

Wastewater Surveillance Using ddPCR Reveals Highly Accurate Tracking of Omicron Variant Due to Altered N1 Probe Binding Efficiency

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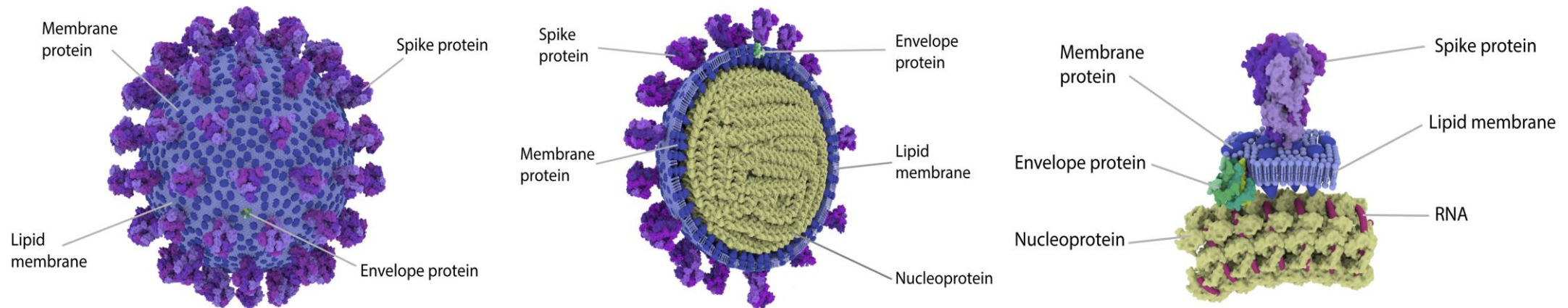
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Outline

1. Underlining Assumptions and Study Purpose
2. Methodology
 - Wastewater Surveillance
 - Experimentation of this study
3. Analyze how wastewater surveillance captures community infection.
 - Compare our laboratory results to standard clinical testing.
3. Conclusions and Future Work

Underlying Assumptions / COVID-19

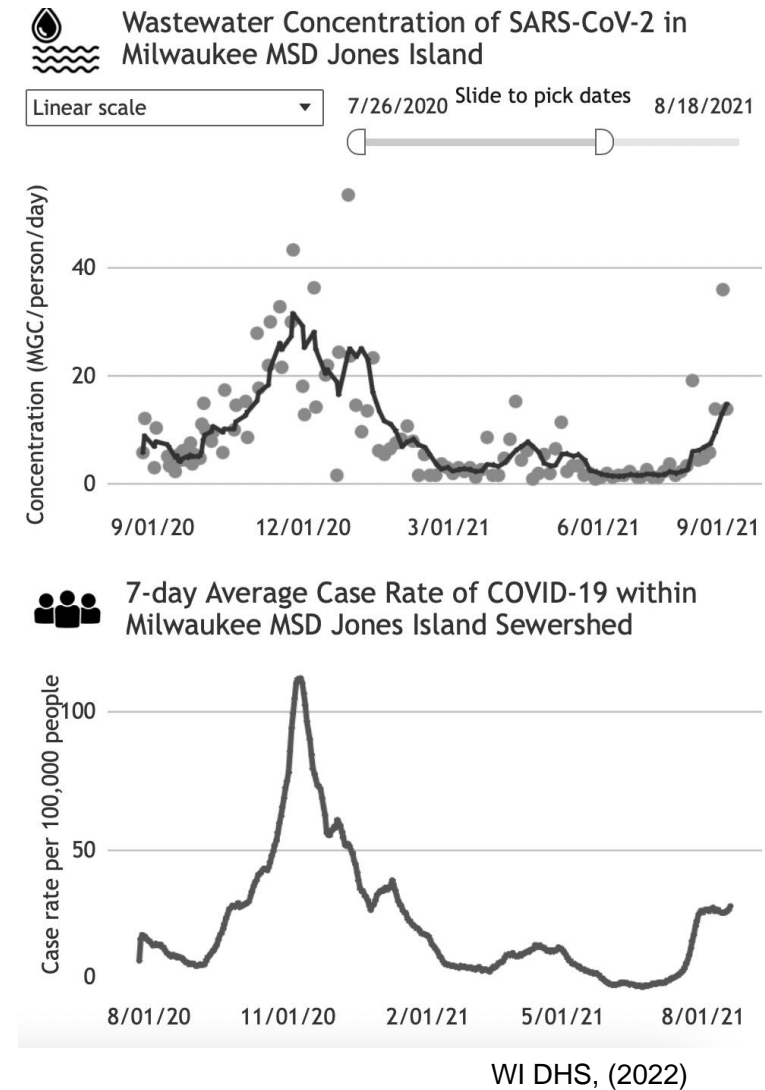
- The COVID-19 pandemic has changed the world as we know it, confining residents to their homes, shutting down business, and challenging national health systems.
- SARS-CoV-2 is the causative agent of COVID-19. It is an enveloped non-segmented positive-sense RNA.



Gaslow Life Sciences, 2020.

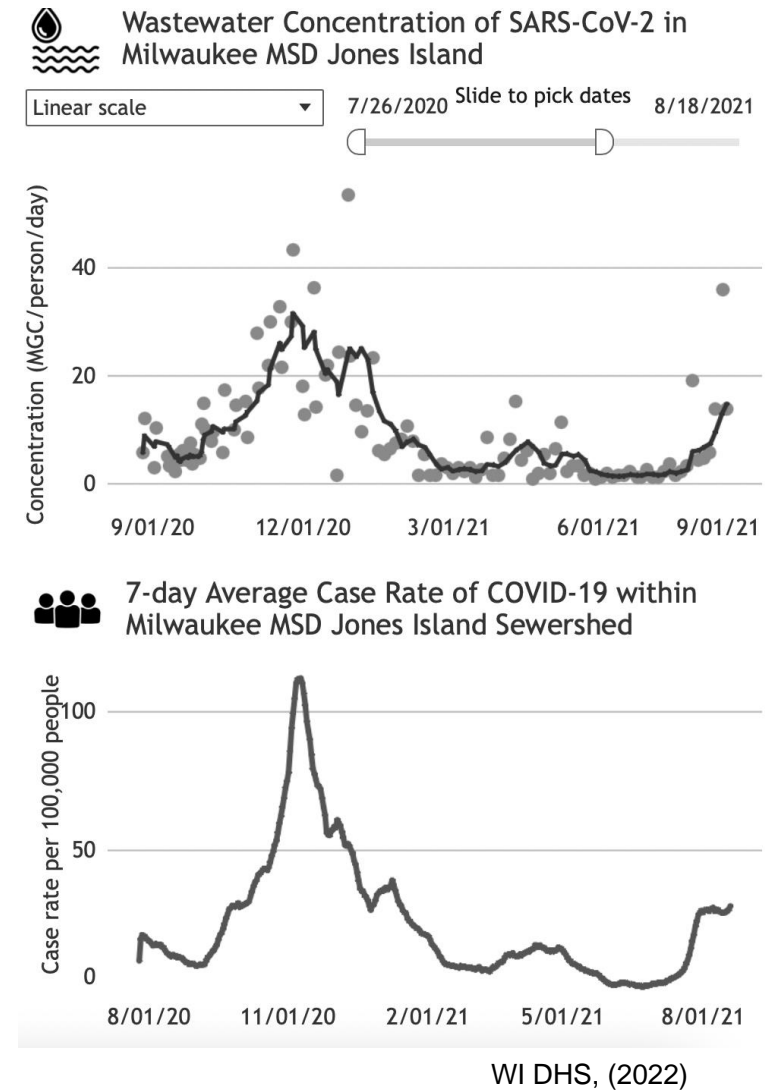
Wastewater Surveillance

- SARS-CoV-2 detected in fecal matter Feb 2020.
(Zhang et al. 2020)



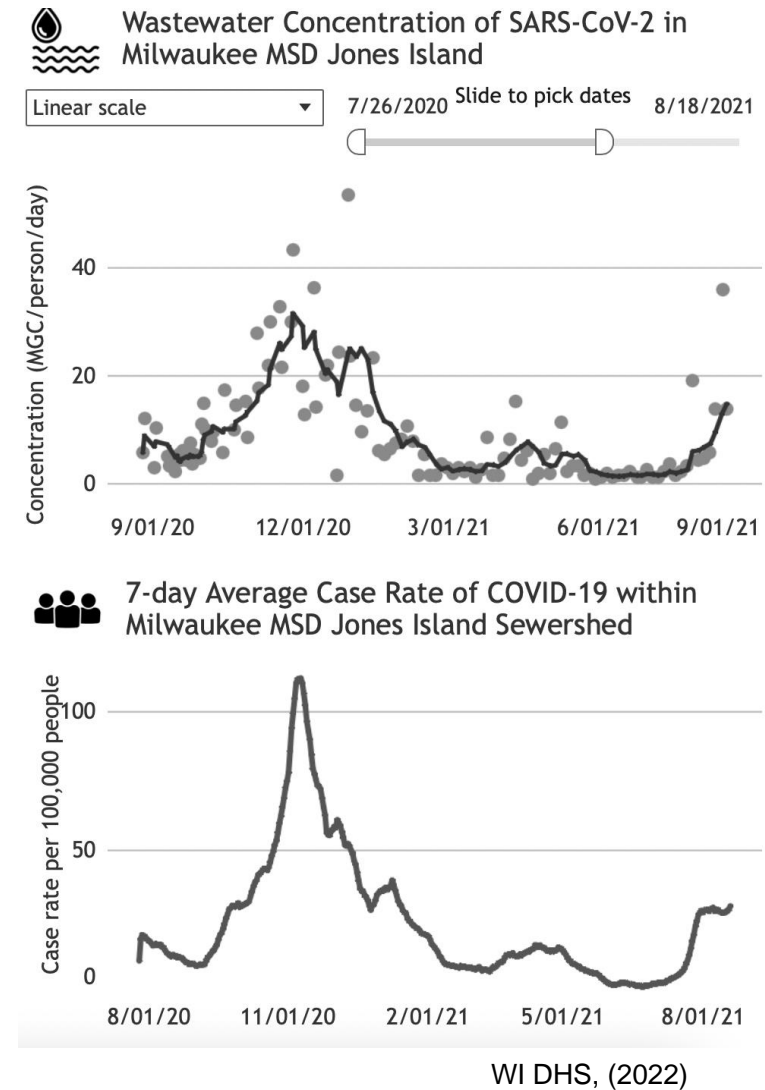
Wastewater Surveillance

- SARS-CoV-2 detected in fecal matter Feb 2020 (Zhang et al.).
- SARS-CoV-2 detected in wastewater April 2020.
(Medema et al. 2020)



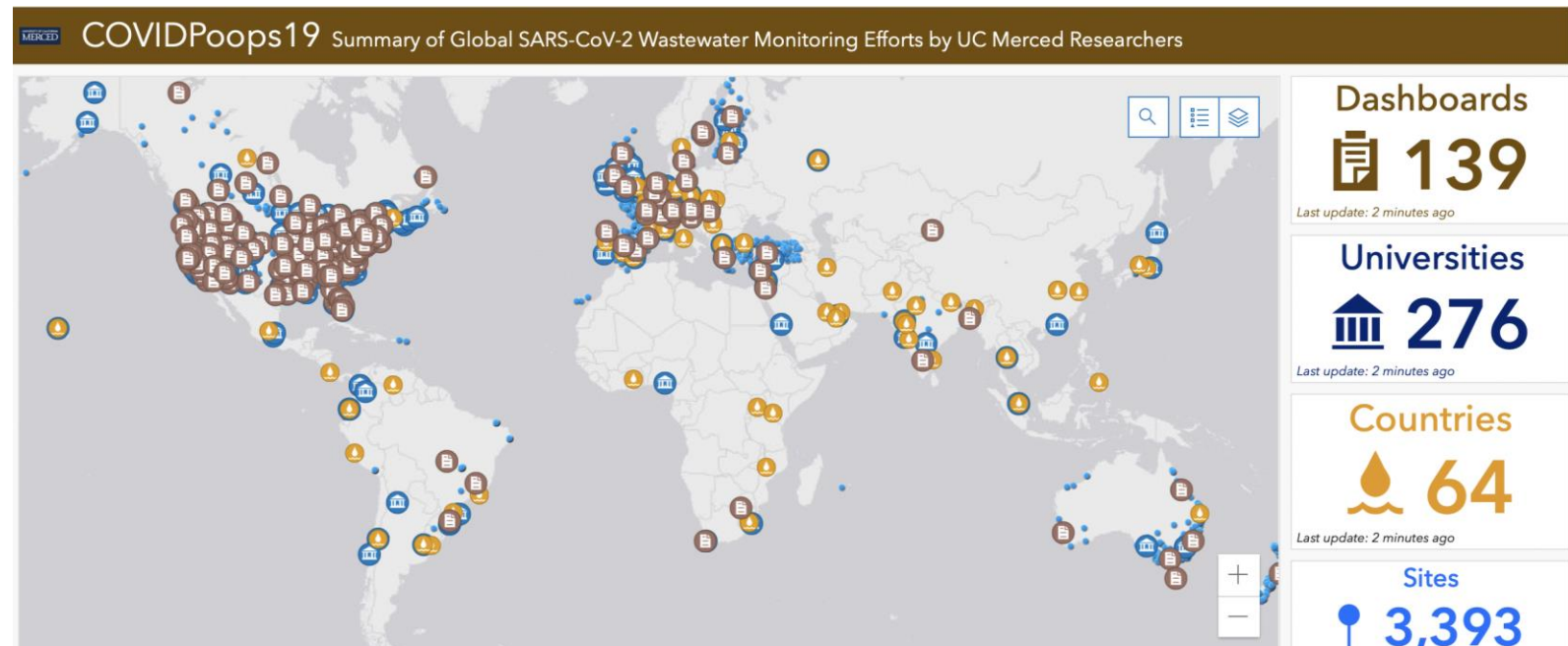
Wastewater Surveillance

- SARS-CoV-2 detected in fecal matter Feb 2020 (Zhang et al).
- Detected in wastewater April 2020 (Medema et al).
- Wastewater surveillance benefits:
 - One sample can be used for many targets.
 - Provides an unbiased reflection of community health.



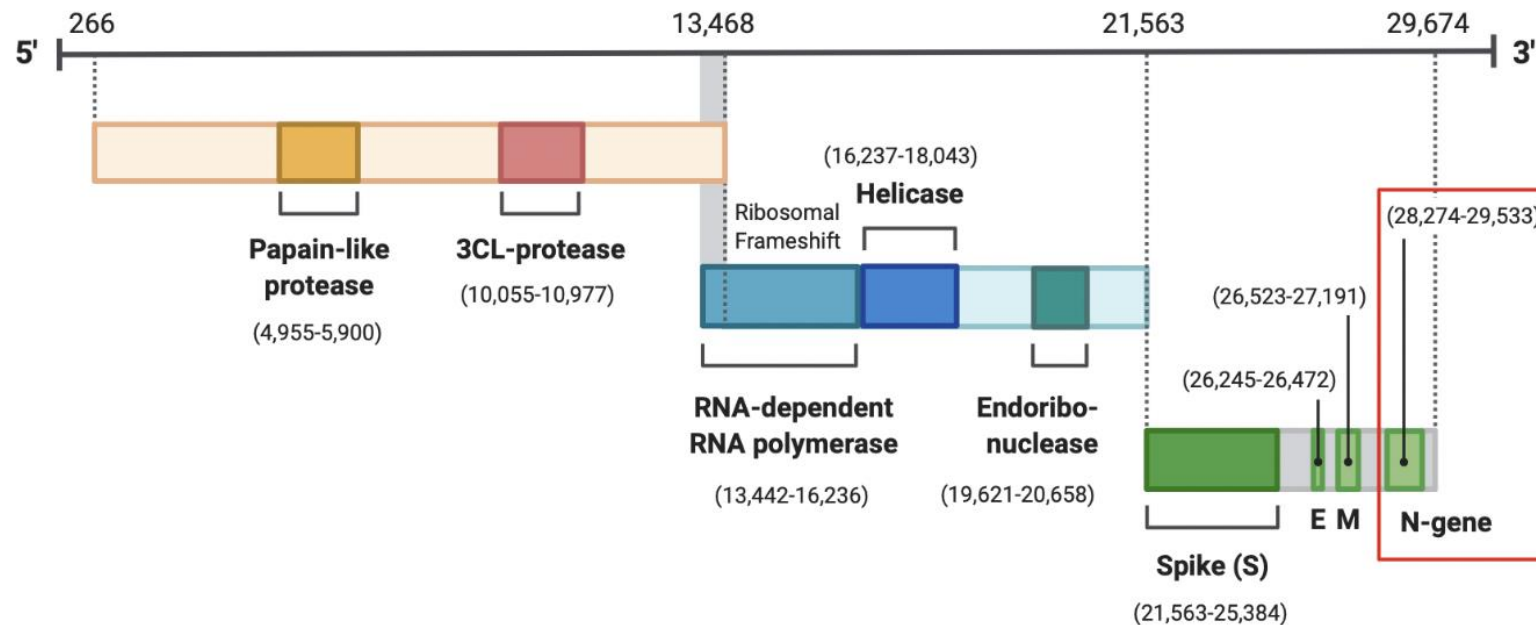
Wastewater Surveillance

- Wastewater surveillance has gained popularity as a public health tool across the world.
- No standard laboratory method for SARS-CoV-2 measurement from wastewater.



Wastewater surveillance

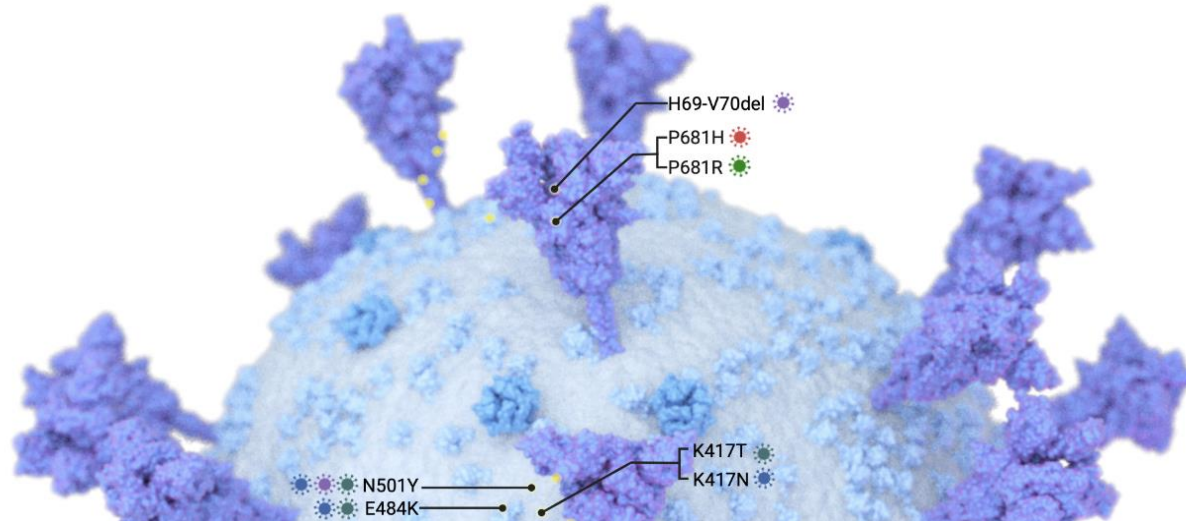
- The CDC designed a primer and probe targeting the N-gene of SARS-CoV-2.



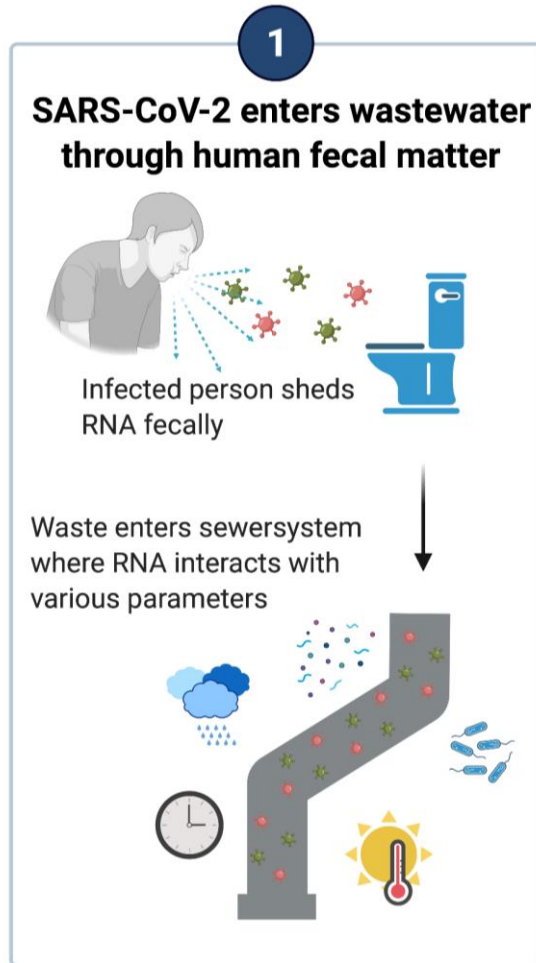
Study Rationale / Background

- The CDC designed a primer and probe targeting the N-gene of SARS-CoV-2.
- Rapid and frequent emergence of variants has pressured continuous method adaptations.

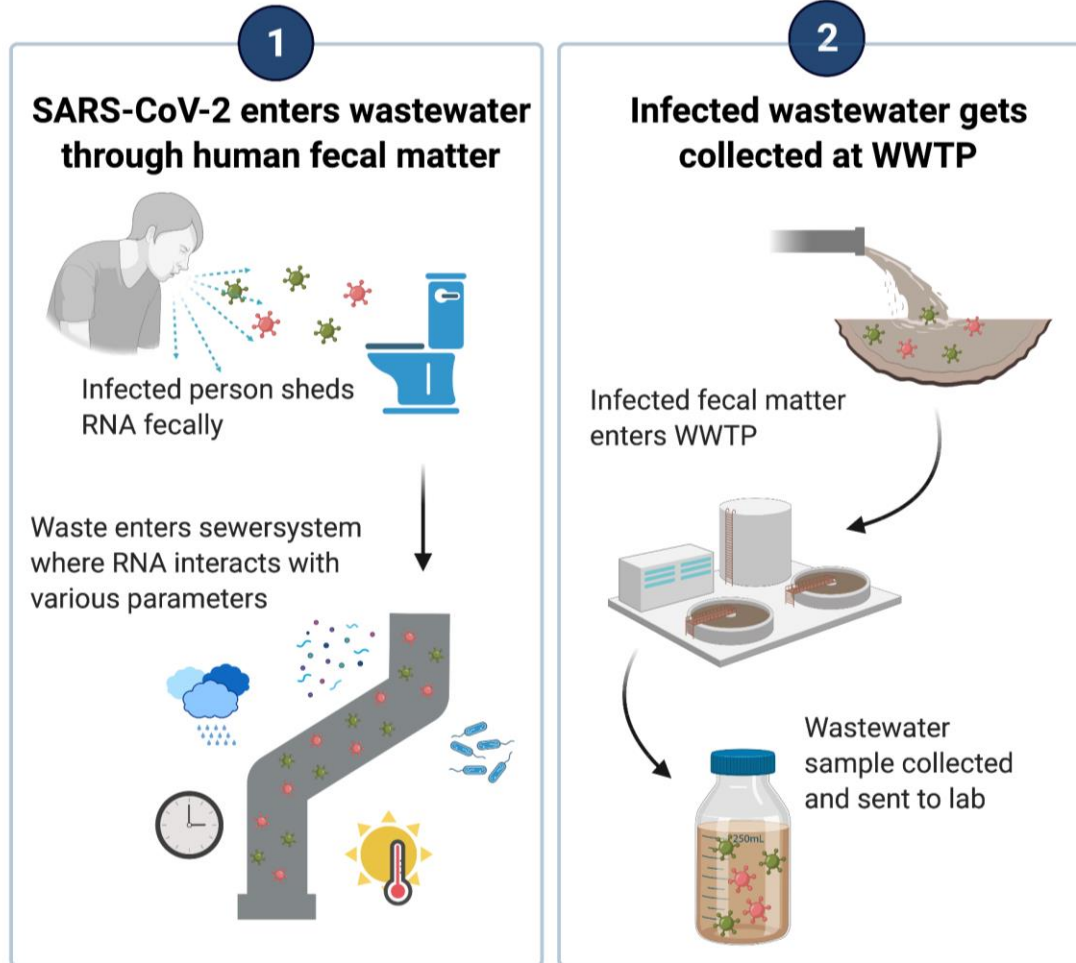
 Alpha Discovered: Sep. 2020	 Beta Discovered: Nov. 2020	 Gamma Discovered: Nov. 2020	 Delta Discovered: Dec 2020	 Omicron Discovered: Nov. 2021
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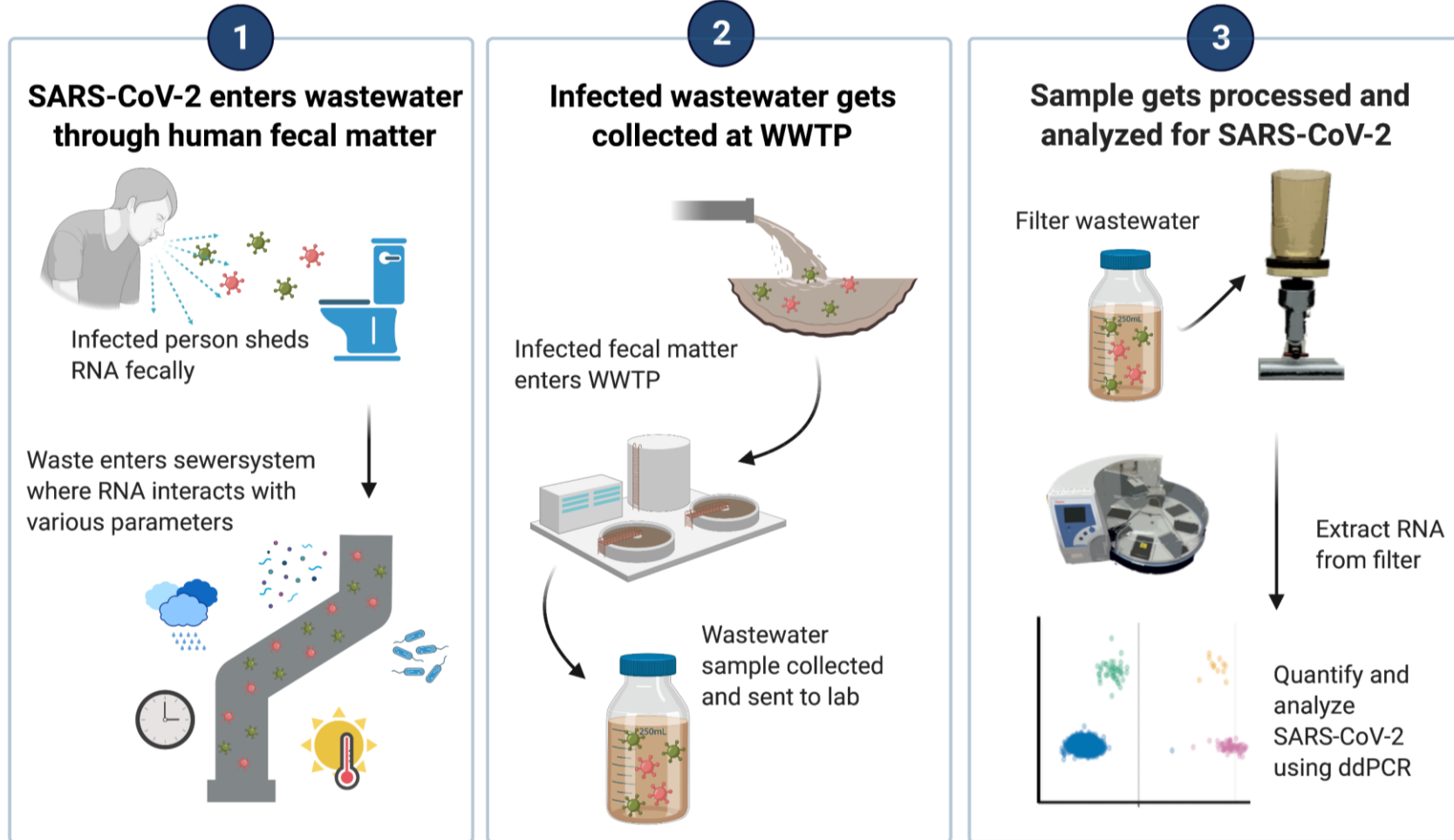
Wastewater Surveillance Methodology



Wastewater Surveillance Methodology

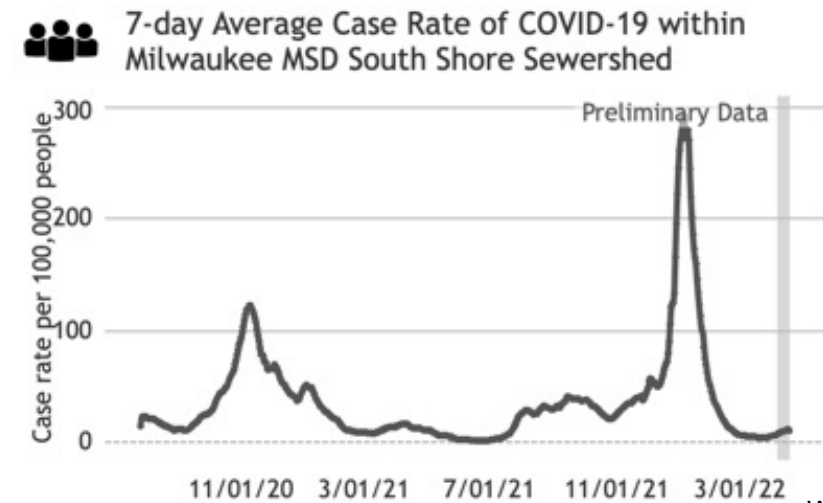
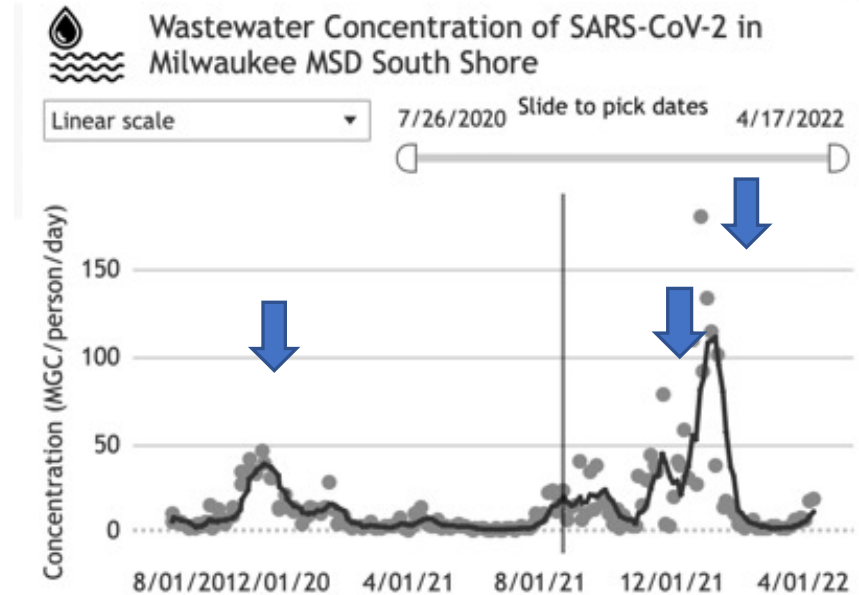


Wastewater Surveillance Methodology



Correlation to Case Data

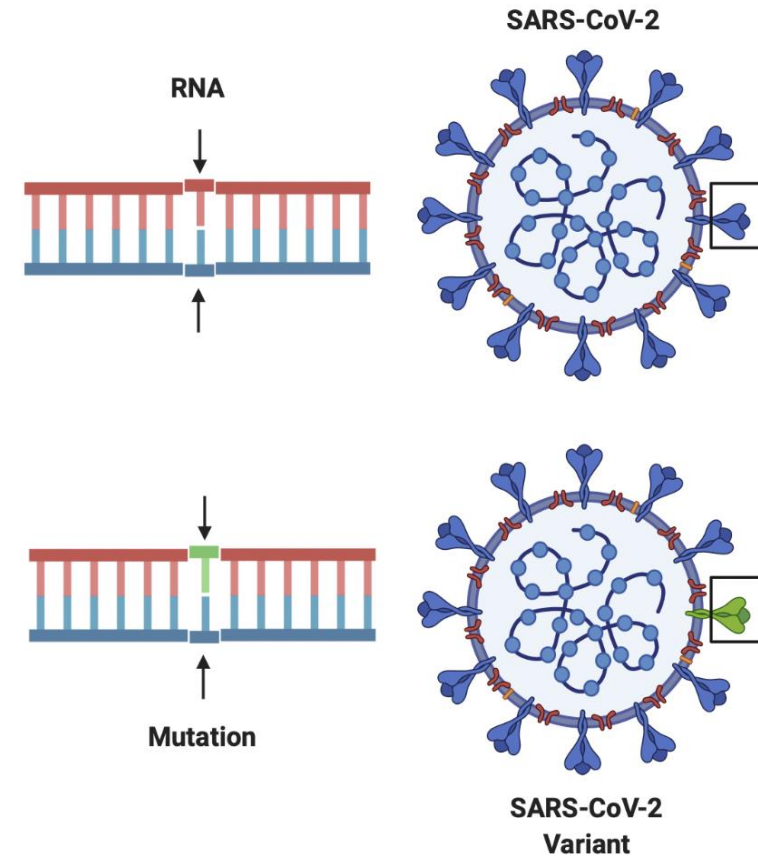
- Wastewater surveillance data of SARS-CoV-2 correlate well to clinical case data.
- Weekly wastewater surveillance of COVID-19 detects variant changes.



WI DHS, (2022)

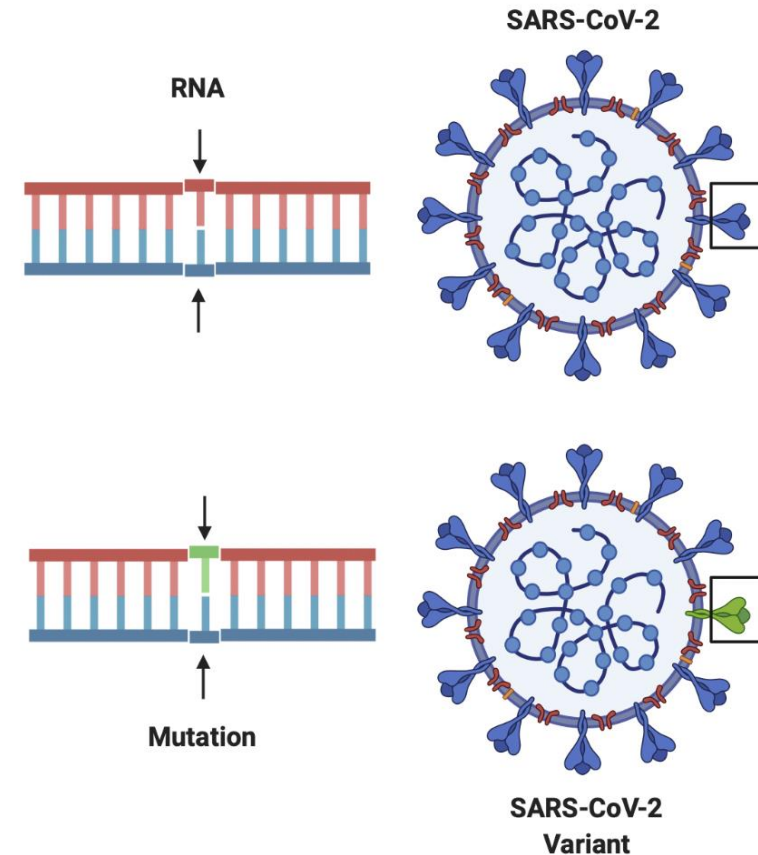
Study Rationale / Background

- Variants are new strains of the same species.
- Omicron contains a mutation in the N gene.



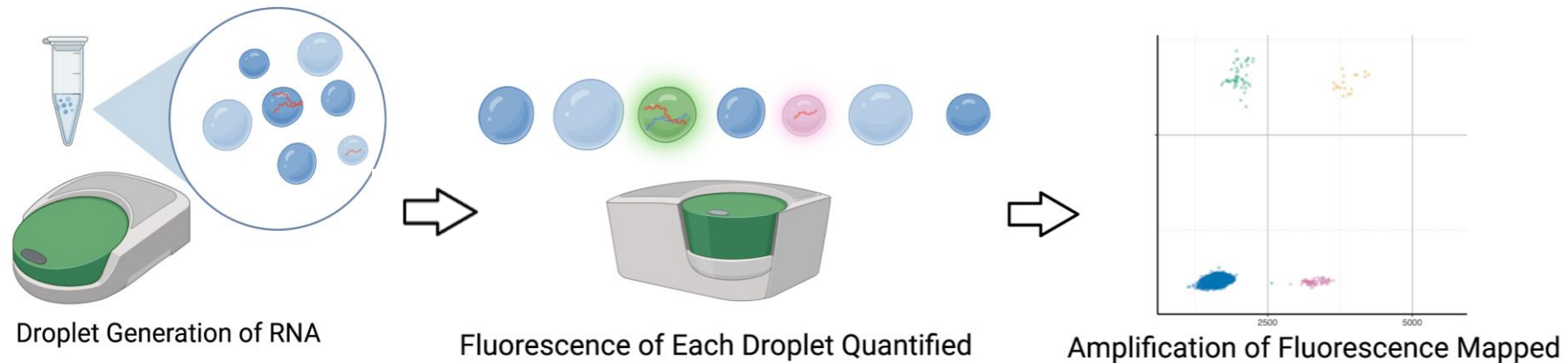
Study Rationale / Background

- The Omicron variant contains a mutation in the N1 gene that corresponds to the probe binding site of the CDC assay.



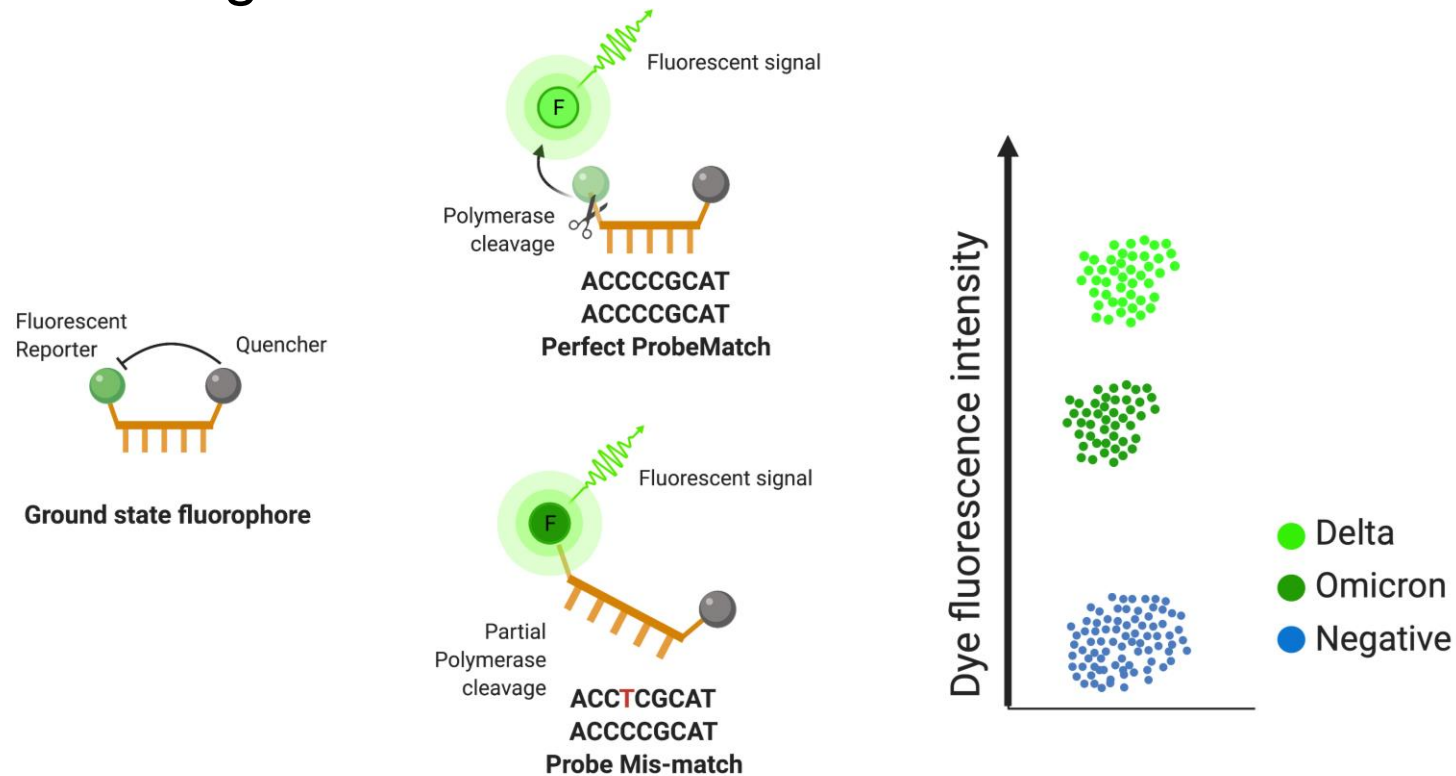
Methods

- Compared Omicron variant tracking in wastewater surveillance to clinical sequenced data on GISAID.
- Used droplet digital PCR to quantify SARS-CoV-2.



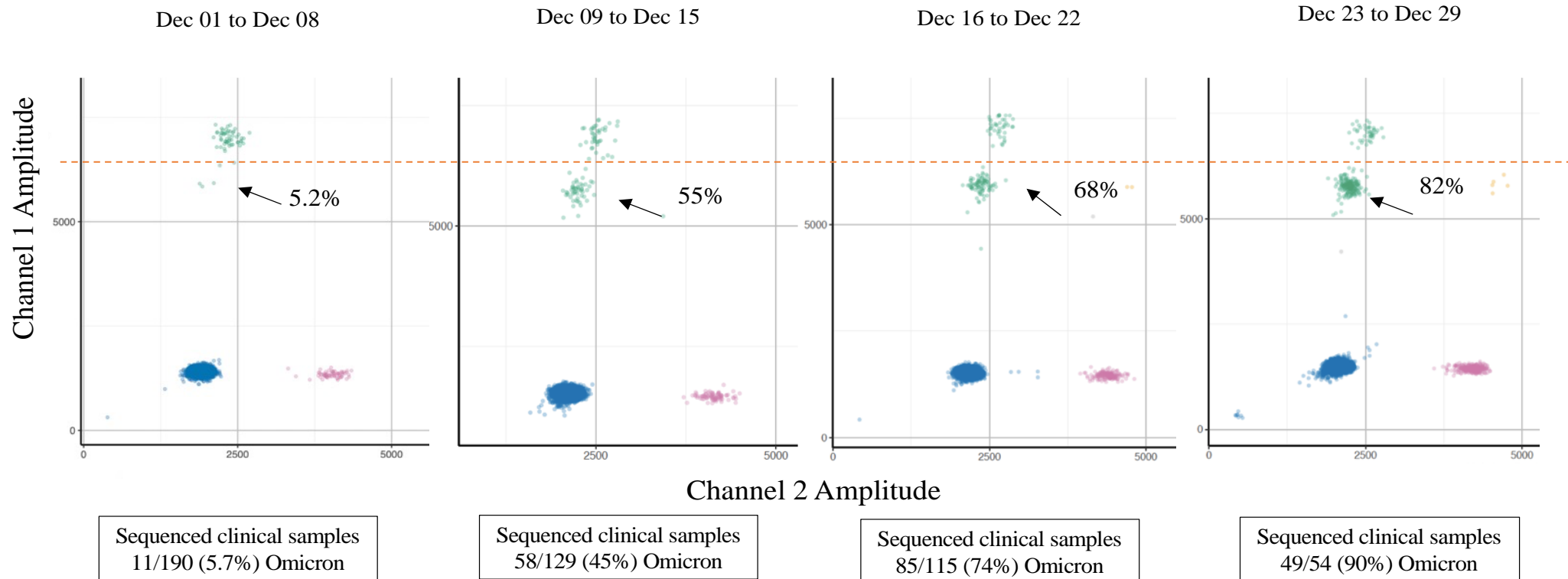
Methods

- N-gene mutation corresponding to CDC N1 probe caused reduced fluorescence in N1 signal of SARS-CoV-2.

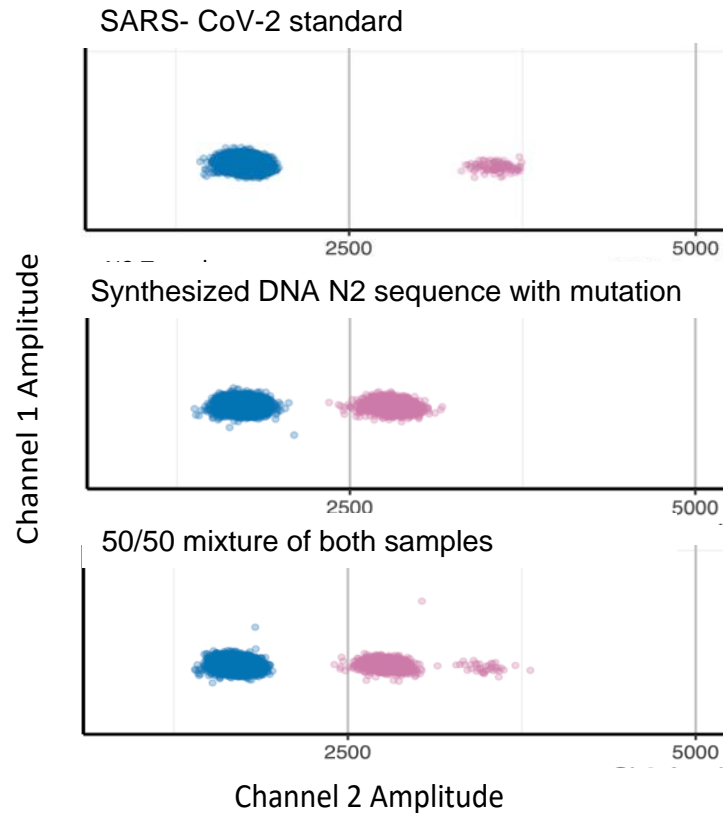


Results

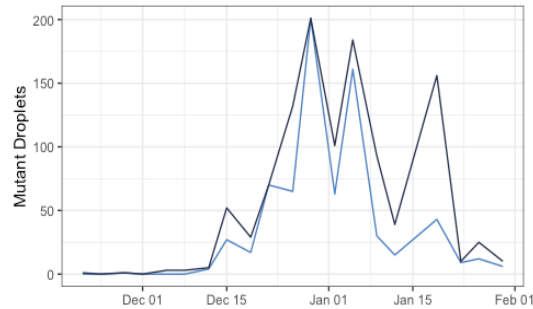
- Distinguished Omicron from circulating Delta variant.



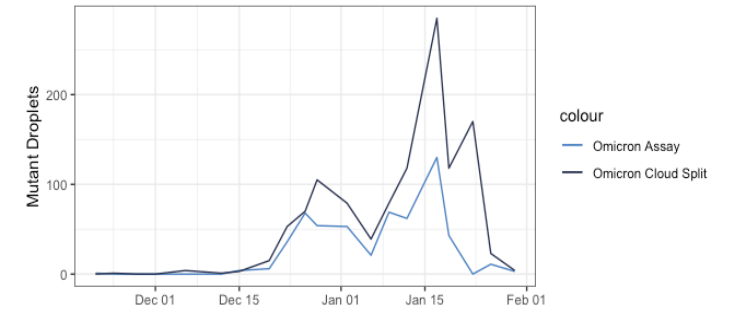
Verification of Cloud Split Method



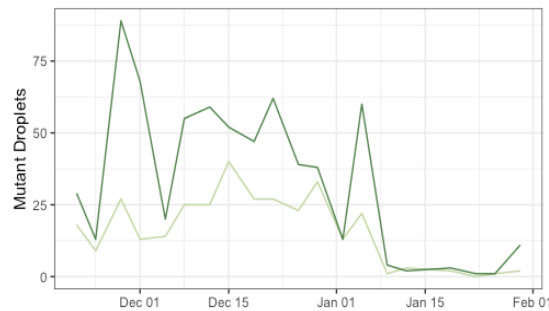
Assay Comparison of Omicron Detection for Milwaukee JI WWTP



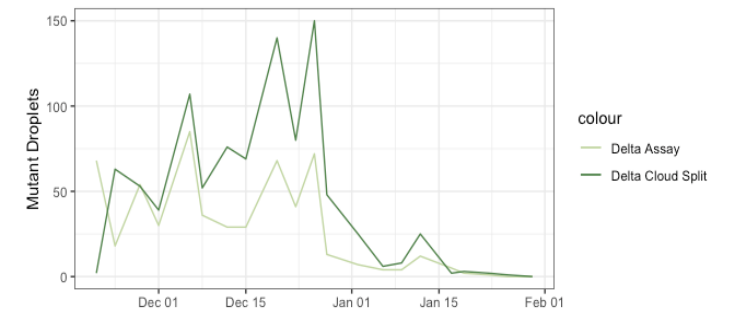
Assay Comparison of Omicron Detection for Green Bay DP WWTP



Assay Comparison of Delta Detection for Milwaukee JI WWTP

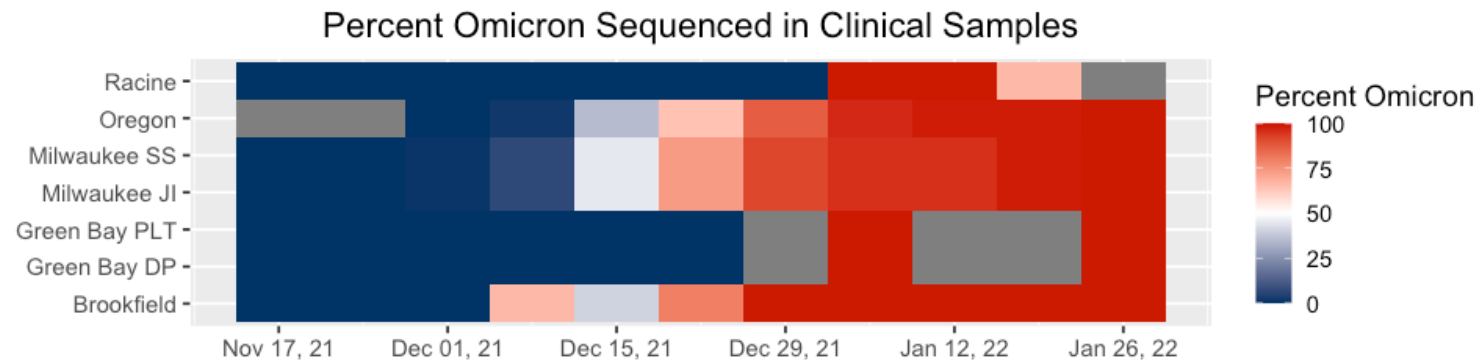
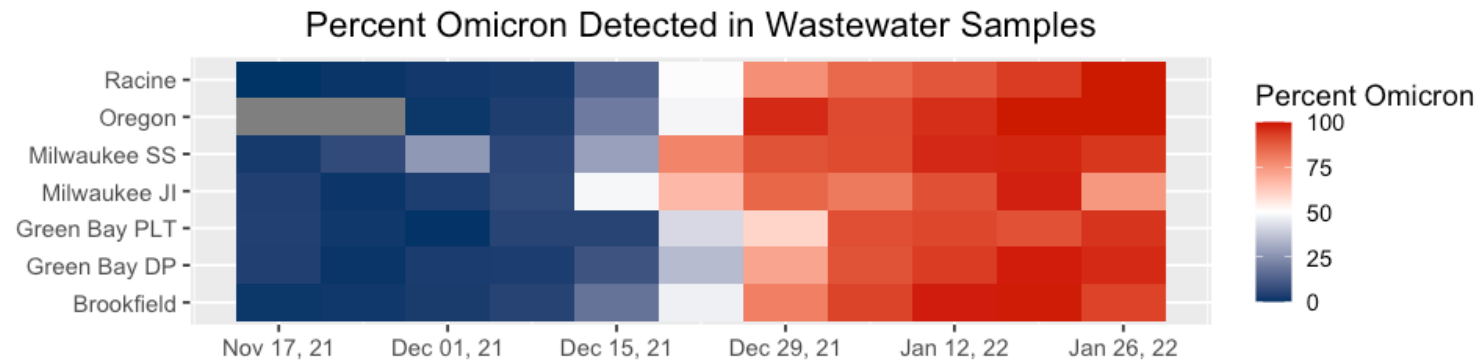


Assay Comparison of Delta Detection for Green Bay DP WWTP



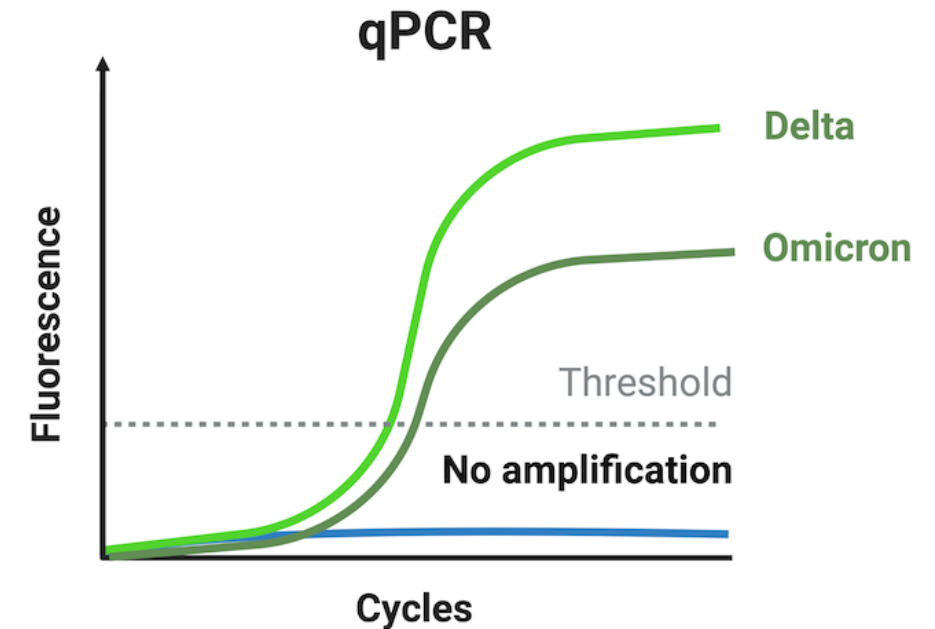
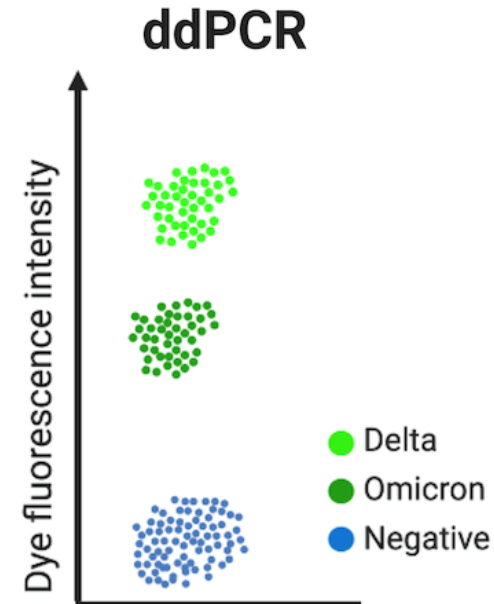
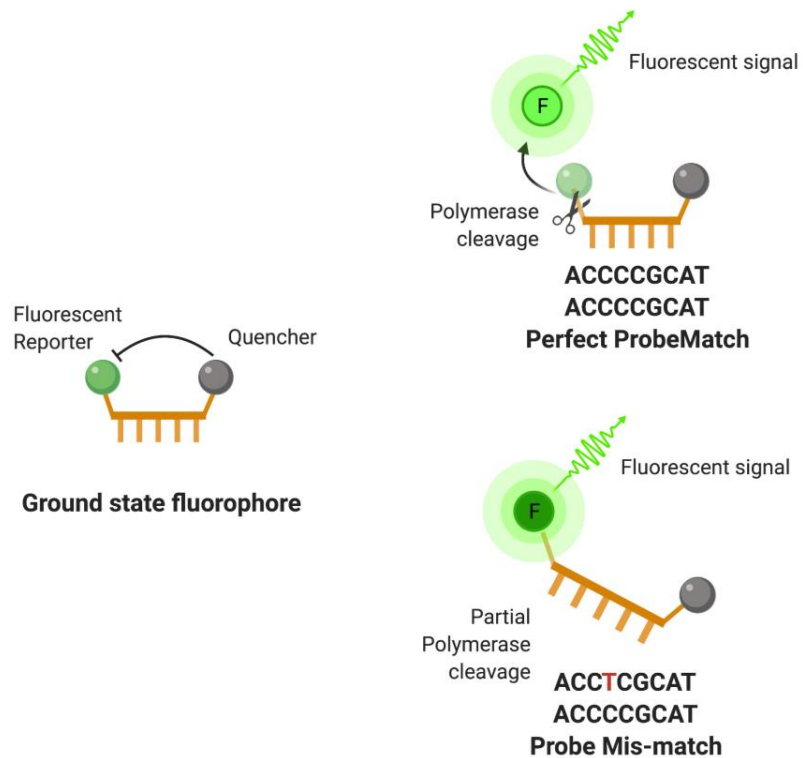
Results

- Omicron was always detected prior to clinical samples.
- The first positive wastewater sample was November 21, 2021.



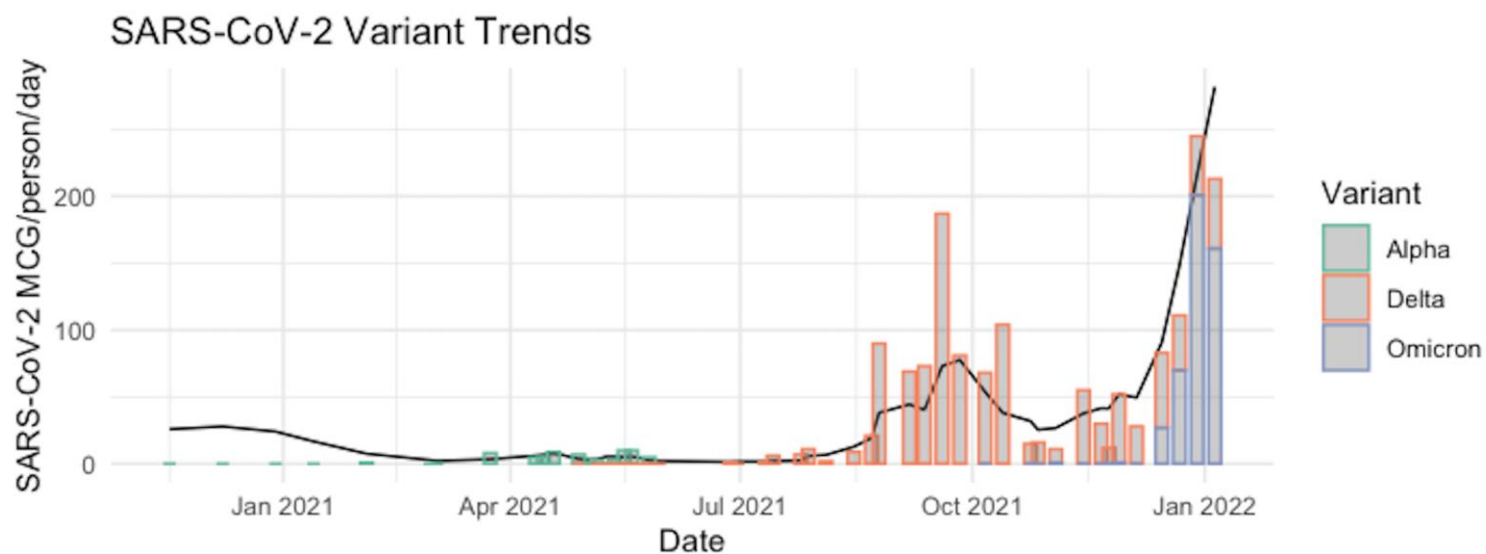
Conclusions

- ddPCR is a preferred platform for wastewater surveillance so that mutations can be detected.



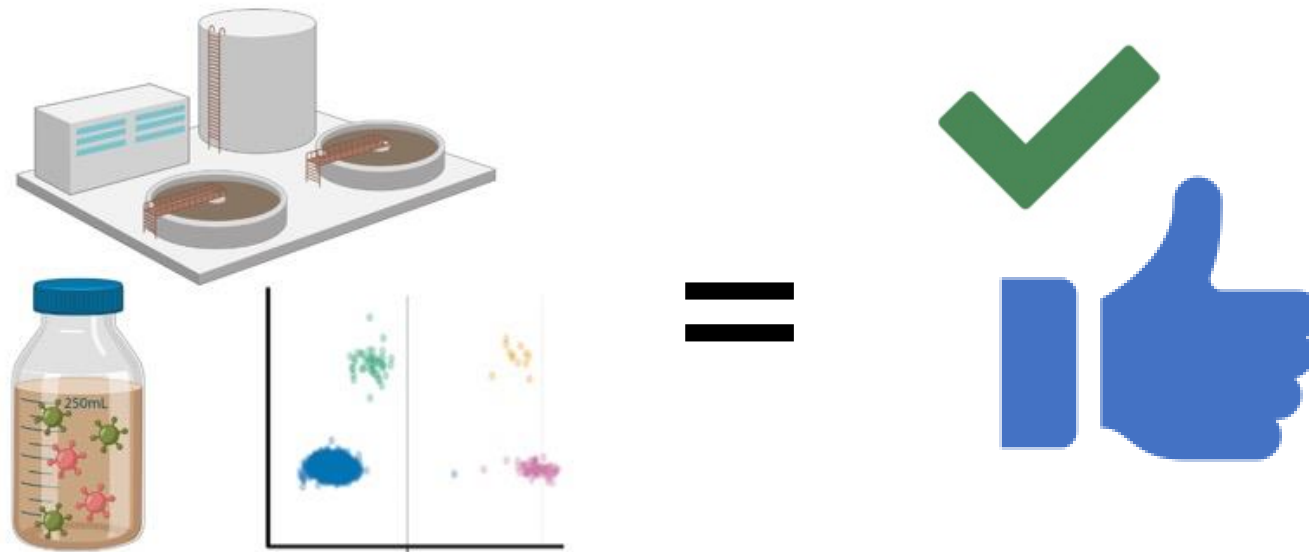
Conclusions

- ddPCR is a preferred platform for wastewater surveillance so that mutations can be detected.
- Appearance and prevalence was easily detected in wastewater even in counties that lacked clinical data.



Overarching Conclusion

- Wastewater surveillance is an accurate population-level measure that can provide more information than traditional public health metrics.



Acknowledgements

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Graphics on slides 4-7, 9, 10, and 11 were created on BioRender.com

Questions?

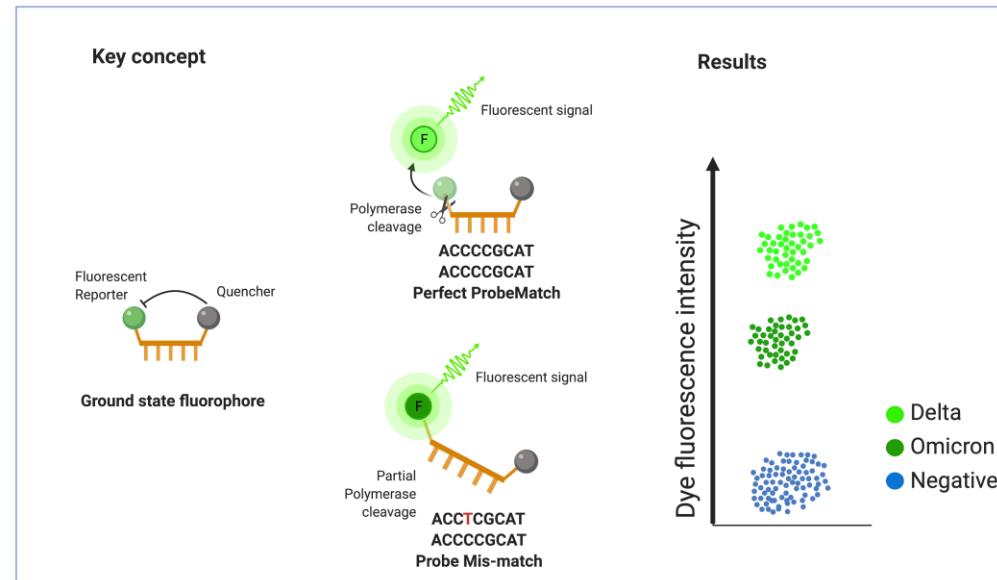
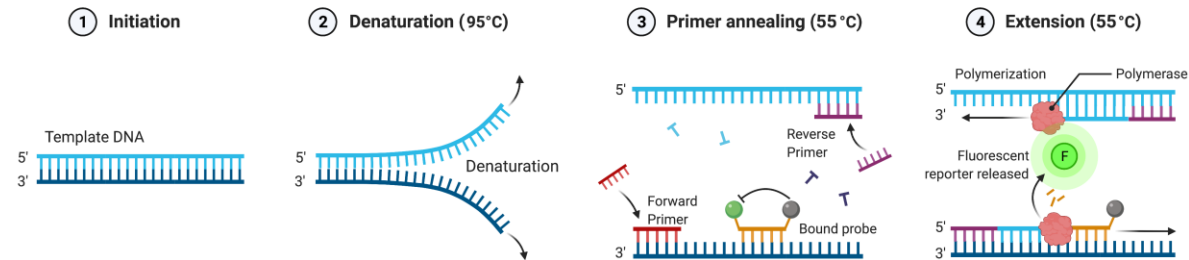
Thank You!

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M. K. Schussman, A. Roguet, A. Schmoldt, B. Dinan, and S. L. McLellan, “Wastewater surveillance using ddPCR reveals highly accurate tracking of Omicron variant due to altered N1 probe binding efficiency,” Public and Global Health, preprint, Feb. 2022. doi: 10.1101/2022.02.18.22271188.



ddPCR – N1 Probe Mutation



Limits of Detection and Quantification

- LOB: maximum value from a 90% confidence interval of the average false positive droplets **(0 droplets)**
- LOD: LOB + 3 positive droplets **(3 droplets)**
- LOQ: the concentration at which the relative standard deviation was $\leq 30\%$ between triplicate assay measures and the difference between the calculated and expected concentrations was $\leq 30\%$ **(10 droplets)**

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