gurion University of the Negev (BGU) has signed an agreement with the Israeli Ministry of Health to detect and monitor for COVID-19 in sewage samples from 14 cities around the country.

**Israeli Scientists Hunt COVID-19 Evidence in Sewage Samples**

**Using sewage to track coronavirus outbreaks**  
Ben Gurion University study seeks to use sewage samples to detect increase in coronavirus morbidity before symptoms appear.  
& Tags: Coronavirus, Ben Gurion University

# Acknowledgments



אוניברסיטת בן-גוריון בנגב  
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