Optimization of Viral Concentration and RNA Extraction Methods for Detecting Viruses in Wastewater Using Real-Time PCR

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Real-time PCR (Quantitative PCR)

PCR inhibitor

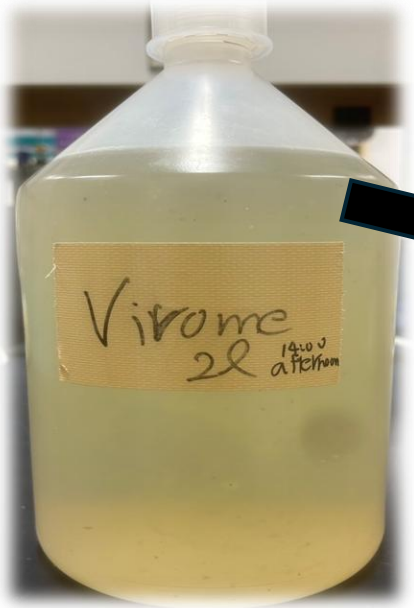
Highly sensitive ≒ Remove PCR inhibitors

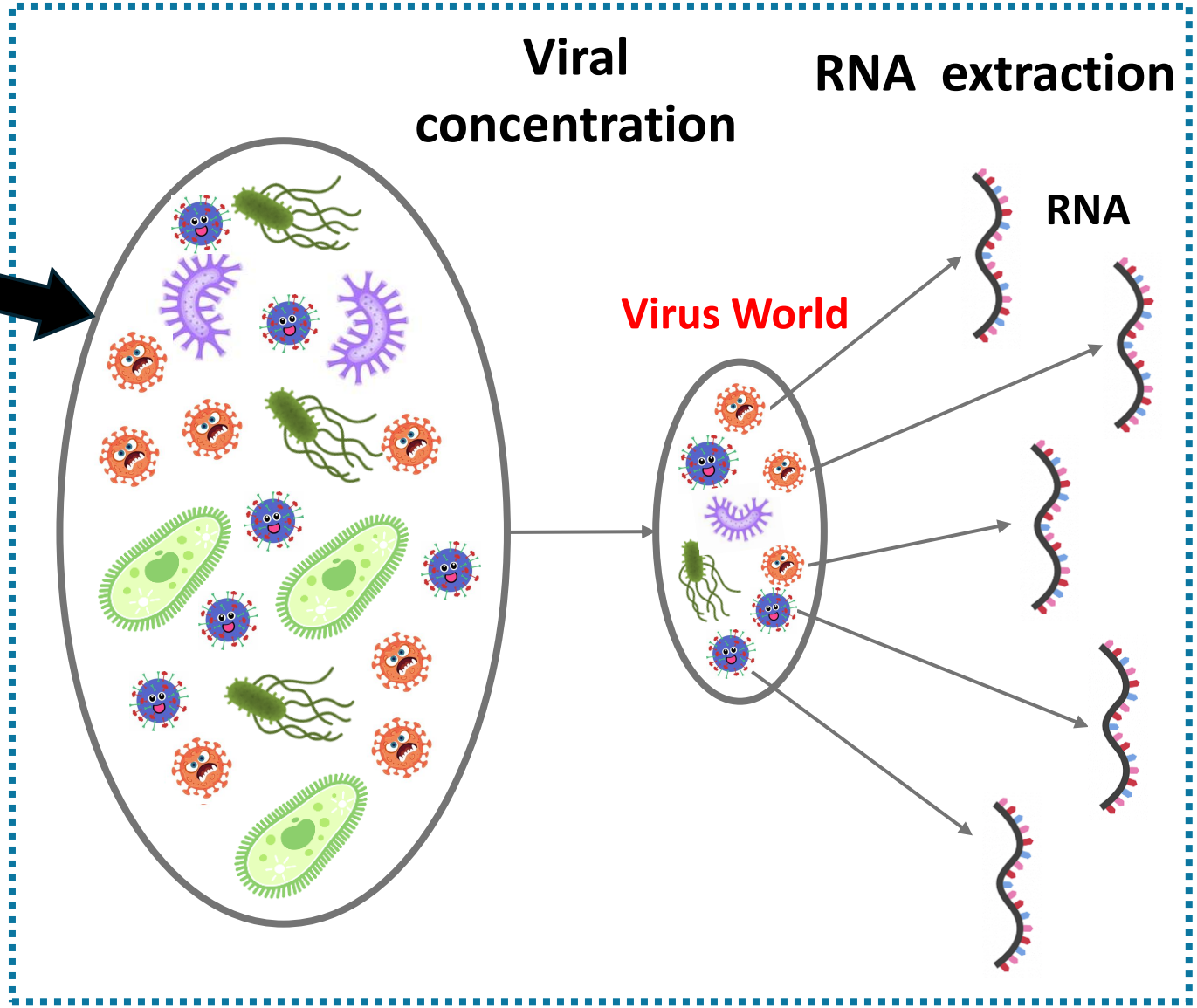
Viral concentration

RNA extraction

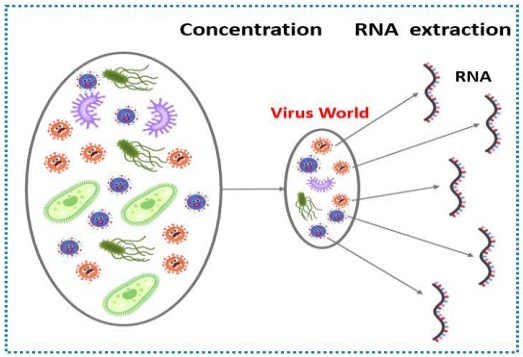
Real-time PCR

Viral concentration & RNA extraction



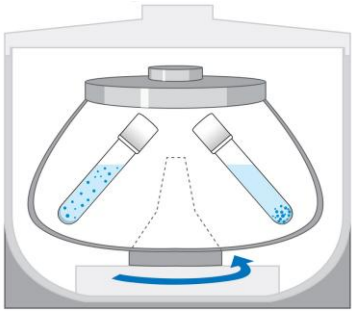


Methodology

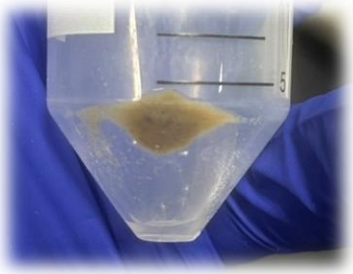


E

Wastewater



Centrifugation

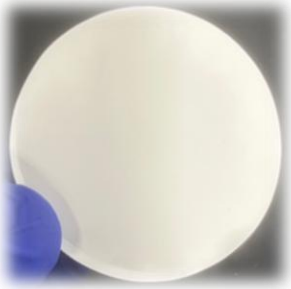


Solid phase

V

Wastewater

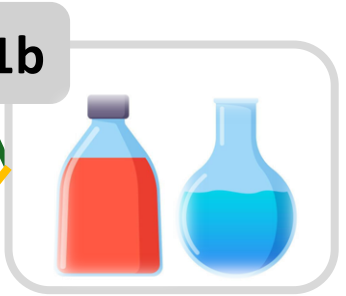
Filtration set



Electronegative Filter

1a

1b

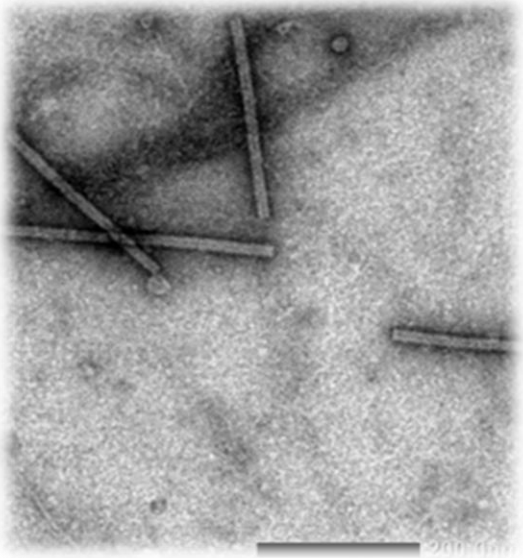


2

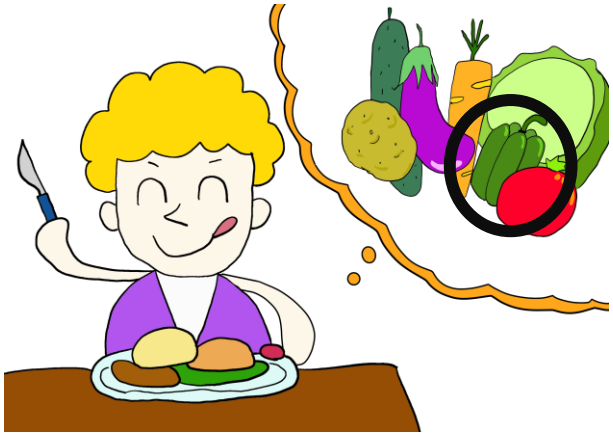
Filtration set: https://www.unic.or.jp/news_press/features_backgrounders/1154/?utm_source=chatgpt.com

Target virus

Pepper Mild Mottle Virus (PMMoV)



https://www.thespruceeats.com/types-of-sweet-peppers-4067589

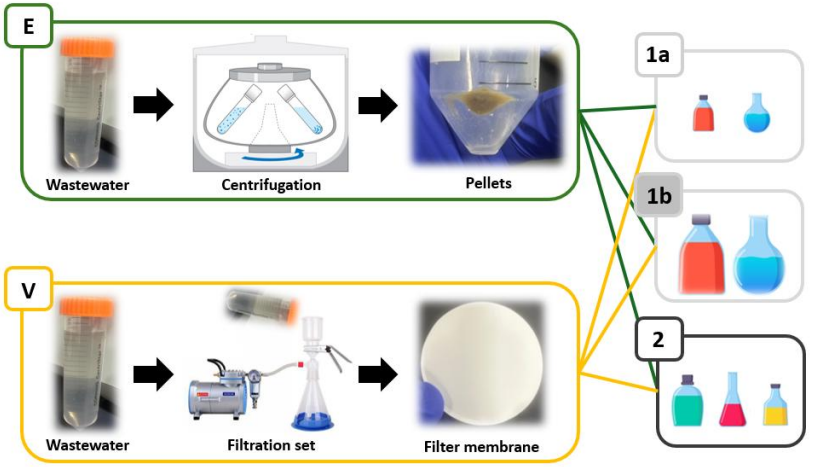


https://www.watercarelabs.co.nz/specialties/water-testing/wastewater/

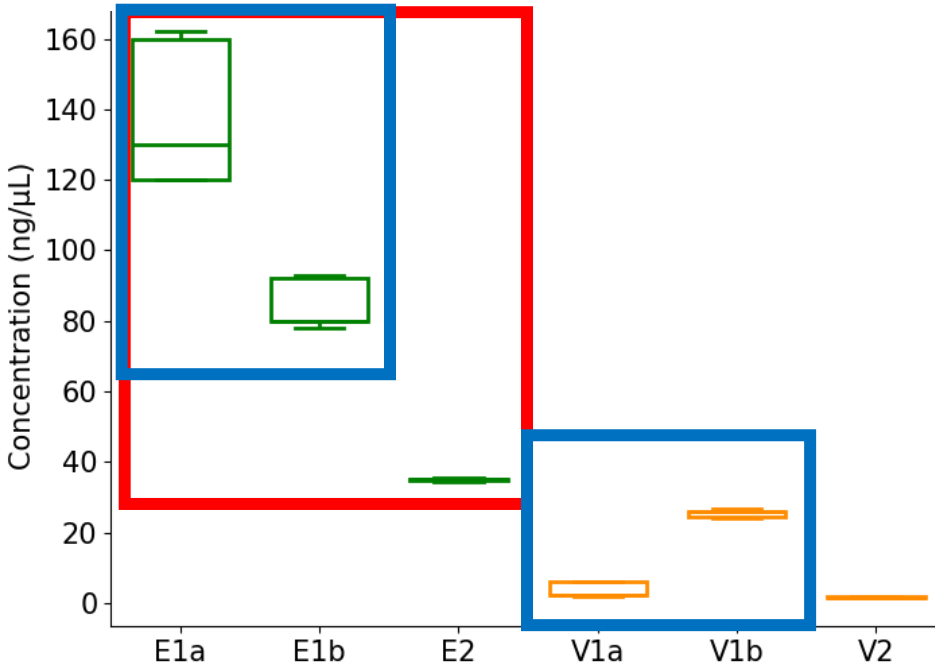
Result

&

Discussion

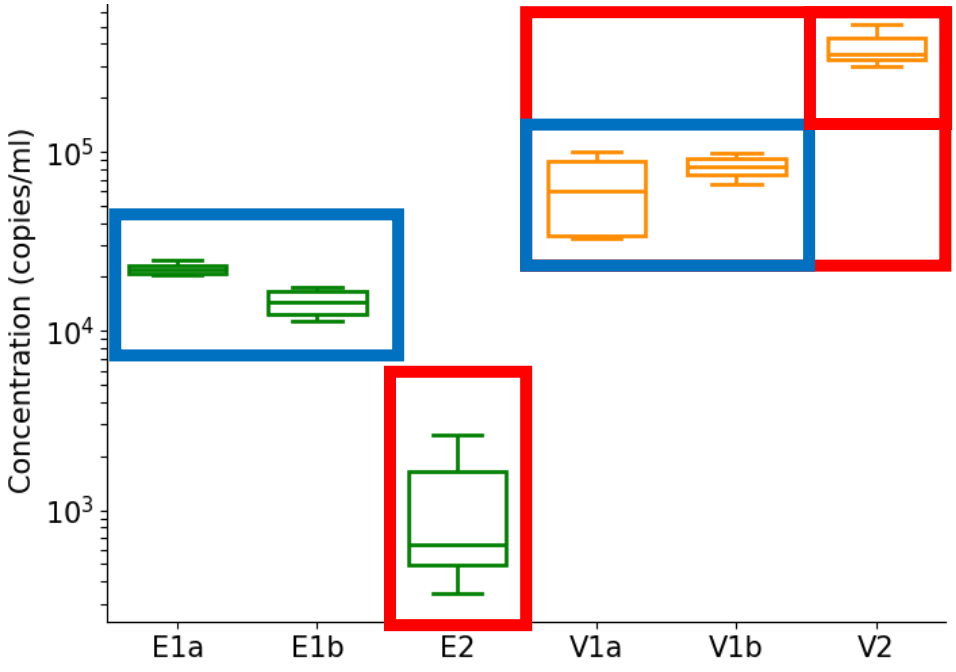


RNA concentration (Qubit)



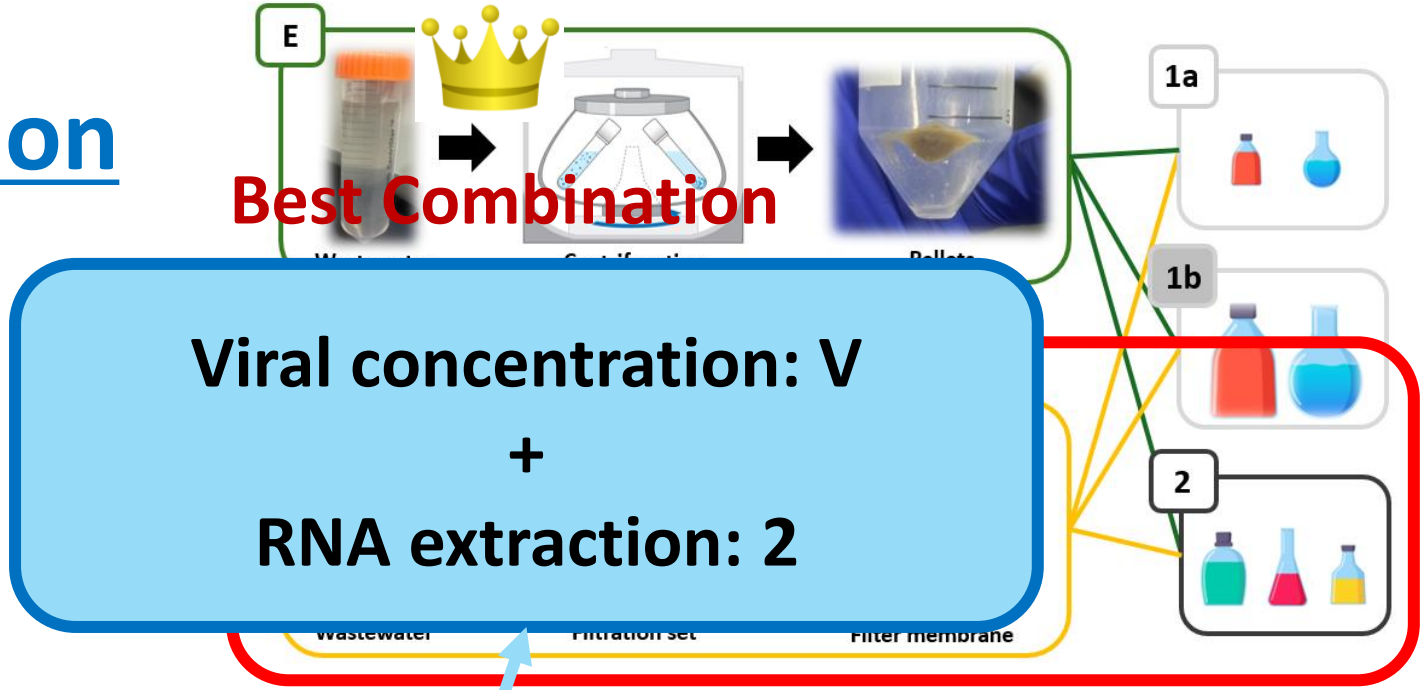
9 replicates

PMMoV concentration (real-time PCR)



4 replicates

Conclusion



Methods	Sensitivity
E1a	△
E1b	△
E2	×
V1a	○
V1b	○
V2	◎

Thank you very much!!!