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VIROLOGY

Large-Scale, In-House Production of Viral Transport Media To Support SARS-CoV-2 PCR Testing in a Multihospital Health Care Network during the COVID-19 Pandemic

Kenneth P. Smith,[a,b] Annie Cheng,[a] Amber Chopelas,[a] Sarah DuBois-Coyne,[a,c] Ikram Mezghani,[d] Shade Rodriguez,[a] Mustafa Talay,[e] James E. Kirby[a,b]

[a]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[b]Harvard Medical School, Boston, Massachusetts, USA
[c]Department of Biochemistry, University of Massachusetts Boston, Boston, Massachusetts, USA
[d]Department of Surgery, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[e]Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA

Amber Chopelas, Sarah DuBois-Coyne, Ikram Mezghani, Shade Rodriguez, and Mustafa Talay contributed equally to this work and are listed alphabetically.

ABSTRACT The COVID-19 pandemic has severely disrupted worldwide supplies of viral transport media (VTM) due to widespread demand for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcription-PCR (RT-PCR) testing. In response to this ongoing shortage, we began production of VTM in-house in support of diagnostic testing in our hospital network. As our diagnostic laboratory was not equipped for reagent production, we took advantage of space and personnel that became available due to closure of the research division of our medical center. We utilized a formulation of VTM described by the CDC that was simple to produce, did not require filtration for sterilization, and used reagents that were available from commercial suppliers. Performance of VTM was evaluated by several quality assurance measures. Based on cycle threshold (C_T) values of spiking experiments, we found that our VTM supported highly consistent amplification of the SARS-CoV-2 target (coefficient of variation = 2.95%) using the Abbott RealTime SARS-CoV-2 Emergency Use Authorization (EUA) assay on the Abbott m2000 platform. VTM was also found to be compatible with multiple swab types and, based on accelerated stability studies, able to maintain functionality for at least 4 months at room temperature. We further discuss how we met logistical challenges associated with large-scale VTM production in a crisis setting, including use of a staged assembly line for VTM transport tube production.

KEYWORDS COVID-19, PCR, SARS-CoV-2, bottleneck, logistics, quality assurance, quality control, supply chain, universal tranport medium, viral transport medium

The COVID-19 pandemic has led to an unprecedented need for diagnostic reverse transcription-PCR (RT-PCR) testing. The ideal specimen type is currently believed to be a nasopharyngeal (NP) swab specimen transported to a molecular microbiology laboratory in viral transport medium (VTM). Starting in March 2020, increasing demand for testing led to a national shortage of both NP swabs and VTM that created significant bottlenecks in large-scale testing efforts.

VTM exists in several formulations, all of which consist of a buffered salt solution, a complex source of protein and/or amino acids, and antimicrobial agents. Its purpose is to preserve virus for later amplification by nucleic acid amplification testing (NAAT) technology and/or viral culture. Although simpler formulations, for example, saline, are technically compatible with RT-PCR, most NAAT assays for respiratory pathogens have

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Address correspondence to James E. Kirby, jekirby@bidmc.harvard.edu.

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been developed and FDA cleared for use with more complex transport media (i.e., VTM and universal transport medium [UTM]). Overgrowth of bacteria may also occur in media lacking antimicrobial agents. Therefore, we chose to reproduce a standard of care transport medium to serve our health care network.

In response to the COVID-19 pandemic, our molecular microbiology laboratory was able to quickly scale up severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RT-PCR testing to approximately 1,000 tests per day to support the needs of our hospital network. However, national shortages of collection materials, including nasopharyngeal swabs and VTM, were projected to limit our ability to continue testing at this level. We discuss a nationwide collaborative effort to produce three-dimensional (3D) printed swabs to address the former bottleneck (1). Here, we describe processes for large-scale, local production of VTM within the framework of a rigorous quality assurance program as a model to address an unmet need for diagnostic supplies in a crisis setting.

MATERIALS AND METHODS

VTM preparation. VTM was prepared using CDC standard operating procedure DSR-052-01 (2), with Hanks' balanced salt solution (HBSS) including phenol red (Gibco, Waltham, MA, or Millipore, Burlington, MA) serving as this medium's balanced salt solution, which was supplemented with fetal bovine serum (FBS; Gibco or Corning, Tewksbury, MA), amphotericin B (HyClone, Waltham MA, Corning, or Sigma, Burlington, MA), and gentamicin (HyClone, Corning, or Sigma) to concentrations of 2%, 0.5 μg ml^{-1}, and 100 μg ml^{-1}, respectively. We modified the CDC recipe slightly by inclusion of 10 mg liter^{-1} of phenol red. Phenol red is a pH indicator that is pink or red at neutral or basic pH and transitions to yellow at acidic pH, providing a visual check on medium pH. Furthermore, phenol red turns purple in the presence of bleach, thereby allowing us to visually confirm that our dispense tubing was appropriately washed after bleach sterilization.

Component solutions were sterile, and all liquid handling steps were performed using sterile technique in a class II biosafety cabinet to ensure sterility of the final VTM. Biosafety cabinets were thoroughly wiped down with 70% ethanol and UV decontaminated before and after use.

A foot pedal- or hand-controlled peristaltic pump was used to dispense medium (Flexipump, model number 562000 [Interscience, Woburn, MA], or Digital MiniPump, model number AR77922-32 [Argos Technologies, Vernon Hills, IL]). Sterilization of pump tubing was achieved by continuous dispensing of ~150 ml of a 10% bleach solution. Tubing was cleared of bleach by two extensive wash steps, each using a different bottle of sterile distilled water. Immediately before aliquoting, the pump was primed by dispensing at least 150 ml of VTM. After sterilization and priming, 3 ml of medium was aliquoted into conical 15-ml centrifuge tubes (Falcon or Corning).

QC. Each day's production of VTM (approximately 3,500 to 4,000 tubes; maximum, 6,000 tubes a day) was assigned a new lot number for independent assessment in our quality control (QC) program to address any potential variability introduced by sterilization of our semiautomated pumping apparatus. Randomly selected tubes (at least 5 per lot) were examined. Rejection criteria for media included cloudiness, presence of any particulates, volume of >3.25 ml or <2.75 ml, and phenol red color suggesting the presence of bleach (purple) or acidity (yellow). Additionally, tubes were inspected and were rejected if there were any signs of physical damage (cracks, crazing, or discoloration) or leaks. Next, 1 ml of medium from a randomly selected tube in each lot was also plated on chocolate agar. The chocolate agar plate was dried in a biosafety cabinet and incubated overnight at 35°C to evaluate VTM sterility.

Finally, a randomly selected tube of medium from each lot was tested for its support of SARS-CoV-2 RT-PCR testing using the Emergency Use Authorization (EUA)-authorized Abbott RealTime SARS-CoV-2 assay run on the Abbott m2000 platform, the same system as used for clinical testing in our health care network. Accuplex COVID-19 reference material (SeraCare, Milford, MA), consisting of recombinant Sindbis virus containing the SARS-CoV-2 RNA amplicon target, was spiked into samples of VTM from the lot under study at 2× the limit of detection (LoD) of the assay (200 copies/ml) to assess amplification of SARS-CoV-2 near the assay limit of detection. QC was considered to pass if both the internal control (IC) and SARS-CoV-2 were amplified with cycle threshold (C_T) values within acceptable limits reflecting the inherent small native variation of this assay. All spiked VTM C_T values were recorded and visualized in aggregate as a Levey-Jennings plot using GraphPad Prism 7.0. Potential contamination of VTM with the SARS-CoV-2 amplicon was also evaluated by testing the VTM lot alone. This QC assessment was considered to have passed if the assay IC demonstrated appropriate levels of amplification in the absence of SARS-CoV-2 detection.

Alternative swab and medium testing. VTM was evaluated for compatibility with various swab types, including NP swabs as well as nonstandard swab types that we considered might be used in place of NP swabs to respond to shortages. Briefly, swabs were removed from packaging, broken or cut with office scissors if no breakpoint was present, and placed in VTM. This step was performed on an open bench with nonsterile gloved hands to approximate the clinical environment. Swabs in VTM were incubated for 16 to 24 h at 4°C to mimic conditions during specimen transport, after which RT-PCR QC was performed as outlined in the QC section. Alternative medium-swab combinations were tested in an

TABLE 1 Swab validation using in-house VTM

Swab type	Manufacturer	Positive control	Negative control
E-Swab	Becton Dickinson	25.07	Not detected
Foam applicator	Puritan	25.11	Not detected
Female cleaning swab	Hologic	25.37	Not detected
Urethral swab	Hologic	24.77	Not detected
FLOQSwab	Copan	25.63	Not detected
NP swab	Diagnostic Hybrids	24.38	Not detected
Disposable sampling swab	Miraclean Technologies	25.50	Not detected
Lesion/other swab	Diagnostic Hybrids	25.68	Not detected

identical manner. See Table 1 for a listing of alternative swabs tested in VTM and Table 2 for a listing of alternative swab-medium combinations.

Accelerated stability testing. Two randomly selected tubes of VTM were incubated at either 56°C or 4°C (CDC-recommended storage temperature) for 12 days. After incubation, visual inspection was performed, and the suitability of aged VTM for RT-PCR testing was evaluated as outlined in the QC section.

Stability of antimicrobial agents was confirmed by a killing study using *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028. Organisms were grown overnight on blood agar (*Escherichia coli*) or Sabouraud dextrose agar (*Candida albicans*) and suspended in 0.85% NaCl to a density of 0.5 McFarland using a Vitek DensiChek handheld colorimeter. For each organism, the 0.5 McFarland suspension was diluted 1:10 into 0.85% NaCl, and 10 μl of this dilution was added to 250 μl of VTM. An aliquot was immediately plated onto medium using the drop plate method to quantify the initial inoculum prior to significant antibiotic exposure (3).

Organisms suspended in VTM were then incubated for 24 h at 4°C as might occur during normal specimen transport and storage in the laboratory prior to testing. CFU were then quantified using the drop plate method, and the percent recovery after VTM incubation was determined. The metric part for acceptability for this part of the accelerated stability study was >99% killing of *E. coli* and *C. albicans* in VTM aged at elevated temperature.

RESULTS

VTM formulation. Broadly speaking, we had two potential options for VTM production: following a recipe released by the CDC or attempting to "reverse-engineer" a recipe to mimic commercial formulations. We selected the CDC VTM for several reasons. First, exact formulations of commercial proprietary VTM are difficult to obtain. In contrast, components of the CDC VTM are explicitly defined. Second, all reagents necessary for the CDC formulation are commonly used for cell culture and were already available in our research division and available to order. Third, these component reagents could be purchased as sterile products, eliminating the time-consuming process and technical difficulties associated with sterile filtration of large quantities of medium (>40 liters per week), especially in light of concurrent shortage of filtration devices.

Infrastructure, team organization, and management. In-house production of VTM is a technical and logistical challenge requiring dedicated space and personnel. Our diagnostic laboratory's staffing and infrastructure were fully committed to addressing diagnostic testing needs and could not accommodate the additional burden of VTM production. However, early in the pandemic, our institution discontinued all nonessential research activities, resulting in availability of space and personnel, which and who could be redeployed for this effort.

Therefore, two tissue culture rooms with 8 class II biosafety cabinets were repurposed for VTM production efforts. In addition, 9 research personnel were redeployed to

TABLE 2 Alternative medium-swab combinations tested

Medium	Swab	Manufacturer	Positive control	Negative control
0.9% saline	None	TekNova	24.54	Not detected
0.9% saline	None	Becton Dickinson	24.59	Not detected
0.9% phosphate-buffered saline	None	Corning	25.02	Not detected
Aptima transport medium	Female cleaning swab	Hologic	24.87	Not detected
Liquid Amies	E-Swab	Becton Dickinson	25.91	Not detected
Viral collection medium	NP swab	Diagnostic Hybrids	24.65	Not detected

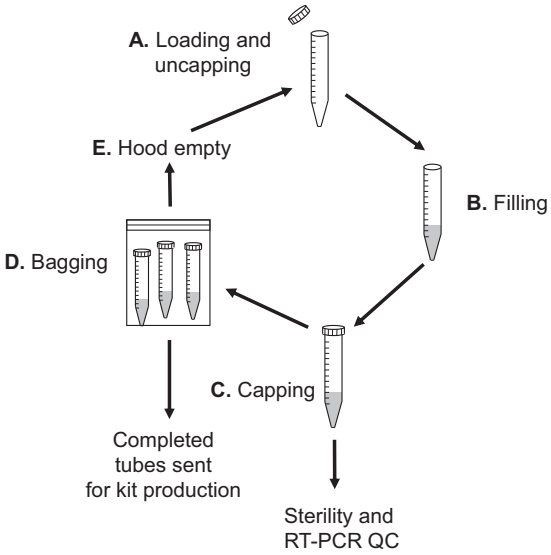

FIG 1 Workflow diagram. Each biosafety cabinet was used for all steps in the VTM production workflow. Personnel and the peristaltic pump rotated between cabinets, but tubes remained in place until packaging. (A) First, tubes were loaded and uncapped. (B) Medium filling was then accomplished through use of a peristaltic pump system moved to each biosafety cabinet in turn on a mobile cart. (C) Filled tubes were capped and random samples were subject to QC. (D) Tubes were then removed from the hood, bagged, and sent for distribution. (E) The now-empty hood was then used to start the next production cycle.

the VTM production team, all of whom had significant laboratory experience, including sterile technique. These personnel were divided into independently functioning teams working in rotations, so that if any team became infected with SARS-CoV-2, the other teams would be able to continue production. Direct oversight of team personnel, supply chain management, and maintenance of the quality control/quality assurance program was delegated to a senior American Society for Microbiology Subcommittee on Postgraduate Education Programs (CPEP) medical microbiology fellow under supervision of the clinical microbiology laboratory director.

For VTM transport tube production, we established a staged assembly line, taking advantage of the multiple available biosafety cabinets (Fig. 1). Each cabinet was used sequentially for multiple production steps. The first biosafety cabinet was used as a staging area where conical tubes were uncapped and arranged in racks. Caps were stored for later use in sterile bags that originally held the tubes. The worker responsible for uncapping then shifted to the next empty biosafety cabinet to start the process again, while a peristaltic pump was brought into proximity on a laboratory cart to fill uncapped tubes. After the tubes in the first biosafety cabinet were filled, a third worker capped the tubes, and finally, a fourth worker bagged and boxed tubes for later transport to a packaging facility. This cycle proceeded continuously during production, with personnel moving sequentially to each of four biosafety cabinets to keep each step of the production line running. In this way, we were able to prepare 3,500 to 4,000 VTM tubes per day.

Importantly, it should also be noted that releasing VTM for clinical use requires collaboration with personnel outside the laboratory. A separate team of 8 to 12 personnel in our materials management and distribution department was tasked with labeling each VTM tube, including with a designated lot number, and pairing it with an NP collection swab to create a complete collection kit. This was an additional labor-intensive effort of critical importance.

Quality control. Quality control was performed on each batch of VTM prior to release for clinical use. QC was incorporated into normal clinical workflow and was performed identically to that for patient specimens. Controls were spiked at 2× the

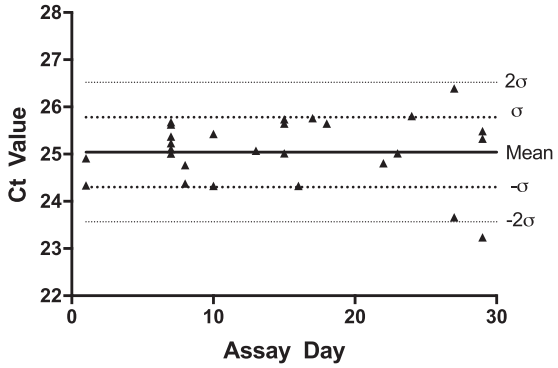

FIG 2 Levey-Jennings plot of VTM quality control data. C_T values for VTM lots spiked at 2× the LoD with the SARS-CoV-2 target were plotted each day of testing. Test dates with more than one data point represent the same batch of VTM evaluated for compatibility with multiple swab types (see Table 1).

limit of detection (LoD) of the assay, stated in the Abbott EUA documentation (200 copies/ml) and independently confirmed in our internal verification studies, to enable detection of impactful deviations in the analytical sensitivity of the assay. In 27 quality control experiments thus far, representing >50,000 VTM tubes, as well as swab compatibility studies, spiked SARS-CoV-2 amplicon was detected in all samples, indicating that the upper bound of deviation in LoD using homemade VTM was no more than 2-fold above the specified LoD and therefore PCR efficiency was no more than minimally affected, if at all.

Furthermore, on average, observed C_T values were 25.0 ± 0.7 for our spiked controls, approximately one cycle below the mean C_T of 26.0 ± 1.0 found for the LoD during our internal verification studies ($n = 80$, tested at the LoD of 100 genome copies/ml) and therefore at the expected C_T value for a spiked sample at 2× the LoD. The mean internal control C_T was 17.1 ± 0.4 for the same reactions. We used coefficient of variation (CV) to evaluate the relative variabilities of spiked target and internal control and found that they were similar, at 3.0% and 2.4%, respectively. The CV for our LoD study was similar, at 4.0%, with slightly increased variance noted closer to the LoD, as expected. We additionally visualized variance in spiked controls using a Levey-Jennings plot (Fig. 2). The majority of C_T values (96.2%) fell within ±2 standard deviations from the mean. A single value (3.8%) was >2 standard deviations from the mean, as is expected for 27 normally distributed data points. We did not observe any obvious trending in the data.

Alternative swab and medium testing. In light of the national shortage of NP swabs, it may become necessary to pair alternative swab types with in-house-prepared VTM. Therefore, multiple swab types were qualitatively evaluated as previously described (1) and then incubated in our VTM at 4°C overnight. The VTM was then tested with the Abbott SARS-CoV-2 assay. Importantly, we found that all C_T values (Table 1) for these samples, spiked with SARS-CoV-2 RNA at 2× the LoD, fell within ±2 standard deviations of the mean previously determined in our QC studies of VTM without swabs, indicating compatibility of multiple swab types with our prepared VTM.

Accelerated stability testing. Unprecedented test volumes for SARS-CoV-2 resulted in a similarly unprecedented need for storage of collection kits. This is complicated by the CDC recommendation that VTM should be refrigerated. Clinical sites were not designed with large-volume refrigeration space. *A priori*, however, there was no obvious reason why VTM would require low-temperature storage. Therefore, we sought to test whether the VTM formulation would remain stable at room temperature for prolonged periods.

In an emergent situation, real-time stability testing is impractical. Therefore, we performed an accelerated-aging study using elevated-temperature incubation (56°C) to predict the effects of extended storage at room temperature (22°C). This was based on

precedent in drug and diagnostic reagent stability testing for application of the Arrhenius equation (4, 5), which can be used to predict the effect of temperature on decay rate of reagents. Importantly, we found that PCR efficiency was maintained in accelerated-aged VTM based on internal control amplification; i.e., the C_T of the internal control remained within the expected mean and standard deviations of nonaged VTM. Antimicrobial ability to inhibit bacterial and fungal growth (gentamicin and amphotericin B) was also preserved. Based on Arrhenius equation calculations (4), maintenance of critical metrics during the tested 2-week incubation at 56°C was used to predict VTM stability for at least 4 months at room temperature.

DISCUSSION

Production of VTM on a scale capable of supporting a hospital network requires extensive infrastructure, including bench space, reagent storage, and multiple biosafety cabinets, which are unavailable in routine clinical microbiology laboratories. Further, dedicated personnel with laboratory experience are required at a time when technologists are needed to address a surge in routine diagnostic work. In this study, we took advantage of our medical center's extensive research division, which was largely shuttered as a response to the pandemic, to repurpose resources for large-scale VTM tube production.

We acknowledge that saline has been advocated by the CDC as an alternative transport medium (6). However, none of our amplification methods were qualified in their EUA or FDA clearance to be used with saline. Furthermore, there were theoretical advantages in terms of potentially sustaining viral integrity and presumably labile RNA viral genome target material through use of a buffered medium with a protein component for stabilization.

Another alternative considered was the use of a guanidinium-based transport buffer. It has an advantage in stabilization of nucleic acid and inactivation of virus (7). However, guanidinium-based transport medium would have necessitated additional validations on FDA-cleared respiratory PCR and SARS-CoV-2 EUA assays on ePlex (Genmark, Carlsbad, CA) and Cepheid (Danaher, Sunnyvale, CA) systems used in our hospital system at a time when SARS-CoV-2 test reagents for these platforms were on strict allocation and with uncertainty in terms of compatibility. Although not described here, we confirmed that our VTM was compatible with the GenMark ePlex respiratory viral panel, Cepheid influenza virus test, and SARS-CoV-2 tests on both platforms (data not shown).

Alternative VTM recipes exist but require weighing and solubilizing materials, followed by filter sterilization. A replication of commercial universal transport medium was tried but became untenable during scale-up. In contrast, all components for CDC VTM are available as sterile solutions, which streamlined the production process and helped ensure lot-to-lot consistency.

Although the CDC formulation of VTM requires only a few ingredients, initial production of VTM was a significant challenge, as components were not stocked in the clinical laboratory and supply delivery during the crisis was unacceptably slow. Initially, therefore, donations of existing supplies were requested through broadcast email to our research community and beyond through social media until sufficient supplies could be obtained from outside vendors. The response was exceedingly positive, and supplies were provided in abundance, with meticulous accounting for donations from research laboratories to later reimburse costs. Laboratory supply companies also quickly placed the reagents used to make VTM on allocation. Although this was meant to prioritize orders from hospitals over research laboratories, it also resulted in these reagents appearing out of stock when ordered through existing channels. We therefore needed to place each order by directly contacting company representatives to ensure that stock would be appropriately released.

After solving logistical issues, we identified several procedural concerns, which initially seemed trivial but became significant hurdles at scale. One of these was tube filling. At capacity, our goal was to produce 3,500 to 4,000 tubes per day, requiring

handling of approximately 50 to 60 liters of medium per week. Manual filling of this number of tubes is not practical. Therefore, we acquired and utilized a semiautomated peristaltic pump that repeatedly dispensed appropriate volumes. The pumps were controlled by a foot pedal or a push button on a dispensing gun, allowing repeatable delivery of 3 ml to each tube much more efficiently than manual pipetting.

Another example of a simple procedure that becomes complex at scale was tube capping. Each of our personnel routinely capped approximately 1,000 tubes per day. This is a demanding process, which invariably resulted in painful blisters and was therefore not sustainable. We also observed that Falcon 15-ml conical tubes, which were used initially, required substantial force to adequately close. Therefore, we switched to Corning tubes with Centristar caps, which proved much easier to manipulate but did not entirely solve the blistering issue. We therefore attempted to protect the fingers of personnel by wrapping them in different types of tape, including surgical tape (Blenderm; 3M, St. Paul, MN), self-adherent wrap (Coban; 3M), or KT blister prevention tape (KTTape, Linden, UT). Each proved suboptimal, being either too thin or too thick or limiting dexterity. Ultimately, we identified rock climbing tape (a donation from Metolius Climbing, Bend, OR) as an ideal solution which eliminated blistering entirely so that capping was fully tolerated by staff.

Aside from logistical issues, we would like also to emphasize the quality control plan put in place. This included testing sterility and PCR efficiency for each lot through assessment of C_T variance of spiked SAR2-CoV-2 and the internal control, results which could be analyzed using in standard Levey-Jennings plots to detect bias and issues with reproducibility.

Our experience with VTM production provides an example of a rigorously controlled effort to provide a critical resource at scale in a crisis. Through collaboration between researchers, clinical microbiologists, and support/logistics personnel, we were able to circumvent the limitations of a routine diagnostic laboratory and address a significant bottleneck that would otherwise limit high-volume COVID-19 testing capacity.

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REFERENCES

1. Callahan CL, Zulauf KE, Tamburello L, Smith KP, Previtera J, Cheng A, Green A, Abdul AA, Yano A, Doraiswami N, Kirby JE, Arnaout R. 2020. Rapid open development and clinical validation of multiple new 3D-printed nasopharyngeal swabs in response to the COVID-19 pandemic. MedRxiv https://doi.org/10.1101/2020.04.14.20065094.
2. Centers for Disease Control and Prevention. 2020. DSR-052-02: preparation of viral transport medium. https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf. Accessed 27 April 2020.
3. Herigstad B, Hamilton M, Heersink J. 2001. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 44:121–129. https://doi.org/10.1016/s0167-7012(00)00241-4.
4. Kirkwood TB. 1977. Predicting the stability of biological standards and products. Biometrics 33:736–742. https://doi.org/10.2307/2529472.
5. Clinical and Laboratory Standards Institute. 2009. Evaluation of stability of in vitro diagnostic reagents. CLSI document EP25-A. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Centers for Disease Control and Prevention. 2020. COVID-19 testing and reporting by laboratories: Q & A. https://www.cdc.gov/coronavirus/2019-ncov/lab/testing-laboratories.html. Accessed 27 April 2020.
7. Daum LT, Worthy SA, Yim KC, Nogueras M, Schuman RF, Choi YW, Fischer GW. 2011. A clinical specimen collection and transport medium for molecular diagnostic and genomic applications. Epidemiol Infect 139:1764–1773. https://doi.org/10.1017/S0950268810002384.