Supporting Information: Wastewater surveillance during mass COVID-19 vaccination on a college campus

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**Detailed Procedure: Nucleic Acid Extraction**

Prior to extraction, 600 µL of PM1 (heated to 55°C) and 6 µL of β-Mercaptoethanol (MP Biomedicals, Irvine, CA, USA) were added to each thawed PowerBead tube and homogenized on a FastPrep 24 bead beating instrument (MP Biomedicals, Irvine, CA, USA) by four 20s rounds at 6.0 m/s. After bead beating, each PowerBead tube was centrifuged at 13,000 x g for one minute and 700 µL of supernatant was transferred into a clean 2 mL centrifuge tube. This supernatant was then extracted per the Qiagen protocol and the resulting nucleic acids were eluted from the silica column using 100 µL of RNase-free water provided with the kit. In a final step, the 100 µL of eluate was centrifuged at 13,000 x g for two minutes, and 80 µL of supernatant was transferred into a 2 mL DNA LoBind tube (Eppendorf, Hamburg, Germany) and stored at -80°C for up to three days until testing by reverse transcription droplet digital polymerase chain reaction (RT-ddPCR).

**Detailed Procedure: RT-ddPCR**

RNA reverse transcription and PCR amplification were performed in a one-step protocol using the One-Step RT-ddPCR Advanced Kit for Probes (1864021, BioRad, Hercules, CA, USA) per the manufacturer’s instructions. Each RT-ddPCR reaction was prepared to a volume of 22 µL consisting of 5.25 µL of 4X reaction Supermix, 2.1 µL of reverse transcriptase, 1.05 µL of dithiothreitol. Undiluted nucleic acid eluate volumes added to each reaction were 2 µL, 4 µL, and 6 µL for PMMoV, BRSV, and SARS-CoV-2, respectively, with the remaining volume consisting of molecular-grade water. Of the 22 µL reaction volume prepared, 20 µL was passed into the droplet generation step performed on the BioRad QX200 Droplet Generator (Hercules, CA, USA). Each RT-ddPCR experiment included duplicates of the appropriate no-template controls, sample blanks, and positive controls associated with each RT-ddPCR assay. SARS-CoV-2 positive control was in the form of the 2019-nCoV_N_Positive Control plasmid (10006625, IDT, Coralville, IA, USA), which was not pretreated in any way prior to use. SARS-CoV-2 RNA triplicate reactions
were merged into a single reaction in QuantaSoft to estimate the RNA copy number and error.

RT-ddPCR results for each assay were thresholded at the same amplitude across all experiments to reduce inter-experiment variability. A digital MIQE\textsuperscript{28} compliant checklist is provided in Table S2. The 95% limit of detection (LOD) for the SARS-CoV-2 N1 assay was previously determined as 3.3 copies/reaction\textsuperscript{24}, so for RT-ddPCR performed in triplicate reactions the expected 95% LOD is 1.1 copies.
Table S1 | RT-ddPCR assays used to detect and quantify SARS-CoV-2, PMMoV, BRSV, and Hep G in wastewater solids samples.

| Virus                        | Gene Target          | Sequences                                      | RT-ddPCR Reaction Concentration | Thermal Cycling Conditions                   |
|------------------------------|----------------------|------------------------------------------------|---------------------------------|----------------------------------------------|
| SARS-CoV-2                   | N1                   | F: 5'-GAC CCC AAA ATC AGC GAA AT-3'             | 1000 nM                         | 50°C 60 min; 95°C 10 min; 40 Cycles: 95°C 30 s, 59°C 60 s 98°C 10 min; 4°C hold |
|                              |                      | R: 5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'       |                                 |                                              |
|                              |                      | P: 5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3' | 250 nM                          |                                              |
| PMMoV (fecal indicator virus)| replicase protein    | F: 5'-GAG TGG TTT GAC CTT AAC GTT TGA-3'       | 900 nM                          |                                              |
|                              |                      | R: 5'-TTG TCG GTT GCA ATG CAA GT-3'            | 900 nM                          |                                              |
|                              |                      | P: 5'-FAM-CCT ACC GAA GCA AAT G-MGBNFO-3'      | 250 nM                          |                                              |
| BRSV (process control)       | nucleoprotein        | F: 5'-GCA ATG CTG CAG GAC TAG GTA TAA T-3'     | 900 nM                          |                                              |
|                              |                      | R: 5'-ACA CTG TAA TTG ATG ACC CCA TTC T-3'     | 900 nM                          |                                              |
|                              |                      | P: 5'-HEX-AC CAA GAC T/ZEN/T GTA TGC TGC CAA AGC A-IABkFQ-3' | 250 nM                          |                                              |
| Hep G Armored RNA (extraction & molecular control) | polypeptide precursor | F: 5'-CGG CCA AAA GGT GGT GGA TG-3'           | 900 nM                          | 50°C 60 min; 95°C 10 min; 40 Cycles: 95°C 30 s, 55°C 60 s 98°C 10 min; 4°C hold |
|                              |                      | R: 5'-CCC GAC GTC AGG CTC GTC G-3'            | 900 nM                          |                                              |
|                              |                      | P: 5'-FAM-AG GTC CCT C/ZEN/T GGC GCT TGT GGC GAG-IABkFQ-3' | 250 nM                          |                                              |
### Table S2 | Digital MIQE Checklist.

| ITEM TO CHECK | PROVIDED | COMMENT |
|---------------|----------|---------|
|               | Y/N      |         |
| **1. SPECIMEN** |          |         |
| Detailed description of specimen type and numbers | Y | Methods "Raw Wastewater Composite Samples" and "Wastewater Solids Separation" |
| Sampling procedure (including time to storage) | Y | Methods "Raw Wastewater Composite Samples" |
| Sample aliquotation, storage conditions and duration | Y | Methods |
| **2. NUCLEIC ACID EXTRACTION** |          |         |
| Description of extraction method including amount | Y | Methods "Nucleic Acid Extraction" |
| Volume of solvent used to elute/resuspend extract | Y | Methods "Nucleic Acid Extraction" |
| Number of extraction replicates | Y | Methods "Nucleic Acid Extraction" |
| Extraction blanks included? | Y | A sample blank also functioned as a extraction blank. |
| **3. NUCLEIC ACID ASSESSMENT AND STORAGE** |          |         |
| Method to evaluate quality of nucleic acids (including molecular weight and calculations when using mass) | N | Not performed. |
| Method to evaluate quantity of nucleic acids | N | Not performed. |
| Storage conditions: temperature, concentration, duration, buffer, aliquots | Y | Methods "Nucleic Acid Extraction" |
| Clear description of dilution steps used to prepare working DNA solution | Y | Methods "RT-ddPCR" |
| **4. NUCLEIC ACID MODIFICATION** |          |         |
| Template modification (digestion, sonication, pre- | N | Not performed. |
| Details of repurification following modification if | N | Not performed. |
| **5. REVERSE TRANSCRIPTION** |          |         |
| cDNA priming method and concentration | N | Not applicable. |
| One or two step protocol (include reaction details for | Y | One step, Methods "RT-ddPCR" |
| Amount of RNA added per reaction | Y | Methods "RT-ddPCR" |
| Detailed reaction components and conditions | Y | Methods "RT-ddPCR" |
| Estimated copies measured with and without | N | Not performed. |
| Manufacturer of reagents used with catalogue and | Y | Methods "RT-ddPCR" |
| **6. RT OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION** |          |         |
| Sequence accession number or official gene symbol | N | Incorporated by reference to Lu et al. 2020 |
| Method (software) used for design and in silico verification | N | Incorporated by reference to Lu et al. 2020 |
| Location of amplicon | N | Incorporated by reference to Lu et al. 2020 |
| Amplicon length | N | Incorporated by reference to Lu et al. 2020 |
| Primer and probe sequences (or amplicon context sequence)** | Y | Table S1 |
| Location and identity of any modifications | Y | Table S1 |
| Manufacturer of oligonucleotides | Y | Methods "RT-ddPCR" |
| **7. RT PCR PROTOCOL** |          |         |
| Manufacturer of qPCR instrument and instrument | Y | Methods "RT-ddPCR" |
| Buffer/kit manufacturer with catalogue and lot | Y | Methods "RT-ddPCR" |
| Primer and probe concentration | Y | Methods "RT-ddPCR" |
| Pre-reaction volume and composition (incl. amount | Y | Methods "RT-ddPCR" |
| Template treatment (initial heating or chemical denaturation) | N | Not performed. |
| Polymerase identity and concentration, Mg++ and dNTP concentrations*** | Y | Manufacturer's specification |
| Complete thermocycling parameters | Y | Table S1 |
| **8. ASSAY VALIDATION** |          |         |
| Details of optimisation performed | N | Incorporated by reference to Bivins et al. 2021 |
| Analytical specificity (vs. related sequences) and limit of blank (LOB) | N | Incorporated by reference to Lu et al. 2020 |
| Analytical sensitivity/LoD and how this was evaluated | N | Incorporated by reference to Bivins et al. 2021 |
| Testing for inhibitors (from biological matrix/extraction) | Y | Methods "RT-ddPCR" |
| **9. DATA ANALYSIS** |          |         |
| Description of qPCR experimental design | Y | Methods "RT-ddPCR" |
| Comprehensive details negative and positive of controls | Y | Methods "RT-ddPCR" |
| Partition classification method (thresholding) | Y | Methods "RT-ddPCR" |
| Examples of positive and negative experimental | Y | Table S4 |
| Description of technical replication | Y | Methods "RT-ddPCR" |
| Repeatability (intra-experiment variation) | Y | Methods "RT-ddPCR" |
| Reproducibility (inter-experiment/user/lab etc. variation) | N | Not performed. |
| Number of partitions measured (average and standard deviation) | Y | Table S5 |
| Partition volume | N | Table S5 |
| Copies per partition (λ or equivalent) (average and standard deviation) | Y | Table S5 |
| qPCR analysis program (source, version) | Y | Methods "RT-ddPCR" |
| Description of normalisation method | Y | Methods "RT-ddPCR" |
| Statistical methods used for analysis | Y | Methods "RT-ddPCR" and "statistical analysis" |
| Data transparency | Method with ID | Methods "RT-ddPCR" |
Table S3 | SARS-CoV-2 Wastewater Concentration Calculation (copy number per gram solids)

| Equation 1 |
|-------------|
| \[
N1 \left( \frac{\text{copy number}}{\text{gram}} \right) = C \times \frac{V_{RXN}}{V_{RNA}} \times D \times \frac{V_{RNA \text{ eluted}}}{S_{WM}} \frac{S_{WM}}{S_{WM} + 1}
\]

- **C**, RNA copy number per reaction (mean and 95% CI from QuantaSoft)
- **V\(_{RXN}\)**, volume of ddPCR reaction (20 µL)
- **V\(_{RNA}\)**, volume of RNA added to reaction (variable 2 µL to 6 µL)
- **D**, dilution factor (22/20) to account for 22 µL preparation and 20 µL reaction
- **V\(_{RNA \text{ eluted}}\)**, volume of RNA eluted during extraction (100 µL)
- **S\(_{WM}\)**, solids wet mass in grams (variable)
Table S4 | Examples of RT-ddPCR positive and negative results

| Assay | Hep G | PMMoV | SARS-CoV-2 N1 | BRSV |
|-------|-------|--------|--------------|------|
| Positive Reaction | ![Graph](image1.png) | ![Graph](image2.png) | ![Graph](image3.png) | ![Graph](image4.png) |
| Negative Reaction | ![Graph](image5.png) | ![Graph](image6.png) | ![Graph](image7.png) | ![Graph](image8.png) |
Table S5 | RT-ddPCR partition volume, partition number, copies per partition summary statistics

| Assay   | Partition volume (nL)* | Partition number per RT-ddPCR reaction (mean ± sd) | RT-ddPCR estimated copies per partition (λ) (mean ± sd) |
|---------|------------------------|----------------------------------------------------|-------------------------------------------------------|
| PMMoV   | 0.848                  | 14,126 ± 1,754                                     | 0.0725 ± 0.254                                       |
| BRSV    | 0.848                  | 14,396 ± 2,378                                     | 0.00569 ± 0.00581                                   |
| N1      | 0.848                  | 43,828 ± 4,709**                                   | 0.0000522 ± 0.000157**                              |
| Hep G   | 0.848                  | 13,361 ± 2,638                                     | 0.0204 ± 0.00244                                    |

* BioRad specified volume per droplet
** RT-ddPCR performed in triplicate reactions and merged
Figure S1 | Recovery efficiency as assessed by BRSV RNA copy number. Individual values, mean, and standard deviation for all wastewater solids samples, samples during the dose 1 period, samples during the dose 2 period, and samples after the end of the semester. P values estimated by Mann-Whitney U test.
Figure S2 | Inhibition as assessed by Hep G RNA copy number. Individual values, mean, and standard deviation displayed for wastewater solids samples and molecular-grade water controls. P values estimated by Mann-Whitney U test.
Figure S3 | Daily COVID-19 clinical surveillance tests administered throughout the study period.

Figure S4 | N1 copy number per gram of wastewater solids and COVID-19 positivity. For detections, the mean and 95% CI are displayed. For non-detections, the LOD in units of copy number per gram is displayed.
Table S6 | Example data pairings for COVID-19 cases and N1 copies per gram of wastewater solids with no lag, 1-day lag, 2-day lag, and 3-day lag of the wastewater data. The first data pair for the Spearman calculation in each case is highlighted. (ND = non-detect)

| Date       | COVID-19 Cases | No lag | 1-day lag | 2-day lag | 3-day lag |
|------------|----------------|--------|-----------|-----------|-----------|
|            |                | N1 copies/g | N1 copies/g | N1 copies/g | N1 copies/g |
| 8-Apr-2021 | 15             | 121    | 1         |           |           |
| 9-Apr-2021 | 4              | 130    | 121       |           |           |
| 10-Apr-2021| 2              | ND     | 130       | 121       |           |
| 11-Apr-2021| 0              | 30     | ND        | 130       | 121       |
| 12-Apr-2021| 10             | 59     | 30        | ND        | 130       |
| 13-Apr-2021| 9              | 29     | 59        | 30        | ND        |
| 14-Apr-2021| 7              | 39     | 29        | 59        | 30        |
| 15-Apr-2021| 7              | ND     | 39        | 29        | 59        |
| 16-Apr-2021| 7              | ND     | ND        | 39        | 29        |
| 17-Apr-2021| 1              | 55     | ND        | ND        | 39        |
| 18-Apr-2021| 2              | 11     | 55        | ND        | ND        |
| 19-Apr-2021| 9              | 68     | 11        | 55        | ND        |
| 20-Apr-2021| 5              | 48     | 68        | 11        | 55        |
| 21-Apr-2021| 3              | 13     | 48        | 68        | 11        |
| 22-Apr-2021| 4              | ND     | 13        | 48        | 68        |
| 23-Apr-2021| 1              | ND     | ND        | 13        | 48        |
| 24-Apr-2021| 1              | 23     | ND        | ND        | 13        |
| 25-Apr-2021| 0              | ND     | 23        | ND        | ND        |
| 26-Apr-2021| 2              | ND     | ND        | 23        | ND        |
| 27-Apr-2021| 6              | 18     | ND        | ND        | 23        |
| 28-Apr-2021| 1              | ND     | 18        | ND        | ND        |
| 29-Apr-2021| 2              | 53     | ND        | 18        | ND        |
| 30-Apr-2021| 0              | ND     | 53        | ND        | 18        |
| 1-May-2021  | 0              | 17     | ND        | 53        | ND        |
| 2-May-2021  | 0              | ND     | 17        | ND        | 53        |
| 3-May-2021  | 1              | ND     | ND        | 17        | ND        |
| 4-May-2021  | 2              | ND     | ND        | ND        | 17        |
| 5-May-2021  | 1              | 27     | ND        | ND        | ND        |
| 6-May-2021  | 2              | 35     | 27        | ND        | ND        |
| 7-May-2021  | 1              | ND     | 35        | 27        | ND        |
| 8-May-2021  | 0              | ND     | ND        | 35        | 27        |
| 9-May-2021  | 0              | ND     | ND        | ND        | 35        |
| 10-May-2021 | 0              | ND     | ND        | ND        | 35        |
| 11-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 12-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 13-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 14-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 15-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 16-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 17-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 18-May-2021 | 0              | ND     | ND        | ND        | ND        |
| 19-May-2021 | 0              | ND     | ND        | ND        | ND        |
Figure S5 | Recovery-corrected N1 copy number per gram of wastewater solids and COVID-19 cases. For detections, mean and 95% CI are displayed. For non-detections, the sample LOD with recovery correction is displayed.

Figure S6 | Recovery-corrected N1 copy number per gram of wastewater solids and COVID-19 positivity. For detections, mean and 95% CI are displayed. For non-detections, the sample LOD with recovery correction is displayed.
Figure S7 | PMMoV-normalized N1 copy number per gram of wastewater solids (both in units of copy number per gram) and COVID-19 cases. For detections, mean and 95% CI are displayed. For non-detections, the sample LOD is displayed.

Figure S8 | PMMoV-normalized N1 copy number per gram of wastewater solids (both in units of copy number per gram) and COVID-19 positivity. For detections, mean and 95% CI are displayed. For non-detections, the sample LOD is displayed.
Figure S9 | PMMoV copy number per gram of wastewater solids (mean and 95%CI), wastewater solids wet mass, and recovery efficiency as observed during the study period.

Figure S10 | Daily wastewater flow as observed during the study period.