**Methods**

**Sample Pooling**

Fifteen mL conical tubes (Cat. No. 89039-666, VWR, Batavia, IL) were manually labelled with unique 1D barcodes and charged with 2 mL of 0.9% saline (VWR Cat # BDH7257-1) using M4 repeater manual dispensers (Cat. No 4982000322, Eppendorf, Enfield, CT) equipped with 50-mL syringes (Cat. No. 0030089693, Eppendorf). Manual filling achieved adequate throughput; however, available automated liquid handling systems (Biomek FXp [Beckman Coulter, Indianapolis, IL], Freedom EVO 200 [Tecan, Switzerland]) were programmed to assist with tube filling for surge capacity and redundancy. Tubes, swabs, and transport bags were supplied to testing sites in bulk; students scanned a conical tube barcode with the SymMon app, collected an observed self-administered nasal swab, and placed the swab into the conical tube. Tubes were placed into sealed plastic bags, collected from testing locations and transported to the processing lab.

Laboratory staff wore personal air purifying respirator (PAPR) hood personal protective equipment (Versaflow, 3M), disposable gowns and double gloves for sample processing. Pre-processing of samples consisted of visual inspection for proper collection and transport; invalid samples (leaked saline, missing swab) were discarded and flagged in the barcode database to trigger student recall for testing. Valid tubes were briefly vortexed, uncapped, and the swab manually removed using metal forceps. After use, forceps were cleaned by immersion in 10% bleach (30 min), rinsing with diH2O, brief immersion in 70% ethanol, and air-drying. Uncapped tubes were placed in SBS-compliant 16 mm tube racks (Cat No A000260, Alpaqua, Beverly, MA) after position recording by barcode scanning into a password-protected MS Excel template containing macros to check for duplicate and missing entries. Timestamped reports were automatically saved in both local and network drives.

Sample pooling was performed on a dual-pod Biomek FXp automated liquid handling workstation (Beckman Coulter) equipped with 8 individually pump-operated syringes (Span-8) using 1-mL conductive disposable tips (Cat No 987935, Beckman Coulter) and controlled using standard Biomek software. Uncapped and barcoded 2-mL tubes were scanned as described above to locate them in a 24-position tube rack with 11 mm inserts (Cat Nos. 373661 and 373696, Beckman Coulter). Samples were pooled five-to-one by taking 0.2 mL/primary sample into 2-mL tubes (Cat. No. 76417-214, VWR). In the event that samples were not a multiple of 5, 15-mL tubes containing only saline were added to obtain 1 mL final volume/pool tube. The Biomek method achieved high throughput by optimizing the following variables: deck layout, movement sequences and range, syringe usage, pod speed and aspirating/dispensing heights and speeds. Aspirating/dispensing speeds that retained pipette accuracy throughout the process were empirically determined. Syringe lines were routinely primed with 15 mL H2O/line before each run. Post-processing consisted of manual recapping of filled 2-mL tubes that were moved into lidded racks for transport to the qPCR team. Primary sample tubes were recapped using plug caps (SAV-IT Closures, VWR Cat # 28296-616) and recycled screw caps cleaned by dipping in 70% ethanol for 30 minutes and air-drying. Primary samples were stored at 4°C with position tracking using barcode scanning into Excel files as described above.

**Laboratory-Developed RTqPCR Method**

SARS-CoV-2 RNA copies per milliliter were determined by a laboratory developed two-step real-time quantitative PCR (RTqPCR) assay. A QIAsymphony SP (Qiagen, Hilden, Germany) automated sample preparation platform along with a virus/pathogen DSP midi kit and the *complex800* protocol were used to extract viral RNA from 800 µL of pooled samples. A reverse primer specific to the envelope gene of SARS-CoV-2 (5’-ATA TTG CAG CAG TAC GCA CAC A-3’) was annealed to the extracted RNA and then reverse transcribed into cDNA using SuperScriptTM III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA) along with RNAse Out (Thermo Fisher Scientific, Waltham, MA). The resulting cDNA was treated with RNase H (Thermo Fisher Scientific, Waltham, MA) and then added to a custom 4x TaqManTM Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA) containing primers and a fluorescently labeled hydrolysis probe specific for the envelope gene of SARS-CoV-2 (forward primer 5’-ACA GGT ACG TTA ATA GTT AAT AGC GT-3’, reverse primer 5’-ATA TTG CAG CAG TAC GCA CAC A-3’, probe 5’-6FAM/AC ACT AGC C/ZEN/A TCC TTA CTG CGC TTC G/IABkFQ-3’). The qPCR was carried out on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) using the following thermal cycler parameters: heat to 50°C, hold for 2 min, heat to 95°C, hold for 10 min, then the following parameters are repeated for 50 cycles: heat to 95°C, hold for 15 seconds, cool to 60°C and hold for 1 minute. SARS-CoV-2 RNA copies per reaction were interpolated using quantification cycle data and a serial dilution of a highly characterized custom DNA plasmid containing the SARS-CoV-2 envelope gene sequence. Mean RNA copies per milliliter were then calculated by applying the assay dilution factor (DF=11.7). The limit of quantification (LOQ) for this assay is approximately 62 RNA copies per milliliter of sample.

The primers and probes used in the above assay are from the Charité/Berlin World Health Organization (WHO) assay and are referred to as “E\_Sarbeco.”

*Reference: Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR, Berlin, 13.01.2020*

**Sample Deconvolution**

For each RT-qPCR-positive pool, the pool barcode was used to identify the five primary samples and their positions located using the inventory files described above. Samples were manually aliquoted into 2-mL cryovials labeled with the same barcode as the original sample. The SymMon database was used to pull information for the generation of a clinical order. For each primary specimen, 1 mL was provided to the Duke Clinical Microbiology Laboratory for assay using standard clinical testing platforms and the remaining sample stored at -80°C. Primary samples from pools that tested negative were discarded after a terminal barcode scan into the SymMon database.