LA-UR-22-27408

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Title:	Monitoring SARS-CoV-2 in wastewater.
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Intended for:	Report
Issued:	2022-07-25

Los Alamos NATIONAL LABORATORY

Monitoring SARS-CoV-2 in wastewater.

Dana Urbatsch B-10

Abstract

The monitoring of SARS-CoV-2 through individual testing is subject to inaccuracies. Wastewater biosurveillance has been used around the world to detect the presence of drugs, viruses, and other chemicals present in a population. Fecal shedding of SARS-CoV-2 implies that wastewater can be analyzed in order to detect varying levels of the virus within a given community. Pepper Mild Mottle Virus is present consistently in human fecal matter, and can be used to normalize the amount of SARS-CoV-2 detected. In this experiment, the amount of SARS-CoV-2 in the wastewater sample was compared to the amount of PMMoV present. Wastewater samples were separated into three fractions: direct wastewater, a solid pellet, and filter paper extract. An RT-qPCR test was used on the extracted RNA from each fraction to detect the relative amount of SARS-CoV-2 and PMMoV copies in each sample. The amount of SARS-CoV-2 was successfully normalized to the amount of PMMoV.

Improvements in SARS-CoV-2 Detection

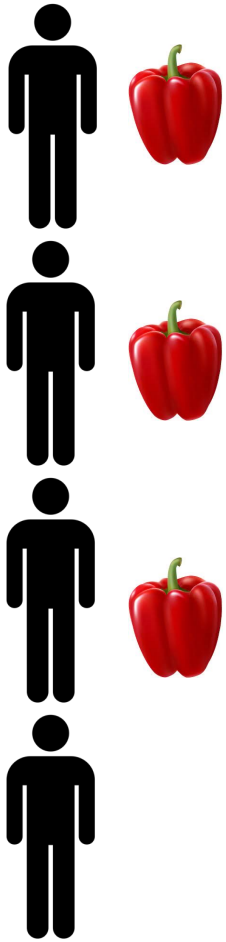
- Wastewater monitoring has a variety of applications
- People shed the virus through fecal matter
- Wastewater monitoring can show fluctuations in the population over time
- Can supplement SARS-CoV-2 tracking

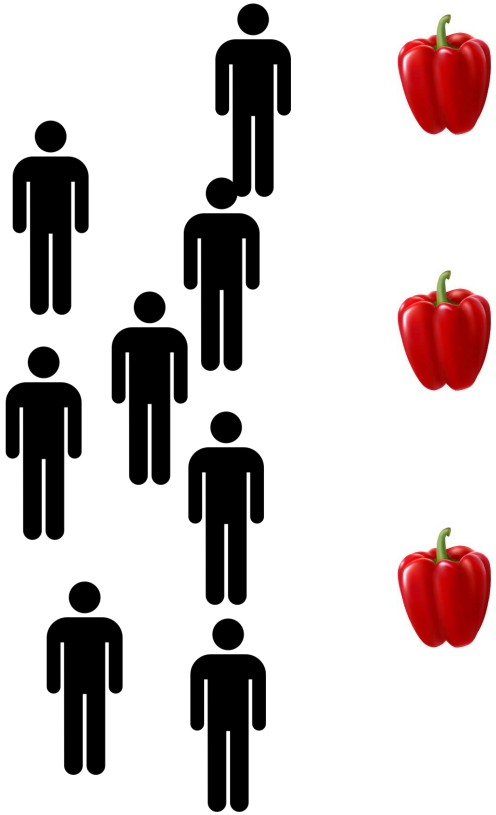
https://www.science.org/doi/10.1126/science.abd0700

Benefits of Wastewater Testing

- Noninvasive
- Anonymous
- Accounts for asymptomatic people
- Is not limited by shortages of tests
- Shows trends several days in advance of individual testing

Normalizing SARS-CoV-2





https://gallery.yopriceville.com/Free-Clipart-Pictures/Vegetables-PNG/Red_Bell_Pepper_Transparent_Clip_Art_Image#.YtbQ28ZMFQI
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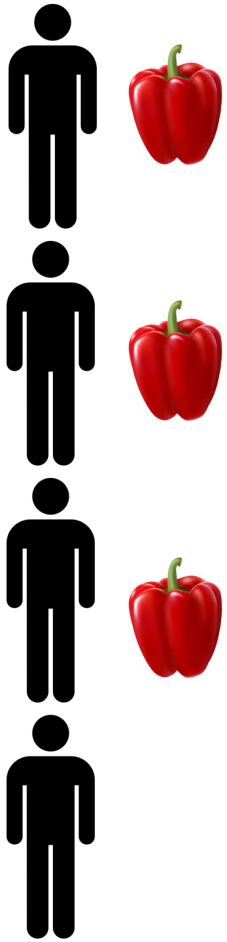
Normalizing SARS-CoV-2



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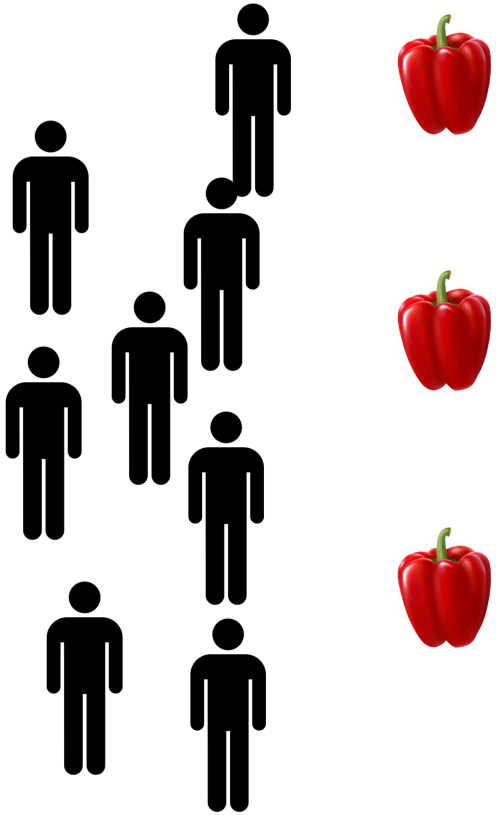

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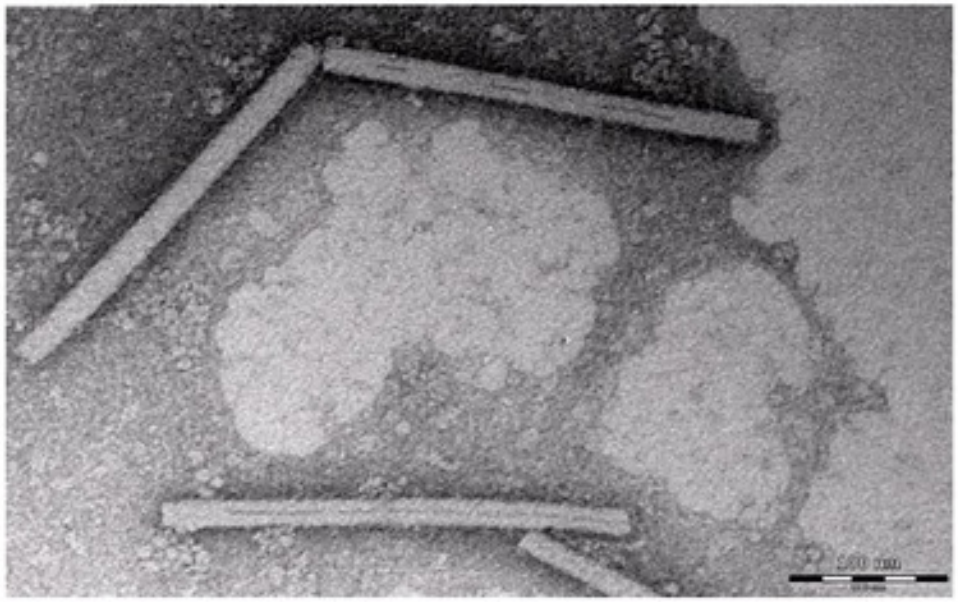
https://gallery.yopriceville.com/Free-Clipart-Pictures/Vegetables-PNG/Red_Bell_Pepper_Transparent_Clip_Art_Image#.YtbQ28ZMFQI
https://commons.wikimedia.org/wiki/File:Person_icon_BLACK-01.svg

Pepper Mild Mottle Virus (PMMoV)

- Can be consumed through peppers or products from peppers, like hot sauce
- Present in human feces, but not animal feces
- Used to show the amount of human feces



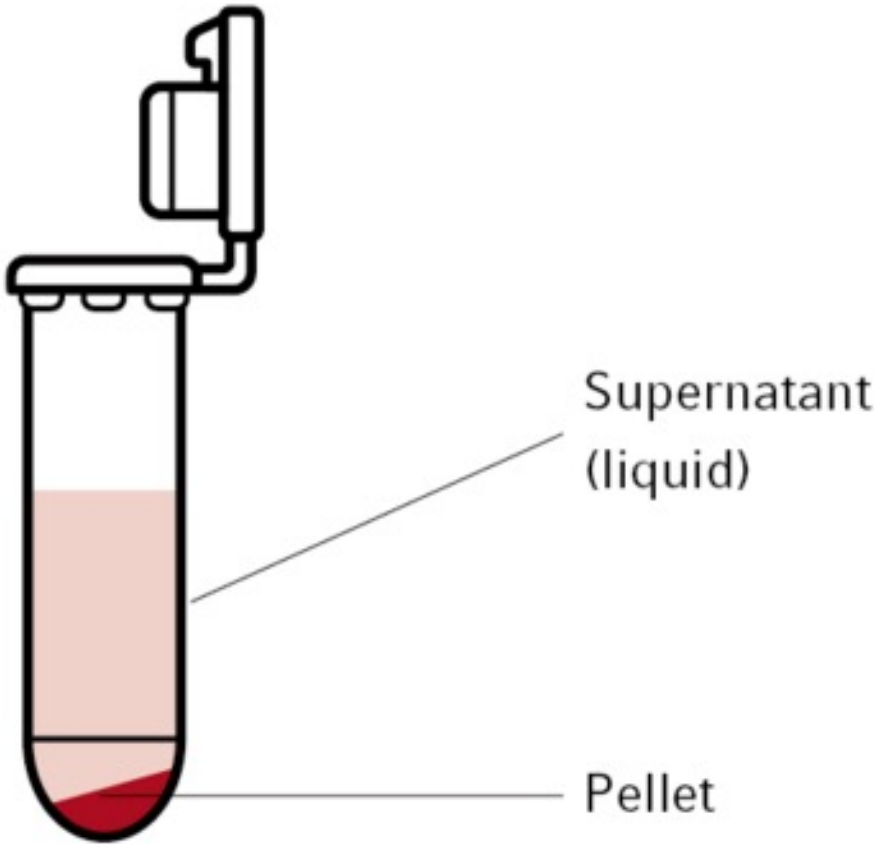
https://upload.wikimedia.org/wikipedia/commons/4/4c/PMMoV_particles.png

Methodology

- Collection
- RNA Extraction
- RT-qPCR

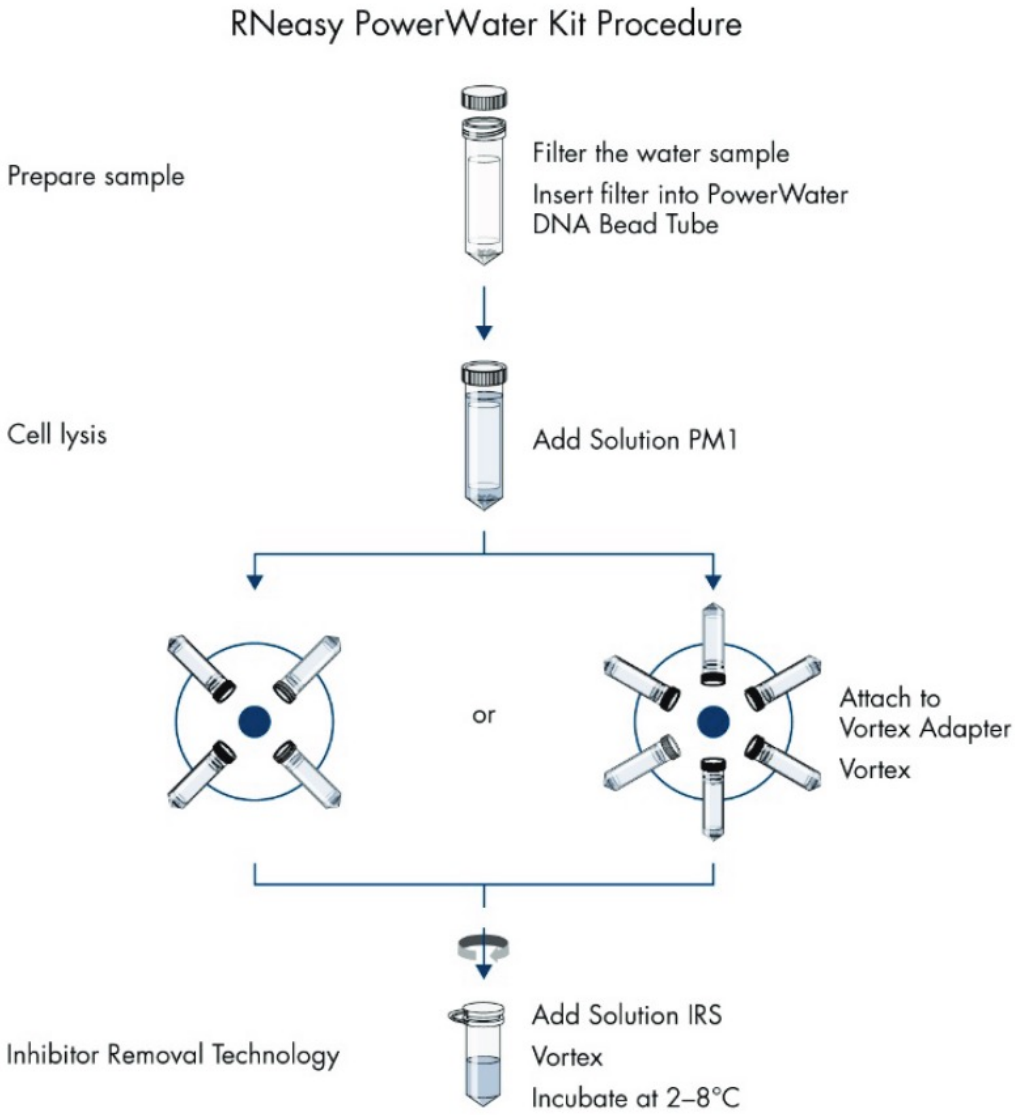
Collection

- LANL Wastewater
- Collection days
- Fractions 1, 2, and 3



https://handling-solutions.eppendorf.com/sample-handling/centrifugation/safe-use-of-centrifuges/basics-in-centrifugation/

RNA Extraction

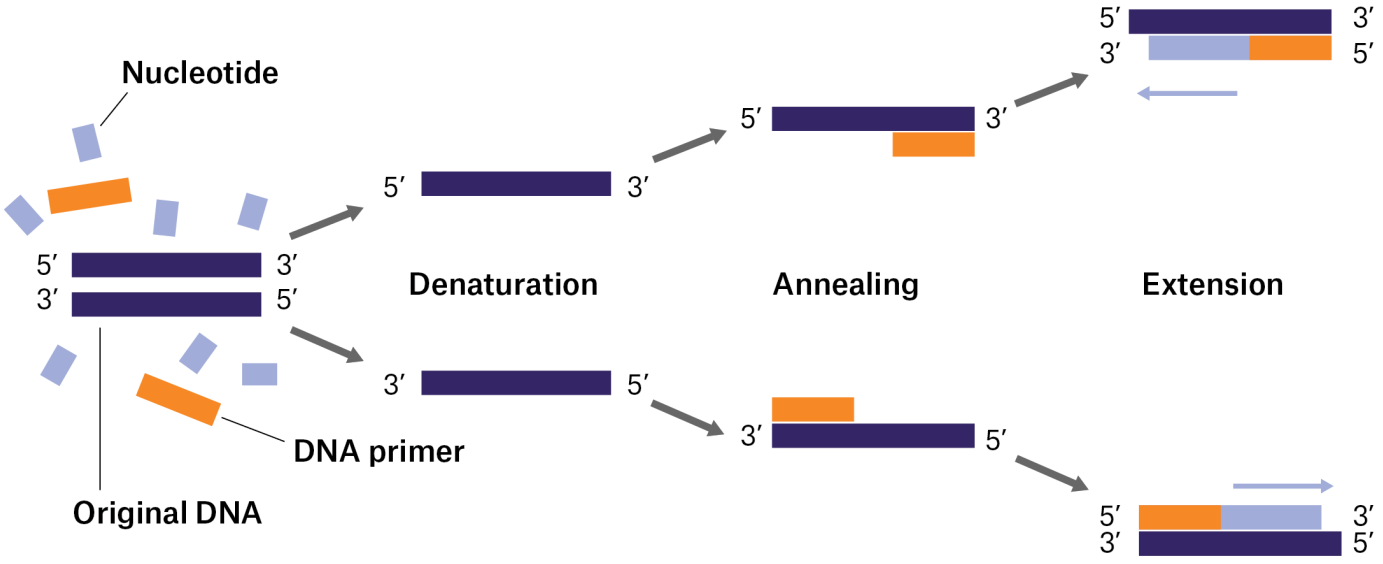


RNA Extraction

Bind DNA	Add Solution PM3 and PM4 Vortex Load into MB RNA Spin Column
DNase I treatment	Wash with Solution PM5 Add DNase I and Solution PM6 Incubate at room temperature
Wash	Wash with Solution PM7 Wash with Solution PM5 Wash with Solution PM4
Elute	Elute with RNase-Free Water

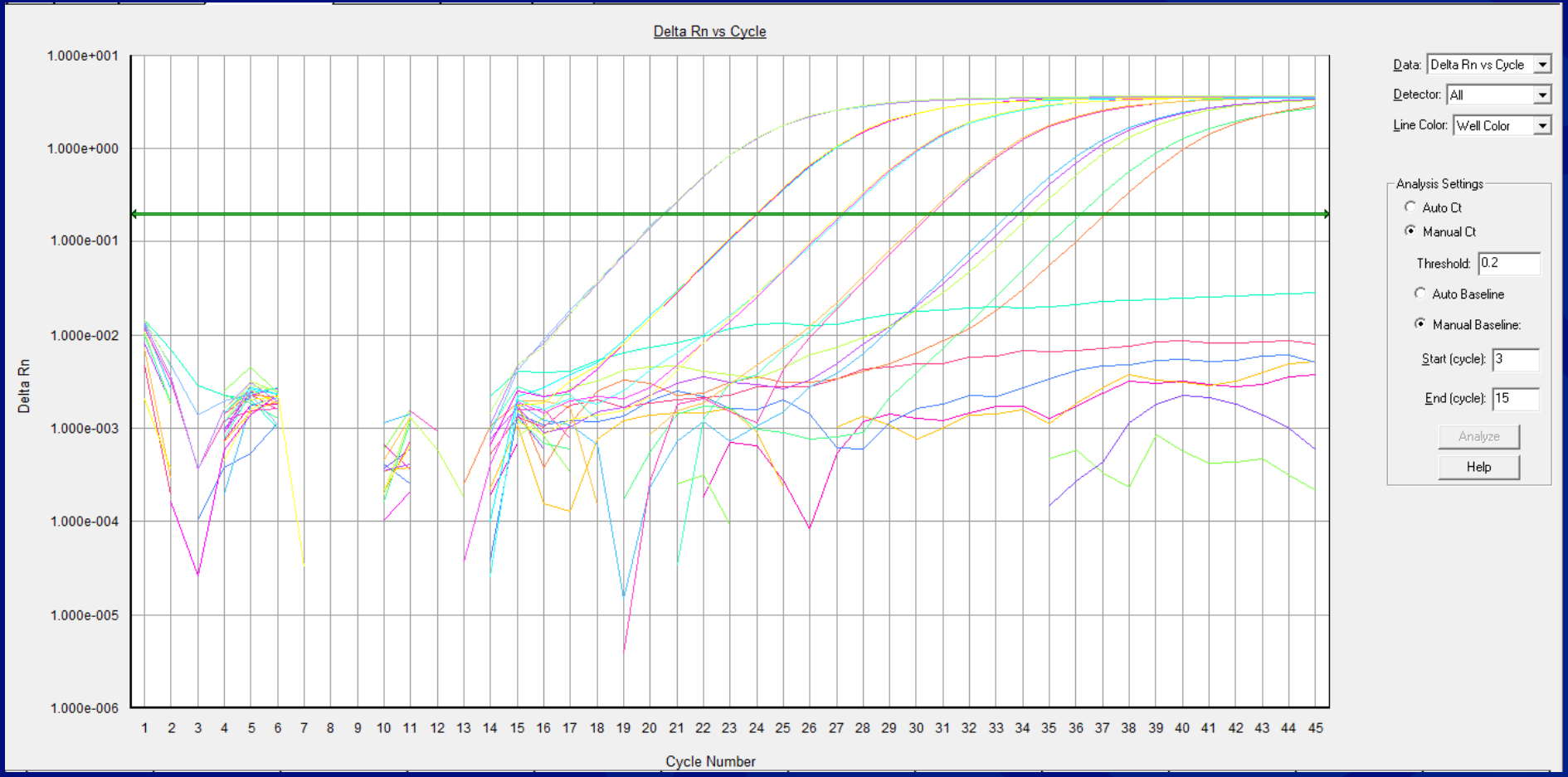
RT-qPCR

- Reverse Transcription Quantitative Polymerase Chain Reaction
- Three Steps: Denaturing, Annealing, Extension
- TaqMan assay vs SYBR Green

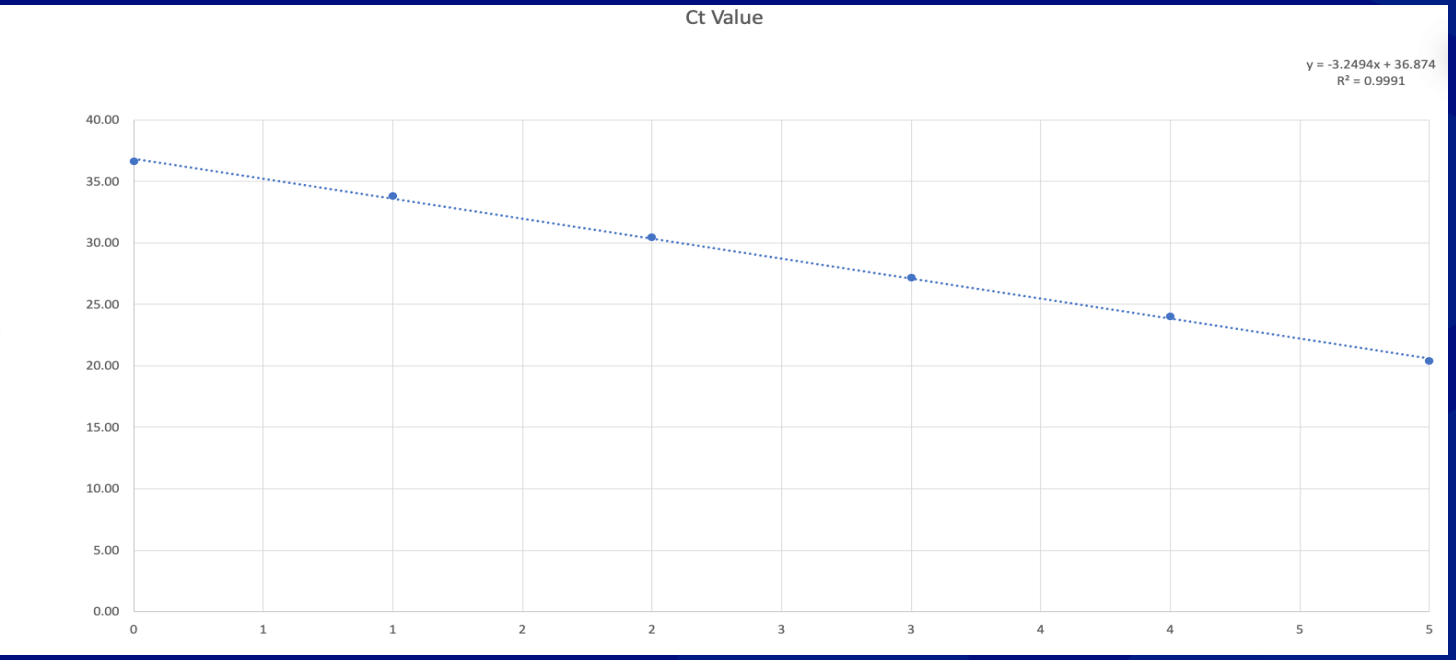


https://blog.labtag.com/a-brief-history-of-pcr-and-its-derivatives/

Amplification Curve – June 9th



Standard Curve – June 9th

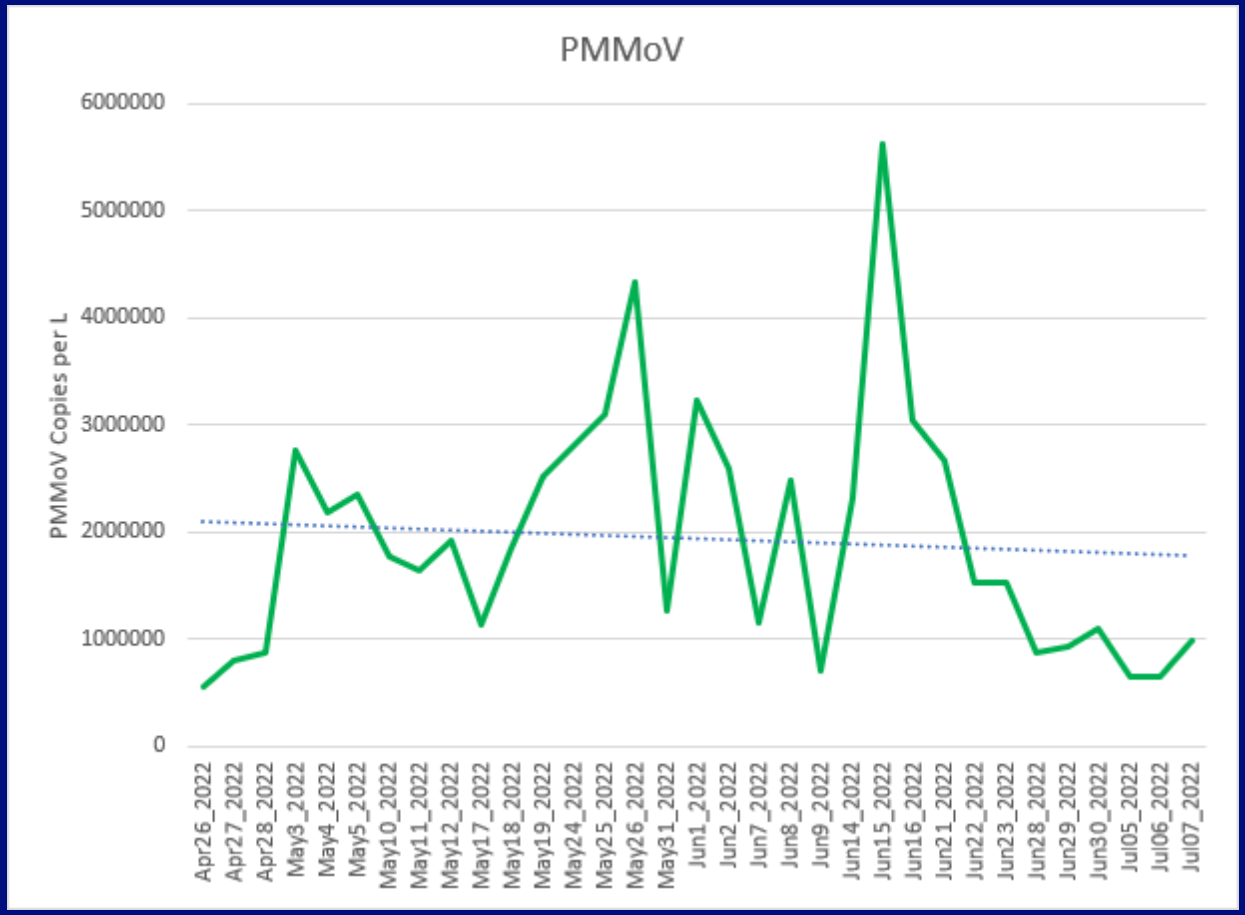


$y=-3.2494x+36.874$
$R^2=0.9991$

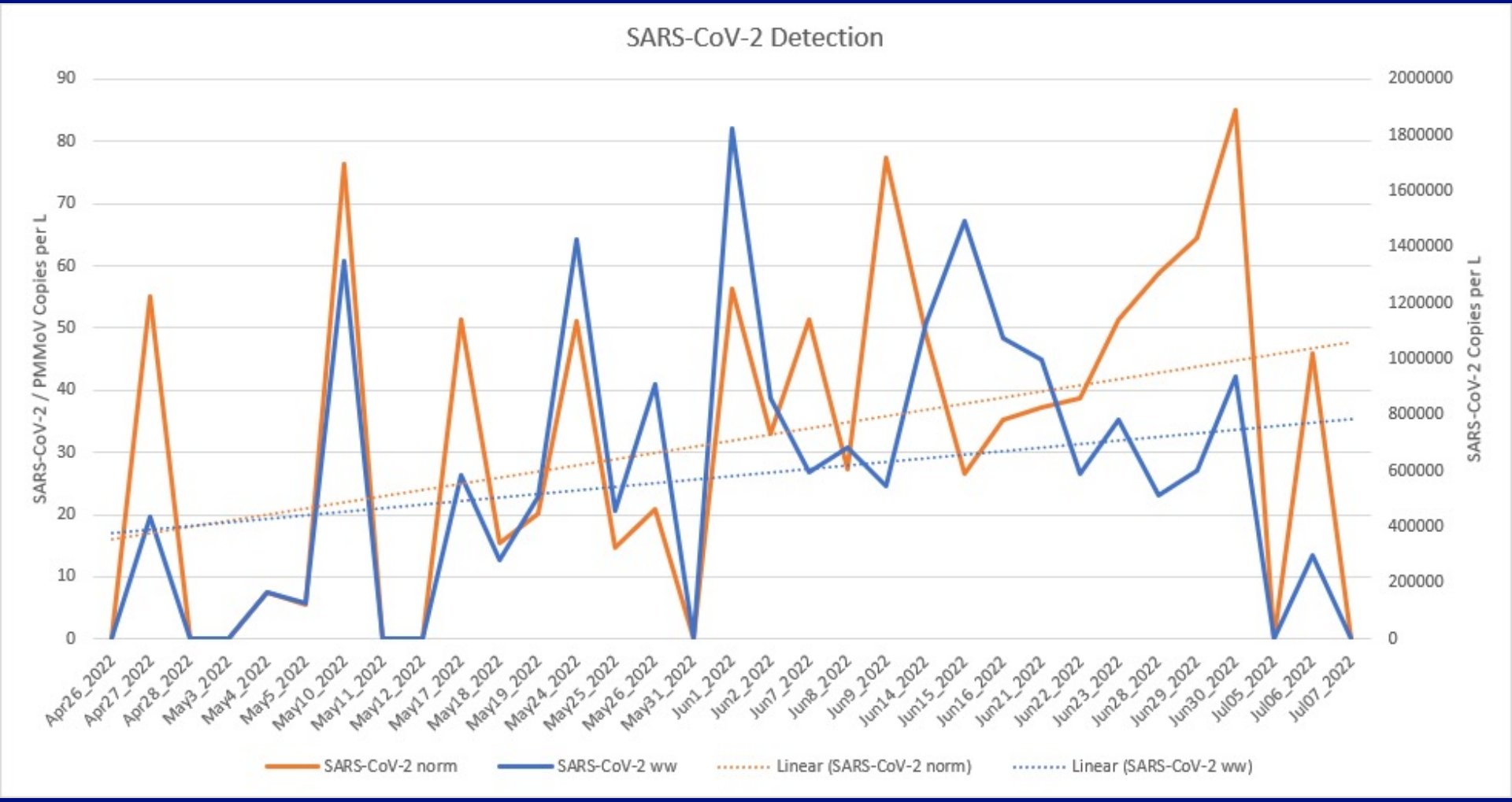
DNA Copies / uL	Log DNA Copies / uL	Ct Value
1	0	36.62
10	1	33.84
100	2	30.45
1000	3	27.17
10000	4	24.00
100000	5	20.43

$32 = -3.2494x+36.874$
$-4.874=-3.249x$
$x=10^{1.5}$
$x=31.6$ copies/uL

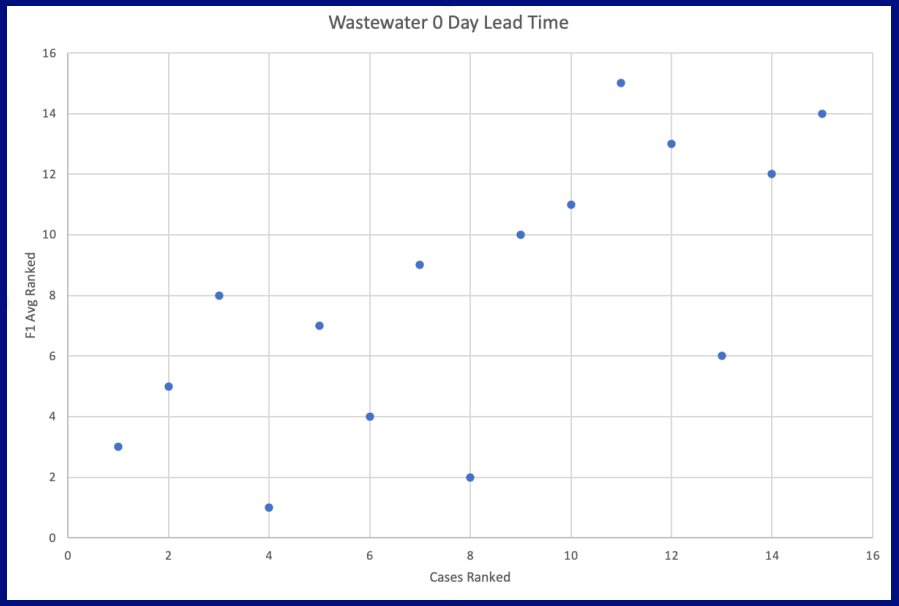
PMMoV Graph


PMMoV
PMMoV Copies per L
6000000
5000000
4000000
3000000
2000000
1000000
0
Apr26_2022
Apr27_2022
Apr28_2022
May3_2022
May4_2022
May5_2022
May10_2022
May11_2022
May12_2022
May17_2022
May18_2022
May19_2022
May24_2022
May25_2022
May26_2022
May31_2022
Jun1_2022
Jun2_2022
Jun7_2022
Jun8_2022
Jun9_2022
Jun14_2022
Jun15_2022
Jun16_2022
Jun21_2022
Jun22_2022
Jun23_2022
Jun28_2022
Jun29_2022
Jun30_2022
Jul05_2022
Jul06_2022
Jul07_2022

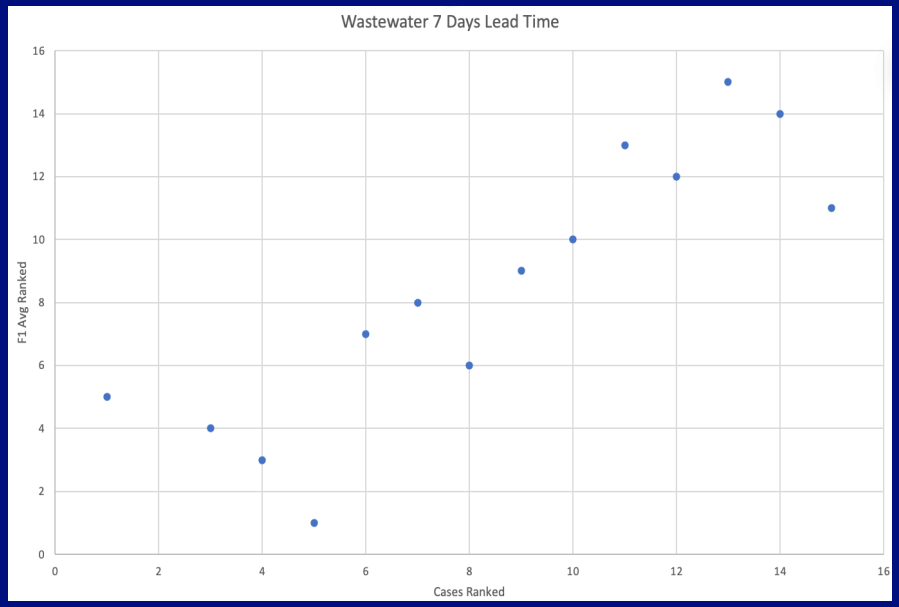
SARS-CoV-2 Detection Graph



Wastewater Detection and Individual Testing Correlation



Spearman Correlation Wastewater 0 Day Lead Time= 0.7



Spearman Correlation Wastewater 7 Day Lead Time= 0.86745

Summary and Future Applications

- Wastewater monitoring
  - Other applications
  - Important for SARS-CoV-2 monitoring
  - Could show earlier trends
  - Widespread implementation
- Normalization using PMMoV to ensure more accurate monitoring

Acknowledgements

- Andy Hatch and Shawn Starkenburg
- Department of Energy

Thank You!

Questions?