The Effects of “Dry Swab” Incubation on SARS-CoV-2 Molecular Testing

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Background: Widespread testing of SARS-CoV-2 has resulted in shortages of collection devices and transport media. We evaluated the stability of flocked swabs inoculated with SARS-CoV-2-containing specimen incubated dry (i.e., without transport medium) at room temperature.

Methods: A pool of SARS-CoV-2 positive specimen was used to inoculate flocked swabs. Five swabs were placed immediately into universal transport media (UTM) following inoculation, and tested immediately (day 0). Fifteen of the swabs were placed into sterile 15-mL conical tubes and incubated at room temperature for 1, 2, or 7 days. Following incubation, swabs were hydrated in separate vials of UTM and tested. This protocol was repeated for viral transport media (VTM) and saline. As a comparison, a series of swabs was prepared and tested in parallel, but stored in the corresponding liquid transport media (UTM, VTM, or saline) and incubated at room temperature. Testing was performed at 1, 2, and 7 days postinoculation in duplicate. All molecular testing was performed using the Roche cobas SARS-CoV-2 assay.

Results: All dry swabs tested on days 1, 2, and 7 provided results that were within 2 cycle thresholds (CTs) of the average CT values for swabs hydrated in the same media and tested on day 0. There was no statistical difference in CT values between swabs incubated in liquid media versus dry swabs incubated at room temperature prior to hydration in liquid media.

Conclusions: The utilization of “dry swabs” may simplify specimen collection, negate the need for liquid transport media, and mitigate safety risks while preserving the accuracy of testing.

INTRODUCTION

Shortages in universal transport media (UTM) and viral transport media (VTM) have forced laboratories and manufacturers to explore alternative approaches to specimen collection and transport for the testing of SARS-CoV-2 (1–3). Some laboratories have developed protocols for making UTM or VTM in house while others are distributing modified collection devices with limited amounts of transport media (4). In some instances, providers may be asked to place the specimen collection device/swab into a limited amount of transport media, shake the swab vigorously, and discard it (5). This process has the potential to generate aerosols and infectious waste, resulting
in a potential biosafety concern, especially in high-
traffic patient care areas. A potential alternative is
to transport the specimen collection swabs in a
tube without liquid transport media (dry swabs),
and hydrate them for analysis in transport media
upon laboratory receipt. The transport of dry
swabs allows for the relegation of aerosol generat-
ning processes to a biological safety cabinet and
eliminates the need to distribute aliquots of col-
lection media to collection sites. This may also ad-
dress the challenge that some transport devices
(VTM) require refrigeration after collection, a logis-
tical challenge, especially at outdoor drive-through
specimen collection sites. Little is known about
the stability of SARS-CoV-2 on a dry swab, or the
impact on testing accuracy. We evaluated the sta-
bility of dry swabs for the testing of SARS-CoV-2
when hydrated in UTM, CDC VTM, and 0.9% saline
following various lag times after collection at room
temperature incubation.

**METHODS**

**Preparation of Positive Swabs**

This quality improvement project was reviewed
by the local Washington University Institutional
Review Board and was determined to not be hu-
man participant research. All swabs used in this
study were flocked swabs (COPAN). To achieve
uniform inoculation, a single pool of SARS-CoV-2
positive UTM from previously tested nasopharyn-
geal swab specimens was used to inoculate
flocked swabs. The pool was tested by the Roche
cobas SARS-CoV-2 assay to confirm positivity prior
to specimen inoculation. Sixty (60) swabs were in-
oculated by submerging them in the UTM pool for
3–5 s. Each swab was then immediately placed in
a sterile 15-mL conical tube. Swabs were incu-
bated and tested as outlined next. On the day of
testing, dry swabs were eluted in 1 mL of liquid
media by submerging for 3–5 seconds without
vortexing. Swabs were left in the liquid media until
immediately prior to testing liquid media. Liquid
media was tested within 12 h of dry swab inocula-
tion in accordance with the manufacturer’s
instructions for the cobas SARS-CoV-2 assay.

**Dry Swab Incubation**

Twenty (20) swabs were used for testing stability
of viral detection in universal transport medium.
Five were placed immediately into UTM (COPAN),
following inoculation and tested immediately (day
0). The remaining swabs were placed into sterile
15-mL conical tubes and incubated at room tem-
perature. On the day of testing, each dry swab
was eluted in 1 mL of UTM by submerging for 3–
5 seconds without vortexing. Swabs were left in
liquid UTM prior to testing. Liquid UTM was tested
within 12 h of dry swab inoculation in accordance
with the manufacturer instructions. This entailed pipetting a 700 μL aliquot of liquid media into a secondary tube and loading the secondary tube into the automated Roche cobas 6800 instrument.

Testing was performed in sets of 5 at 3 different time points (day 1, day 2, and day 7). This protocol was repeated for VTM (Hardy) and saline (Becton Dickinson) (5 replicates at day 0, day 1, day 2, and day 7).

**Liquid Media Incubation**

To compare dry swab incubation to incubation in liquid transport media, additional swabs were inoculated into the pooled positive specimen, immediately hydrated in liquid media (UTM, VTM, or saline) and incubated at room temperature. Testing was performed on the liquid media at 1, 2, and 7 days postinoculation. This protocol was performed in duplicate for each liquid media.

**Molecular Testing**

All molecular testing was performed using the cobas SARS-CoV-2 assay (Roche). The cobas SARS-CoV-2 assay targets ORF1ab gene and E gene sequences of SARS-CoV-2. Both targets were utilized for comparison of CT results.

**Viral Culture**

Viral culture was used to determine the viability of SARS-CoV-2 following each incubation. Culture was performed on the initial SARS-CoV-2 pool used to inoculate swabs. Culture was also performed in triplicate from each media used to hydrate swabs following dry incubation at the day 0, day 1, and day 2 time points. Samples were serially diluted in DMEM supplemented with 2% FBS, then added to Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. All plates included serial dilutions of a positive control sample with known amount of virus to ensure successful detected live virus. Cells were then overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were incubated for 30 h before fixation using 4% PFA in PBS for 1 h at room temperature. Cells were washed then sequentially incubated with anti-SARS-CoV-2 CR3022 antibody (1 μg/mL) and an HRP-conjugated goat antihuman IgG (Sigma) in PBS supplemented with 0.1% (w/v) saponin (Sigma) and 0.1% BSA (6). TrueBlue peroxidase substrate (KPL) was used to develop the plates before counting the foci on a BioSpot analyzer (Cellular Technology Limited).

**RESULTS**

**Testing of Dry Swabs**

The positive pool used to inoculate swabs was positive with CT results of 19.4 and 19.9 for the ORF1ab and E gene, respectively. Testing of inoculated samples revealed qualitative positive percentage agreement by molecular testing of 100% for each set of 5 replicates at day 0, 1, 2, and 7 (Fig. 1). All dry swabs tested at days 1, 2, and 7 were within 2 CT of the average CT values for dry swabs tested at days 1, 2, and 7 on the cobas SARS-CoV-2 assay. Dry swabs were hydrated in the indicated media (UTM, VTM, or saline) at days 0, 1, 2, and 7 following inoculation into a pooled positive specimen. CT values for the ORF1ab gene (closed symbols) and E gene (open symbols) are shown as an average of 5 replicates. The standard deviation is indicated by the error bars. No statistically significant difference was observed (one-way ANOVA with Bonferroni correction) between groups within each gene target.
swabs hydrated in the same media and tested on day 0. No statistically significant difference was observed across all dry swab replicates based on one-way ANOVA with Bonferroni correction between groups within each gene target. The stability in liquid media was similar, with all day 1, 2, and 7 replicates within 2 CT of the average day 0 result (Fig. 2). Similar to dry swab replicates, no statistically significant difference was observed across all liquid media replicates between groups within each gene target. There was no statistical difference in CT results between swabs incubated in liquid media versus dry swabs incubated at room temperature prior to inoculation into liquid media. All dry swabs tested at days 1, 2, and 7 were within 2 CT of the average CT values for swabs tested following the same incubation time in liquid media.

**Viral Culture Results**

Positive and negative controls for all viral cultures yielded acceptable results. The only specimen positive by culture was the original pool used to prepare the swabs. Culture results for all dry swab replicates from the day 0, 1, and 2 specimens hydrated in UTM, VTM, and saline were negative. This was despite moderately low CT values, (between 23 and 26 for both assay targets). Culture results from liquid controls were also negative.

**DISCUSSION**

The ability to obtain robust CT values from dry swabs stored for a week at room temperature despite culture negativity has important implications for SARS-CoV-2 testing. Specimen collection has the potential to be simplified by removing the need for liquid transport media. Liquid media may not need to be included with specimen collection kits and providers would not have to hydrate swabs at the point of collection. This latter step may generating unnecessary waste and aerosols depending on swab type and media container used, therefore being able to perform hydration and testing away from specimen collection has important safety ramifications. Since many communities may have
limited amounts of specimen collection sites, simplification of specimen collection may allow better accessibility to patients. Since transport from limited collection sites may be prolonged, the data indicating that dry swabs are stable for at least a week are encouraging.

Our data indicate that rather than being utilized for specimen transport, liquid media could be used to simply hydrate swabs and release them of virus prior to testing following receipt at the testing laboratory. This allows these steps to be performed in a controlled environment within a biosafety cabinet. If the liquid media is being used to only hydrate the swab it may not need to include all the components necessary for long term stability. Our data showing a similar result from swabs hydrated in saline compared to UTM and VTM supports this. For assays utilizing their specific elution or lysis/inactivation buffer, there may be potential to directly inoculate the dry swab into assay specific buffer, bypassing the need for liquid media altogether and potentially alleviating any dilution of target that may occur in the process of eluting the swab in liquid media.

The use of dry swab specimens has important safety benefits as well. While specimens maintained robust CTs even following 7 days of dry incubation, virus was not cultivable. The inability to culture virus may have been secondary to dilution brought about by our experimental technique, though the robust CT values suggest the inability to culture was in part related to viral stability. This suggests a dry swab approach may have an ability to maintain diagnostic accuracy while minimizing exposure of laboratory workers to viable virus (7).

Our study has several limitations. We only evaluated stability around one specific CT value, so although our CT values did not show significant change this relationship may differ at higher or lower values. Furthermore, we did not evaluate extremes in temperature or atmosphere or other conditions that may affect stability, nor did we evaluate swabs other than the commonly used flocked versions. Finally, our swab specimens were prepared by preparation from a positive patient pool previously incubated in UTM. This technique was needed in order to assure uniform inoculation of swabs for comparator purposes. However the stability of patient specimens collected directly from the nasopharynx or oropharynx may differ and warrant additional investigation. Any of these variables could dramatically affect the performance of testing from “dry swabs” and require additional study prior to implementing this method.

Herein we describe a novel technique for the collection and transport of specimens for SARS-CoV-2 testing. The utilization of “dry swabs” has the potential to simplify specimen collection, negate the need for liquid transport media, and mitigate safety risks, all while preserving the accuracy of testing.

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References

1. Rogers AA, Baumann RE, Borillo GA, Kagan RM, Batterman HJ, Galdzicka MM, Marlowe EM. Evaluation of transport media and specimen transport conditions for the detection of SARS-CoV-2 by use of real-time reverse transcription-PCR. J Clin Microbiol 2020;58:e00708–20.

2. Binnicker MJ. Emergence of a novel coronavirus disease (COVID-19) and the importance of diagnostic testing: why partnership between clinical laboratories, public health agencies, and industry is essential to control the outbreak. Clin Chem 2020;66:664–6.

3. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, et al. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol 2020;58.;

4. Smith KP, Cheng A, Chopelas A, DuBois-Coyne S, Mezghani I, Rodriguez S, et al. 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. J Clin Microbiol 2020;58:e00913–20.

5. Quidel. Lyra SARS-CoV-2 assay specimen collection guide. PCM120000EN00.

6. Yuan M, Wu NC, Zhu X, Lee CD, So RTY, Lv H, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science 2020;368:630–3.

7. Huang C-G, Lee K-M, Hsiao M-J, Yang S-L, Huang P-N, Gong Y-N, et al. Culture-based virus isolation to evaluate potential infectivity of clinical specimens tested for COVID-19. J Clin Microbiol 2020;58:e01068–20.