Swab-Free Transport as an Optimized Preanalytical Workflow for SARS-CoV-2 Molecular Testing

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The emergence of SARS-CoV-2 (COVID-19) and the global ramp up of molecular diagnostic testing has resulted in an enormous strain on both testing supply and personnel resources. Early into the pandemic, consumables such as swabs, pipette tips, and transport media became challenging to obtain, thereby necessitating changes in test protocols and laboratory workflows. Clinical laboratories and diagnostic companies have stepped up to the challenge by validating alternative transport media and devices, developing alternative types of swab, evaluating unique specimen types and pooling methods, and utilizing nontraditional automation systems to meet the enormous need for testing (1–3). While these efforts have allowed for testing to expand, the methods in place are not always those most optimal for laboratory workflows.

In this issue of *The Journal of Applied Laboratory Medicine,* Lockwood et al. describe the requirement for manual preanalytical specimen processing steps that are typical of many molecular SARS-CoV-2 assays with Emergency Use Authorization (EUA) (4). Manual processing requirements demand extreme care to avoid cross-contamination and specimen errors and involve repetitive motions, generally within a restrictive biosafety cabinet. While not individually time consuming (~1 minute per sample), for laboratories performing large-scale testing these manual activities can increase personnel requirements considerably. Lockwood et al. estimate that this extra processing adds 1 FTE per 500 samples tested. If a laboratory is fully utilizing some of the common high-capacity molecular instruments, this could result in up to 3 additional people needed per instrument per day.

High-capacity molecular instruments with automated liquid handling systems have decreased hands-on time and streamlined workflows of traditional swab-based molecular assays for pathogens such as *Neisseria* and *Chlamydia.* However, manual specimen processing requirements exist for many EUA SARS-CoV-2 assays run on the same test instruments. Depending on specimen type, these requirements include removal of collection swabs from transport media (Abbott m2000, Abbott Alinity m) or aliquot of specimen into a swab-free assay tube (Hologic Panther Fusion, Hologic Aptima, Roche 6800) before placement on the instrument (5). These requirements are included because the presence of a swab, and the size or shape of a transport container may create an obstruction to the automated systems. This interference may result in mechanical damage to the instrument, loss of sample, or inaccurate test results. As an example, the Roche

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6800 Instructions for Use require an aliquot to an instrument compatible secondary container for specimens received in Universal Transport Medium, Universal Viral Transport, 0.9% physiological saline, and cobas® PCR Media. The aliquot step is necessary for a variety of reasons including: potential for pipetting obstruction due to glass beads, potential for tube geometry interference with tube handling (ex. rotation and aspiration steps), and potential for increased clot detection due to the presence of mucus/swab material in the primary specimen. Specimens collected with the cobasPCR Media Uni Swab Sample Kit and cobasPCR Media Dual Swab Sample Kit can be placed directly on the instrument with no swab removal or aliquot. Direct testing can occur because the collection kit and included collection swabs have been verified by the vendor (personal communication with Roche Diagnostics).

With such a wide variety of collection products in use, it is challenging for vendors to ensure acceptability of instrument performance with all swab and transport container combinations on the market. Some vendors' branded specimen collection devices with known instrument compatibility are available but not in sufficient supply to meet demand. If vendors cannot validate or supply collection kits necessary to avoid additional processing steps, then the laboratory is left with few alternatives. If swab removal (and not aliquot) is the necessity, one option is to obtain transport media containers with swab capture caps. These caps grasp the swab allowing the cap and swab to be removed and discarded as one unit. Unfortunately, these transport containers also remain in short supply. Without capture caps, swab removal requires using forceps or some other sterile method to remove and discard the swab from each specimen container. Due to the size and shape of swabs, containers, and forceps, swab removal may be prone to contamination or not easily performed. If swab removal is not possible then aliquoting becomes necessary.

As an alternative to laboratory-based processing, Lockwood et al. suggest a simple point of collection protocol where the swab is rotated for 10 seconds in a transport media with viral inactivation properties. Since elution of the sample from the swab into transport media occurs at the point of collection the swab could be immediately discarded on site. Point of collection elution is not the current practice for swab-based infectious disease culture or molecular sample processing. Swab-based testing typically employs a vortex step during specimen processing that is thought to be necessary to release material captured in the swab and to generate an even suspension. Due to aerosol exposure risk, significant manipulation of samples including pipetting or vortexing at the point of collection is undesirable. However, as suggested by the authors, the use of a transport media with inactivation properties could reduce the safety risk. These types of product contain chemicals or detergents that may significantly decrease infectious virus levels (6).

To evaluate the potential for point of collection processing, Lockwood et al. first examined elution of contrived SARS-CoV-2 swab specimen into Hologic lysis buffer for 5, 10, 15, 20, and 25 seconds. No significant change was seen in the level of positivity over the elution time course. The authors also examined the elution from contrived positive swab specimens into media containing lysis buffer only, lysis buffer plus normal nasal swab matrix, and lysis buffer plus a high level of mucus. Cycle thresholds (Ct), which correlate to the quantity of RNA present in the samples, were compared between the original sample and the sample diluted in the 3 matrices. After correction for dilution, a negligible difference in the Cts between the original samples and the contrived lysis buffer samples was observed. This analysis addressed mucus present in eluent solution but does not mimic a real-world scenario where mucus and other human material is present on the swab itself.

To examine the elution from swabs containing human matrix, 28 patient volunteers were asked to collect 2 nasal swab specimens. One swab was placed into transport media following the standard laboratory protocol, and one swab was rotated in lysis buffer for 10 seconds before discarding at the collection site. For the 2 patients positive with SARS-CoV-2, there was not considerable difference in the cycle threshold values between collection methods (+2.5 Ct for one patient and −2.9 Ct for the second patient). The remaining 26 samples were negative, so the impact of the change in processing method cannot be evaluated. Following the same protocol 6 healthy volunteers self-collected paired nasal swabs and PCR analysis of a human material in the specimen (RNase P) was performed. Little difference in the levels of RNaseP was seen when comparing specimen processing methods. Although limited numbers of positive patient samples were examined, preliminary studies with the setup used by these authors (Hologic lysis buffer, Puritan flocked swab, 10 second rotation) suggest that the transport of the swab to the laboratory and the additional laboratory personnel needed to remove the swabs may be unnecessary.

As the authors note, evaluating swab-free transport would be complex for laboratories employing multiple different swab types, collection devices, and testing instruments. Swab composition and physical properties may impact the amount of specimen collected and released, which then may affect the ability to eliminate the laboratory-based processing steps (7). Flocked swabs were used for the analysis described in this article and are traditionally the swab of choice for respiratory virus sampling. Flocked swabs have short perpendicular fibers covering the swab tip like a brush, rather than traditional spun material that is tightly wrapped around the tip. The flocked conformation is thought to provide a better specimen collection and better release of specimen into liquid media for testing (8, 9). Laboratories accepting traditional spun, flocked, and sponge swabs from multiple vendors would need to perform extensive verifications to ensure acceptability with all options. Additionally, given that many molecular based SARS-CoV-2 (COVID-19) assays do not contain a human specimen control, it is important that laboratories visually confirm the presence of a swab. In the absence of a swab, there is no guarantee that a specimen has indeed been collected and is consistent with the specimen selection on the test order.

Given the shortages in skilled clinical laboratory workforce, decreasing the amount of manual processing is important for all areas of the laboratory (10). Eliminating the necessity for swab removal and specimen aliquoting allows for best use of the automated instruments that laboratories have historically relied on to provide high throughput testing with minimal employee intervention. Vendors struggling to meet the demand for test reagents must also keep in mind that the production of collection supplies compatible with their instruments is critical to efficient workflows. If adequate elution of sample material into transport buffer can be safely and consistently achieved at the point of collection, it would be a welcome change for laboratories utilizing technologies that necessitate swab removal.

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