TITLE PAGE:

Development of a Reproducible Method for Monitoring SARS-CoV-2 in Wastewater

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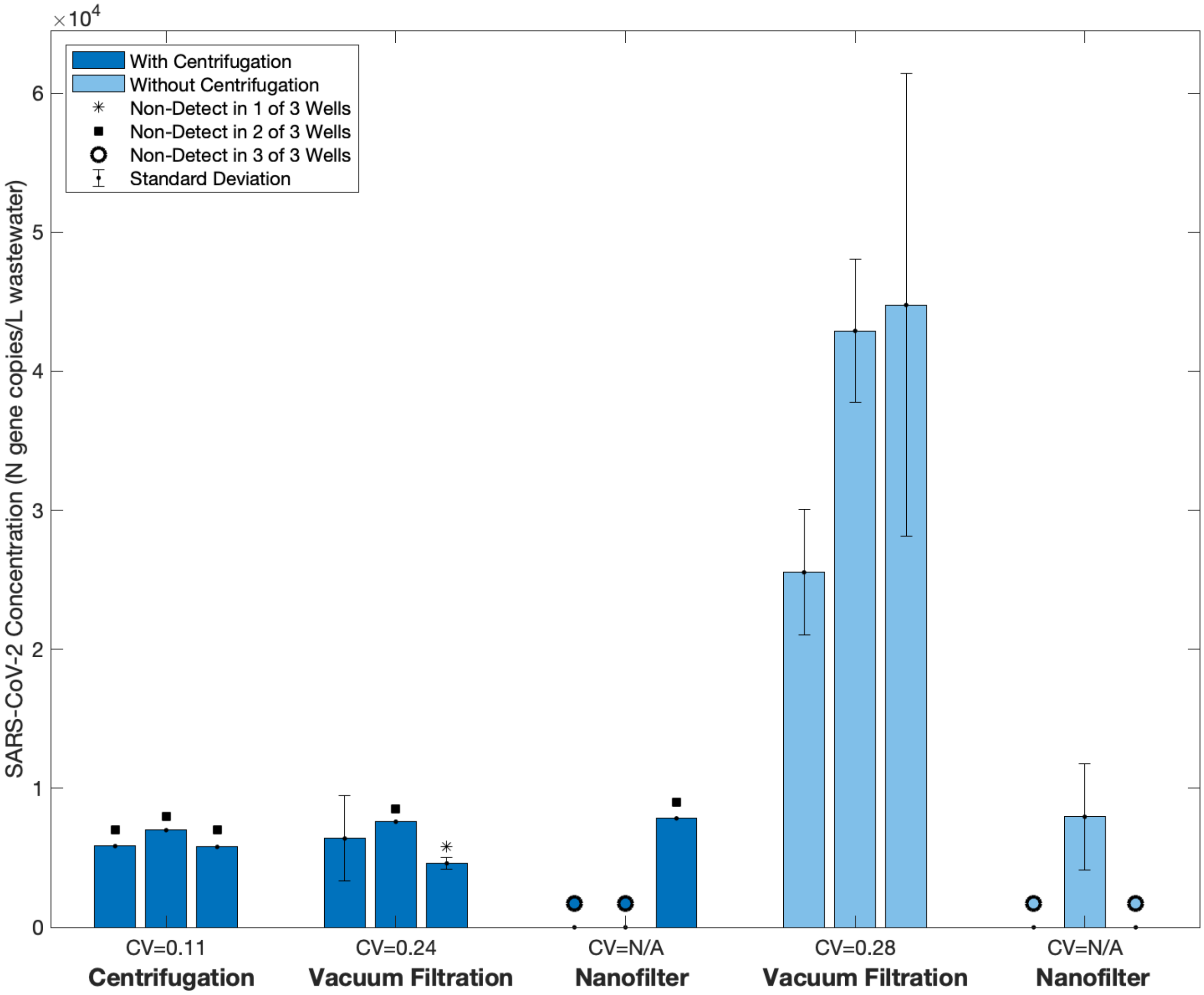
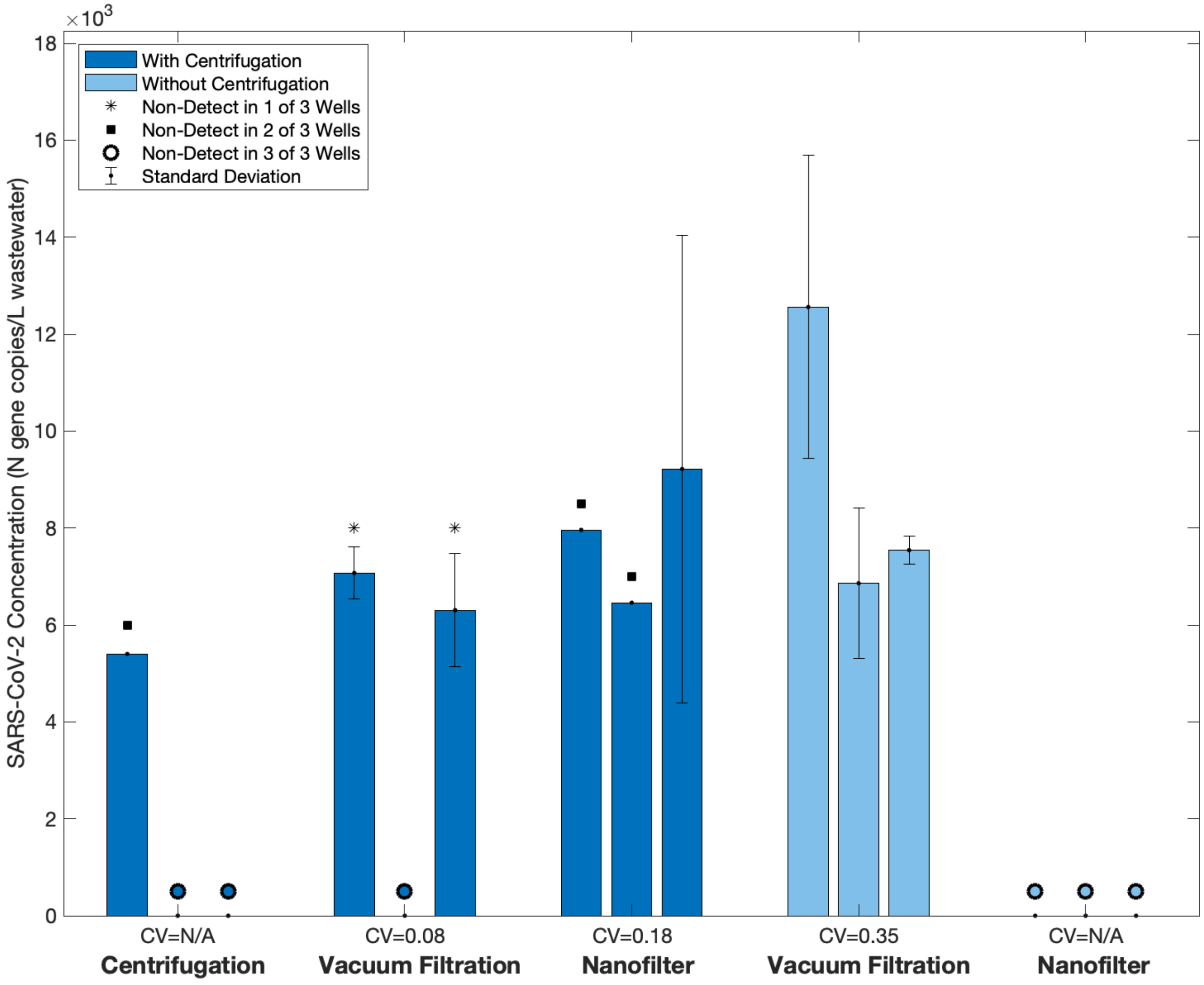
Supplementary Information

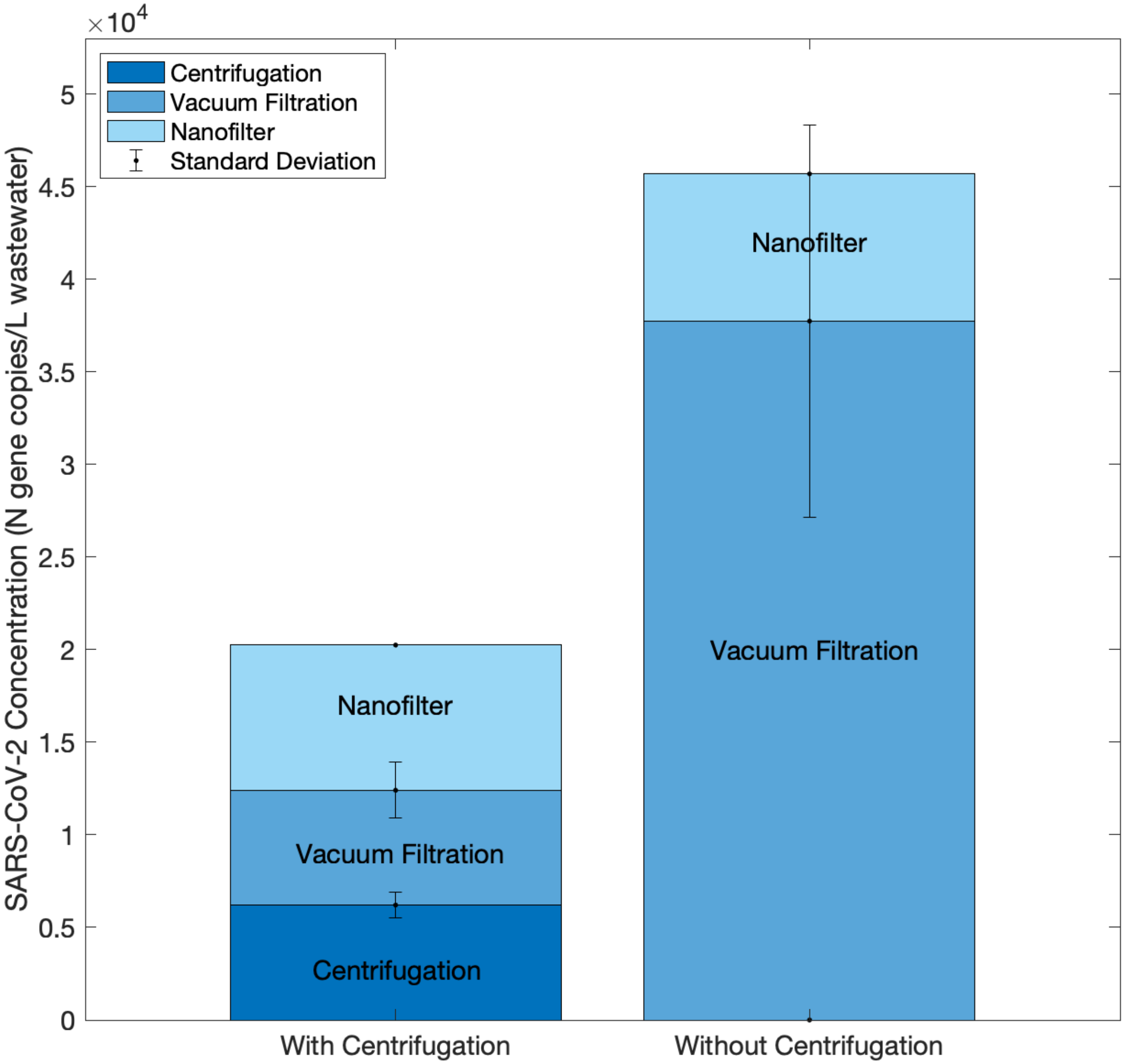


SI Figure 1: Concentrating SARS-CoV-2 from wastewater samples via centrifugation or vacuum filtration without centrifugation. WWTP: Walnut Creek (WAL); Sampling location: Primary Clarifier Effluent (PCE); Sample details: one composite sample was retrieved; Pasteurization: yes; Concentration Method: centrifugation (where the supernatant was then processed by vacuum filtration and the filtrate was processed by nanofiltration) vs. vacuum filtration without centrifugation (where the filtrate was then processed by nanofiltration). RNA Extraction Method: RNeasy with garnet lysis beads. No samples were processed using the final selected workflow. Each column represents one aliquot of wastewater processed from the sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation of the triplicate RT-qPCR reactions; the coefficient of variation (CV) normalizes the standard deviation to the average SARS-CoV-2 concentration. A CV value noted as "N/A" indicates that two of the triplicate aliquots processed yielded triplicate non-detect RT-qPCR results. The

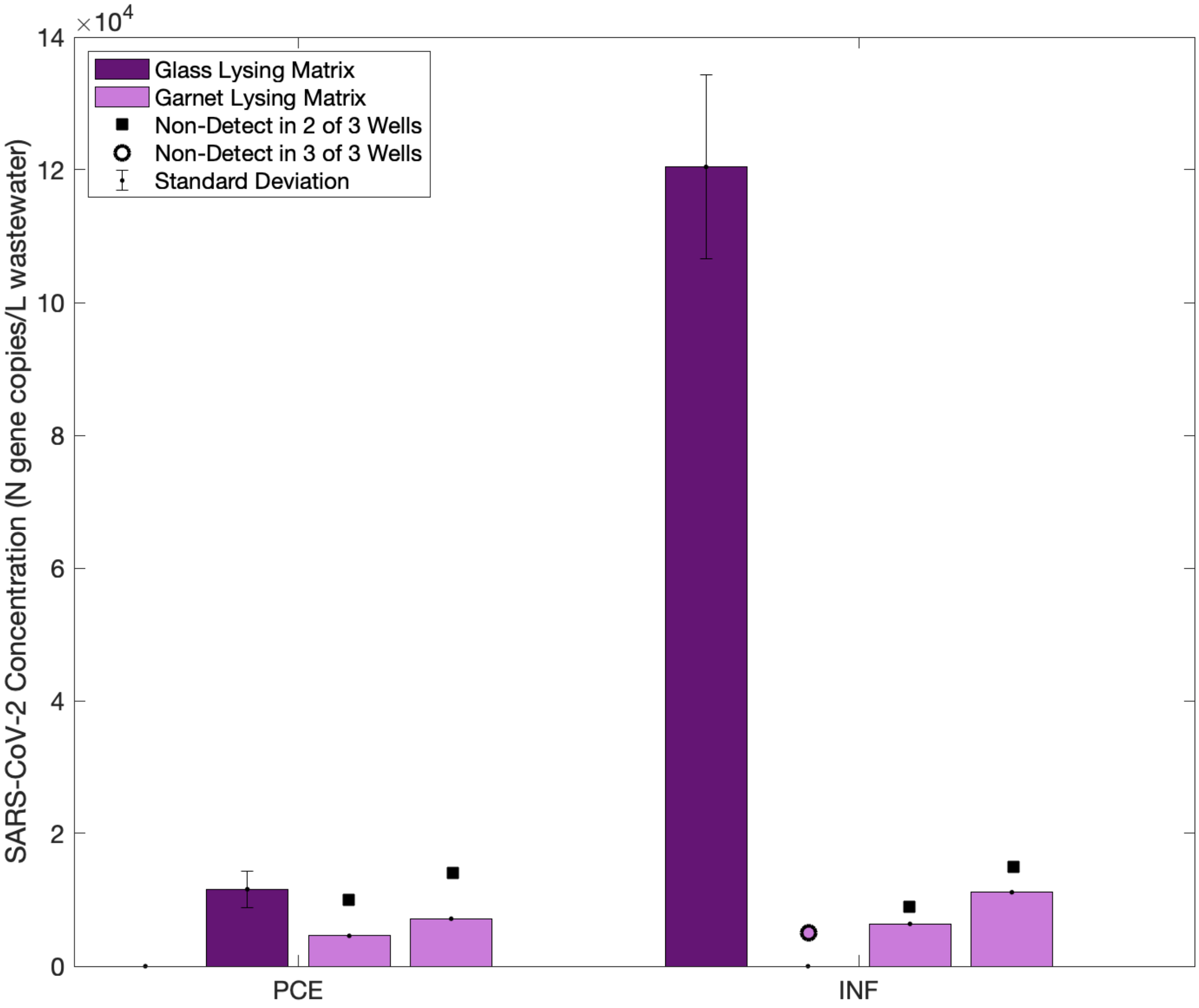
SARS-CoV-2 RNA concentrations for the nanofilter filtrate are not shown because all replicates yielded triplicate non-detect RT-qPCR results.



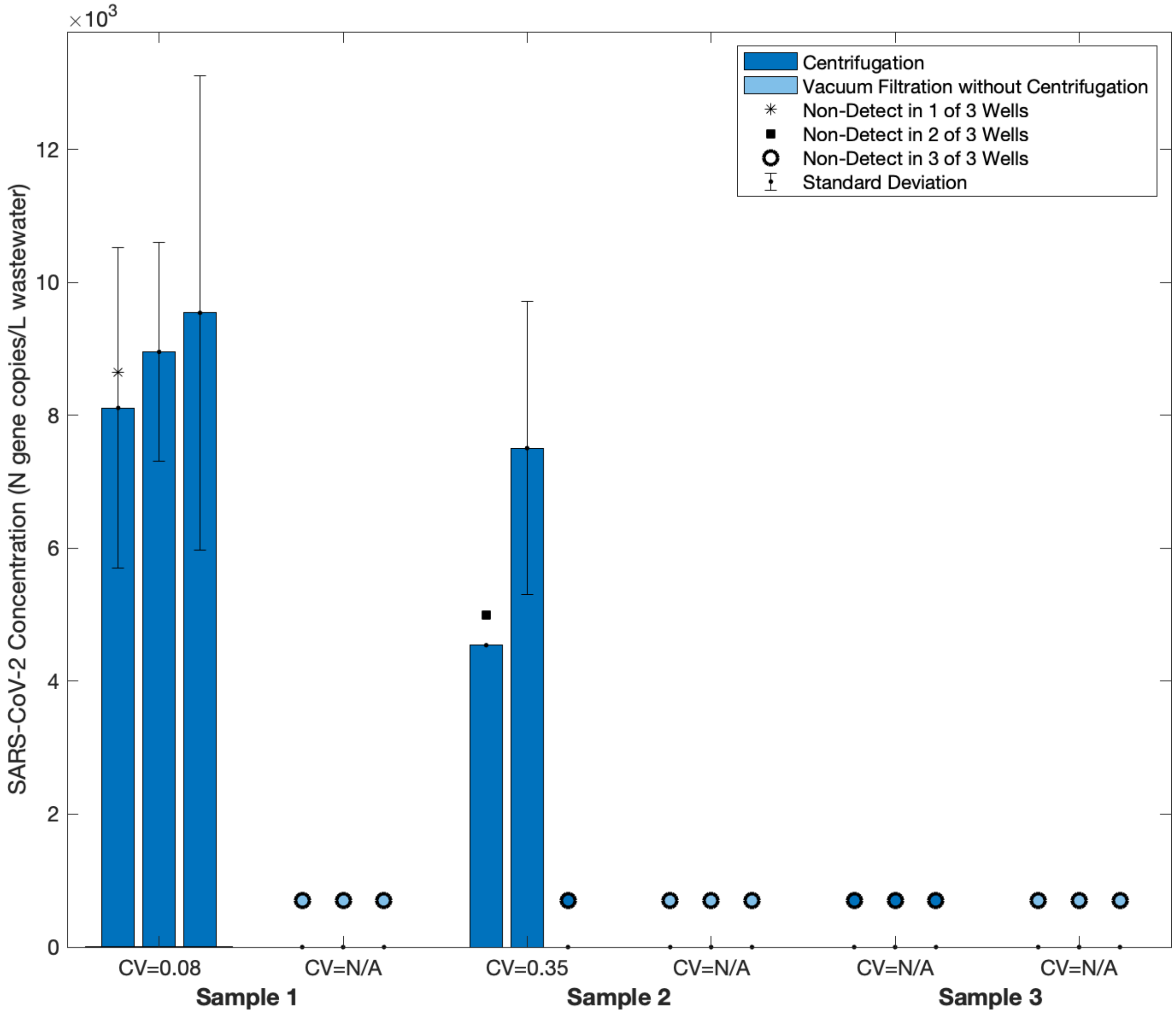
SI Figure 2: Concentrating SARS-CoV-2 from wastewater samples via centrifugation or vacuum filtration without centrifugation. WWTP: Walnut Creek (WAL); Sampling location: Influent (INF); Sample details: one composite sample was retrieved; Pasteurization: yes; Concentration Method: centrifugation (where the supernatant was then processed by vacuum filtration and the filtrate was processed by nanofiltration) vs. vacuum filtration without centrifugation (where the filtrate was then processed by nanofiltration). RNA Extraction Method: RNeasy with garnet lysis beads. No samples were processed using the final selected workflow. Each column represents one aliquot of wastewater processed from the sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation of the triplicate RT-qPCR reactions; the coefficient of variation (CV) normalizes the standard deviation to the average SARS-CoV-2 concentration. A CV value noted as "N/A" indicates that at least two of the triplicate aliquots processed yielded triplicate non-detect RT-qPCR reactions. The SARS-CoV-2 RNA concentrations for the nanofilter filtrate are not shown because all replicates yielded triplicate non-detect RT-qPCR results.



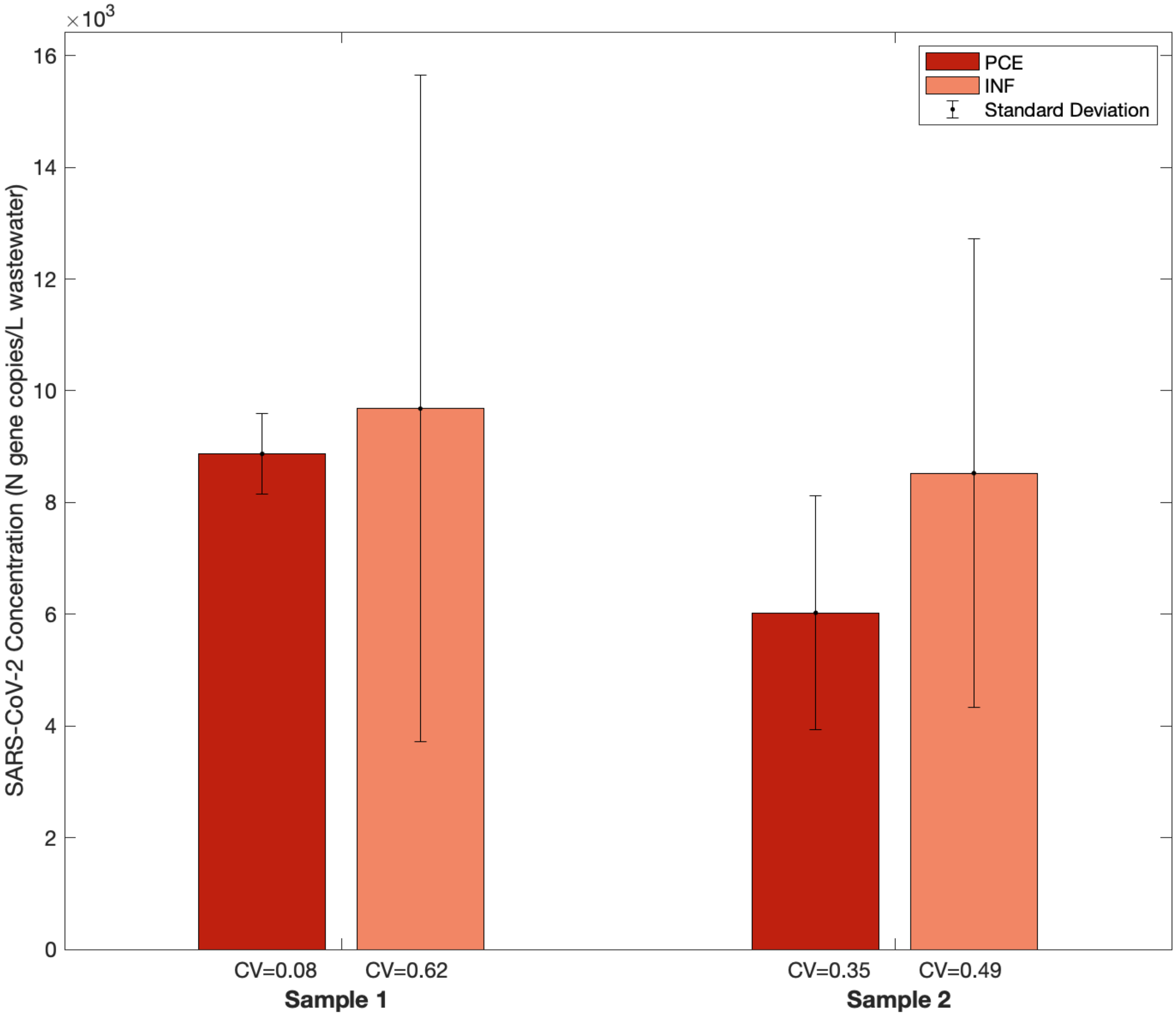
SI Figure 3: Total SARS-CoV-2 measured from reserved components of wastewater samples concentrated with or without centrifugation. <u>WWTP</u>: Walnut Creek (WAL); <u>Sampling location</u>: Primary Clarifier Effluent (PCE); <u>Sample details</u>: one composite sample was retrieved; <u>Pasteurization</u>: yes; <u>Concentration Method</u>: centrifugation (where the supernatant was then processed by vacuum filtration and the filtrate was processed by nanofiltration) vs. vacuum filtration without centrifugation (where the filtrate was then processed by nanofiltration). <u>RNA Extraction Method</u>: RNeasy with garnet lysis beads. No samples were processed using the final selected workflow. Each section of each stacked column represents the average SARS-CoV-2 concentration recovered across the triplicate aliquots of each reserved component, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation among the aliquots. The SARS-CoV-2 RNA concentrations for the nanofilter filtrate are not shown because all replicates yielded triplicate non-detect RT-qPCR results.



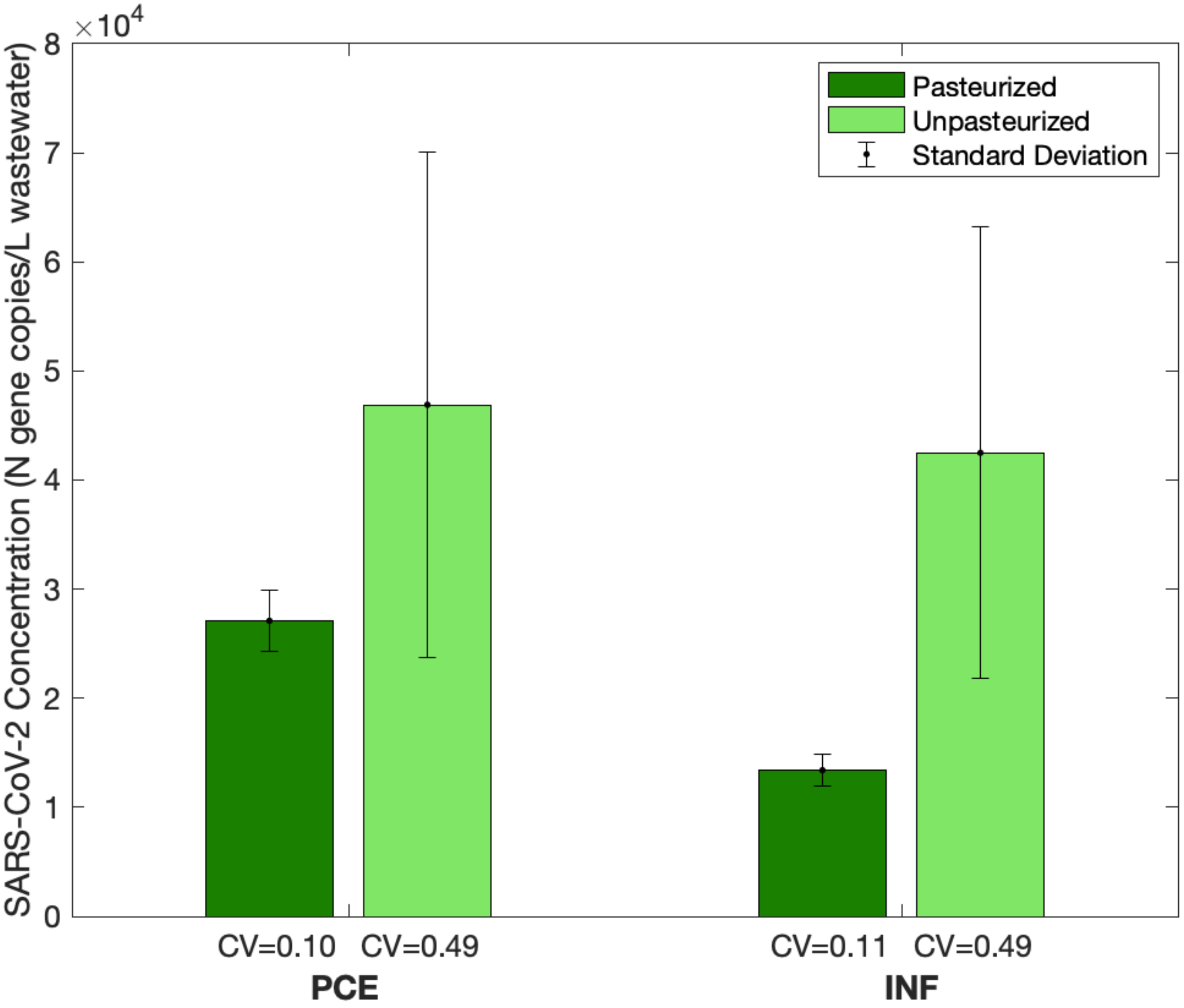
SI Figure 4: Lysing wastewater sample concentrates using a glass or garnet lysing matrix. <u>WWTP</u>: Walnut Creek (WAL); <u>Sampling location</u>: Primary Clarifier Effluent (PCE) and Influent (INF); <u>Sample details</u>: one PCE and one INF composite sample were retrieved; <u>Pasteurization</u>: yes; <u>Concentration Method</u>: centrifugation. <u>RNA Extraction Method</u>: RNeasy with glass or garnet lysis beads. No samples were processed using the final selected workflow. Each column represents one aliquot of wastewater processed from a sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation of the triplicate RT-qPCR reactions. One aliquot of PCE and one aliquot of INF were processed with the glass lysing matrix. Two aliquots of PCE and three aliquots of INF (with one yielding three non-detect qPCR reactions) were processed with the garnet lysing matrix.



SI Figure 5: Concentrating SARS-CoV-2 from wastewater samples via centrifugation or vacuum filtration without centrifugation. WWTP: Walnut Creek (WAL); Sampling location: Primary Clarifier Effluent (PCE); Sample details: three composite samples were retrieved; Pasteurization: yes; Concentration Method: centrifugation or vacuum filtration without centrifugation. RNA Extraction Method: RNeasy with glass lysis beads for samples concentrated via centrifugation or garnet lysis beads for samples concentrated via vacuum filtration without centrifugation. No samples were processed using the final selected workflow. Each column represents one aliquot of wastewater processed from a sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation of the triplicate RT-qPCR reactions; the coefficient of variation (CV) normalizes the standard deviation to the average SARS-CoV-2 concentration. When all three of the triplicate aliquots of a sample processed in the same way yielded triplicate non-detect RT-qPCR results, the CV is listed as “N/A”.



SI Figure 6: Concentrating SARS-CoV-2 from wastewater samples taken following primary treatment (PCE) or preliminary treatment (INF). WWTP: Walnut Creek (WAL); Sampling location: Primary Clarifier Effluent (PCE) and Influent (INF); Sample details: two pairs of PCE and INF composite samples were retrieved on two different dates between July and August 2020; Pasteurization: yes; Concentration Method: centrifugation; RNA Extraction Method: RNeasy with glass lysis beads. No samples were processed using the final selected workflow. Each column represents the average of triplicate aliquots processed for a sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation among the aliquots; the coefficient of variation (CV) normalizes the standard deviation to the average SARS-CoV-2 concentration.



SI Figure 7: Concentrating SARS-CoV-2 from wastewater samples with and without pasteurization at 60°C for 90 min. WWTP: Walnut Creek (WAL); Sampling location: Primary Clarifier Effluent (PCE) and Influent (INF); Sample details: one PCE and one INF composite sample were retrieved on the same day; Pasteurization: some samples were pasteurized (see legend); Concentration Method: centrifugation; RNA Extraction Method: RNeasy with glass lysis beads. No samples were processed using the final selected workflow. Each column represents the average of triplicate aliquots processed for a sample, where triplicate RT-qPCR reactions were run for each aliquot; error bars represent the standard deviation among the aliquots; the coefficient of variation (CV) normalizes the standard deviation to the average SARS-CoV-2 concentration.