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Chapter

The Utility of Mechanical Homogenization in COVID-19 Diagnostic Workflows

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Abstract

The use of mechanical homogenization in patient sample preparation for COVID-19 diagnostics has proven impactful in the face of the global pandemic caused by SARS-CoV-2. Through methods including bead beating and shaker mill homogenization novel approaches to viral detection have been developed and improvements have been made to existing diagnostic workflows for the improvement of throughput and automation capacity. The application of mechanical homogenization techniques has enhanced the sensitivity and methodology for many molecular based approaches to COVID-19 detection and from a variety of sample types ranging from saliva to nasopharyngeal swabs. Additionally, this technology has been used to help increase laboratory safety during sample processing through efficient viral lysis. Herein, the many benefits of mechanical homogenization for COVID-19 detection will be discussed in the context of the many diagnostic workflows currently utilizing the technique.

Keywords: PCR, viral diagnostics, viral detection, antigen diagnostics, antigen detection, molecular diagnostics, COVID-19, homogenization, sample preparation, bead mill, shaker mill, bead beater

1. Introduction

Traditionally the process of mechanical homogenization has been employed in the laboratory setting for the disruption of animal or plant tissues in preparation for downstream molecular applications [1]. However, in the face of a global pandemic this technology has been adapted to increase efficacy and efficiency in viral detection in a variety of COVID-19 diagnostic workflows [2–4].

As the global community began to respond to the spread of SARS-CoV-2, the expansion of public health surveillance programs and community testing protocols became critical objectives [5]. However, the need for rapid expansion in testing capacity caused a tremendous strain on the supply chains providing the equipment and reagents traditionally needed for respiratory virus PCR-based testing [6]. As in most cases, necessity drove innovation. Given the large number of research and academic laboratories equipped to assist in PCR testing, many groups began to offer their assistance in processing patient samples while others began examining novel approaches to viral detection which circumvented the supply chain bottle
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necks. During the development of these novel testing protocols laboratory safety, diagnostic assay sensitivity and specificity became top priority [5–7]. In an attempt to utilize common laboratory equipment to safely speed up testing efforts, the use of mechanical homogenization was proposed to inactivate the SARS-CoV-2 from nasopharyngeal swabs as a method of increasing safety during processing [2–4, 8].

In brief, mechanical homogenization is the process of using shearing forces applied via mechanical grinding media and rigorous repetitive motion to dissociates a given sample [1]. The parameters at which a sample is processed will impact the degree to which it is dissociated and the quality of the targeted product for downstream applications [1]. In the case of SARS-CoV-2, the goal of mechanical homogenization was to disrupt the viral envelope while still maintaining the integrity of its RNA [2, 3]. This allowed for a reduction in infective potential in the laboratory setting, while preserving the accuracy of polymerase chain reaction (PCR) based diagnostic assays [2, 3].

Following the initial application of mechanical homogenization to COVID-19 swab-based PCR protocols, this technology was adapted to process saliva samples for both antigen and PCR detection workflows [2–4]. Through mechanical homogenization, high viscosity saliva samples were sufficiently processed to allow for automation integration, paving the way for the widespread application of this novel methodology [4, 9].

In this chapter we will further explore the applications of homogenization in response to the COVID-19 pandemic and the multiple diagnostic methodologies this technology has been implemented in and its impact on laboratory safety and overall testing efficiency.

2. Direct-to-PCR testing with shaker mill homogenization of nasopharyngeal swabs

During the late spring of 2020, while SARS-CoV-2 was spreading exponentially and uncontrollably across the globe, testing for this disease was focused entirely on RT-qPCR detection of the virus using US CDC or WHO approved primers [5, 10]. The traditional method for these types of RT-qPCR tests involved two major components. First, the process of virus inactivation and RNA extraction completed through a series of chemical reactions that resulted in purified viral RNA from the provided patient sample [5, 6, 10]. The extracted RNA was then utilized in the second half of this method, amplification and detection [5, 6, 10]. Through RT-qPCR, the purified RNA from the patient sample was combined with the preapproved primers for attempted amplification of the targeted genes, indicating the presence or absence of SARS-CoV-2 depending on the level of amplification seen [5, 6, 10]. The RNA amplification was quantified and reported out as a Cq value, with any Cq less than 40 qualifying as a COVID-19 positive sample per the US CDC and WHO guidelines [10].

The necessity of testing drove up demand for all reagents, machines, and plastics utilized in the RT-qPCR testing method, overstressing the supply chain for these products [5, 8, 9]. Additionally, the need for cold storage of reagents involved in the extraction process and the high price tag on the automated machinery needed to complete both the extraction and detection phases of the traditional testing method, furthered the gap between resource challenged areas and the industrialized regions when it came to COVID-19 testing infrastructure [11, 12]. Areas with the capital needed to create multimillion dollar testing facilities were able to do so, improving their public health response to the pandemic, while those lacking that investment and infrastructure were left with reduced testing capabilities [11]. A critical need
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arose for a cost efficient, yet safe and effective testing methodology that could be implemented in these resource challenged settings [8, 11].

While the utility of mechanical homogenization in COVID-19 testing was already established as an effective adjunct to the extraction process, improving sensitivity through efficient viral lysis, this process was expanded upon in an attempt to remove the extraction process entirely allowing for direct detection of SARS-CoV-2 from lysed patient samples [2, 3]. The direct-to-PCR approach for COVID-19 testing arose out of necessity to reduce the use of costly reagents in a period where the strain on the supply chain made them difficult to come by [2, 3, 8]. Additionally, this proposed method dramatically reduces cost when compared with the fully automated extraction machinery [2, 3, 8].

In the direct-to-PCR method for viral detection, shaker mill mechanical homogenization was proposed to provide sufficient viral lysis off nasopharyngeal swabs to expose adequate amounts of RNA for RT-qPCR detection [2, 3]. This method was shown to lyse greater than 95% of virus off a nasopharyngeal swab, allowing the resultant lysate to be placed directly into the RT-qPCR reaction as denoted in Figure 1 [2, 3].

Through proof-of-concept testing with a close relative of SARS-CoV-2, human coronavirus 229E (HCoV-229E), and direct comparison studies between the traditional extraction-based method and the direct-to-PCR method, it was shown that the two methods had above a 94% agreeability in the detection of positive samples [2, 3]. Utilizing the direct-to-PCR method diagrammed in Figure 1, shaker mill homogenization was proven to be a viable alternative to the traditional extraction-based method for RT-qPCR detection of SARS-CoV-2 off nasopharyngeal swabs [2, 3].

In addition to the quality of the matched proven efficacy with the traditional, extraction-based methodology, the direct-to-PCR method described utilizing mechanical homogenization also reduces the total cost and time per swab processed [2, 3, 8]. The traditional model for nasopharyngeal swab viral testing cost $10 - $40 USD per swab, when taking into account the extraction kits, automation equipment for extractions, and the RT-qPCR set up [8]. Compared to $3 - $5 USD per swab with the homogenization methodology, given that this workflow does not require additional reagents for viral nucleotide extraction and purification, the only reagent costs are associated with the final RT-qPCR testing [8]. The homogenization equipment utilized in this workflow is sold at a fraction of the cost of the large fully automated extraction machinery.

Along with reducing cost per sample the homogenization workflow reduces the total processing time per sample from approximately 3 hours to 1 hour and 15 minutes [2, 3]. This is accomplished through replacing the extraction and purification steps of the traditional workflow with a 30 sec homogenization step preceding the RT-qPCR [2, 3]. Further supporting the implementation of this workflow into

![Figure 1](image)

*Figure 1.*  The direct-to-PCR viral detection methodology using shaker mill homogenization off nasopharyngeal swabs.
the COVID-19 testing repertoire to assist in increasing access to cost effective and timely viral detection methods that maintain sensitivity and specificity when compared to the traditional testing methodologies [8].

3. PCR detection of COVID-19 from saliva utilizing bead beating homogenization

After months of nasopharyngeal swabbing for COVID-19 diagnostic testing, there was a push to look for equally sensitive testing methodologies which provided a more pleasant patient experience during sample collection [4, 6, 9]. By improving the patient experience with testing, the hope was to gain public cooperation with viral surveillance efforts [9, 13]. The high concentration of SARS-CoV-2 particles found throughout the upper respiratory tract led researchers to begin examining the utility of oral swabs or saliva in the current RT-qPCR testing strategies [13, 14].

Saliva samples were shown to have adequate viral loads for reliable RT-qPCR detection, however the high viscosity of the samples made them difficult to pipette preventing the utilization of the fully automated extraction machinery already in place in many large public health testing facilities [9, 13, 14]. Mechanical homogenization in the form of bead beating homogenization was introduced to saliva samples to break up the viscous structure and expose the viral particles [4, 15]. The bead beating strategy utilized ceramic bead media within a 2 mL screw capped sample tube and a mechanical homogenizer to apply rigorous kinetic energy to the saliva sample for 30 seconds to achieve complete dissociation (Figure 2) [4, 15]. It was shown that the kinetic energy transferred from the bead beating media homogenized in a sigmoidal pattern was highly effective in dissociating the sample to allow for pipettable lysate that could then be implemented into fully automated extraction-based PCR testing workflows [1, 4, 15]. With the addition of bead beating homogenization to this workflow, the throughput and sensitivity of the assay were dramatically increased [4, 15]. Prior to the implementation of bead beating homogenization, saliva-based testing demonstrated a sensitivity in the mid to low 80% range and throughput was limited to a few hundred samples per day via manual processing [4, 15]. Currently, saliva-based PCR testing utilizing mechanical homogenization prior to extraction procedures demonstrated a 95% sensitivity and 99% specificity, closely matching that of nasopharyngeal swab-based testing for COVID-19 [4, 13–15]. Additionally, with the capability of full automation integration, throughput of sample processing increased from hundreds to thousands of samples per day with the utilization of bead beating homogenization equipment [4, 15].

Figure 2.
The methodology for saliva-based testing utilizing bead beating mechanical homogenization for adequate sample dissociation and viral lysis followed by RNA extraction for RT-qPCR viral detection.
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Given the similar sensitivity and specificity for SARS-CoV-2 testing, with the improvement in patient experience during sample collection, this method was implemented at universities across the state of Georgia as a community surveillance program [4, 15]. The difference in patient experience from obtaining a nasopharyngeal swab versus a saliva sample for weekly surveillance measures dramatically improved community compliance with testing, validating saliva testing as a viable public health surveillance strategy for COVID-19 propagation in a community [4, 6, 13–15]. Similar entities have now implemented saliva-based testing that utilize front-end mechanical homogenization across the world to improve compliance with public health testing efforts [4, 6, 15].

4. Viral antigen detection from saliva

The United States’ Food and Drug Administration (FDA) has approved the use of antigen testing for the detection of SARS-CoV-2 in the summer of 2020 to increase the national testing capacity [6, 16]. Antigen tests are immunoassays that are commonly used in the diagnosis of respiratory pathogens such as influenza [17]. Antigen test are designed to detect the presence of a specific viral antigen, which is defined as a toxin or other foreign substance which induces an immune response [16, 17]. Antigen test are currently approved for nasopharyngeal swab specimens however studies suggest saliva samples may be just as effective in detecting viral antigens [6, 16, 18]. When compared to PCR test, antigen testing is relatively inexpensive, and most test results are available in 15 minutes or less. Antigen test in general are less sensitive than RT-PCR test as well as other nucleic acid amplification test (NAATs) [19]. Alternatively, RT-PCR can amplify and detect minute levels of nucleic acid that cannot be cultured which in this case suggest the presence of viral nucleic acids does not signify contagiousness [20]. Both antigen and nucleic acid tests are optimal when the patient is at their viral load peak [6, 16, 20]. More data is needed to guide the use of antigen tests on asymptomatic individuals and to determine if those who were at one time diagnosed positive for SARS-CoV-2 remain infectious.

The advantage of antigen testing is its convenience and accessibility in the use screening high-risk congregate settings such as primary or secondary educational environments, as well as correctional facilities [16, 21]. Repeat testing could quickly identify infection, therefore allowing implementation of patient quarantine and other preventive measure. However, health care professionals need to understand the limitations of antigen testing [19, 20]. Specifically, the testing factors and analytical performance characteristics, such as sensitivity, specificity, and accurate positive and negative predicted values. The “Holy Grail” for SARS-CoV-2 testing remains to be RT-PCR or some form of nucleic acid amplification testing [21]. Nucleic acid testing should be used to confirm an antigen test to avoid inconsistent and inaccurate results test performance may vary based on specimen choice, quality of specimen, the presence of transport medium, and the amount of time required for transport [20, 21]. Since antigen test are typically less sensitive than NAAT testing, negative results can occur while RT-PCR tests may return a positive result [19]. This may occur is specimen sample is collected early before symptom onset or late in the infection [19, 20]. The specificity of antigen tests is as high as NAAT testing, reducing the likelihood of false positives [16, 21]. False positive will still occur, particularly in communities where prevalence of infection is low [16, 20, 21]. The CDC recommends testing professionals establish infection prevalence for antigen testing based on a rolling average, using the positivity rate of their own SARS-CoV-2 testing over the previous 7–10 days, while considering the clinical and epidemiological context of the person or community being tested [6, 16].
Despite the debated advantages and disadvantages of antigen testing, the concept of saliva-based antigen testing for SARS-CoV-2 detection gained traction due to the ease of use for the patient and potential for rapid turn around time in laboratory processing to support public health efforts. However, as noted in the previous section on saliva-based PCR testing, working with such a viscous material posed difficulty in automation integration prior to the addition of homogenization into the workflow. Antigen testing faced similar difficulties when using saliva for large scale testing, the viscous patient samples required further processing prior to automation integration.

In an attempt to mitigate variations in saliva viscosities and allow for sample integration into high-throughput liquid handler reliant workflows, several protocols were developed to dissociate the saliva samples while maintaining intact antigen for detection [6, 22]. These protocols recommend various combinations of heating and enzyme digestion; heating greater than 60 degrees centigrade for as long as an hour or incubating with Proteinase K as an enzymatic digestion [23]. Reports have found these techniques to be somewhat effective in permitting antigen detection from saliva samples, however inconsistencies have also been scored [18, 20, 23]. Heating can denature the viral proteins and RNA, rendering them undetectable, and enzymes such as Proteinase K is very costly as well as cause degradation of targeted proteins through excessive digestion [20]. Not to mention the suggested incubation periods as great as an hour extends the amount of time required to have a patients’ result.

Just as with saliva-based PCR testing, homogenization was proposed as a method for efficient sample disruption [24]. Viscosity in homogenized saliva samples has been shown to be greatly reduced to amounts that are similar to those found in water. Allowing for ease in pipetting and increase throughput using automation and liquid handlers [15]. The various forces found in homogenization are only required for small amounts of processing time, as short as 5 seconds per sample without generating any extra heat during the processing, maintaining the integrity of the antigens targeted. In contrast to other proposed methods for saliva processing in antigen detection, additional enzymes are not required, saving costs and without any needed incubation steps, also saving valuable time during testing.

5. Improving laboratory safety with homogenization

During the COVID-19 global pandemic, safety of all individuals involved in the care of COVID-19 patients as well as laboratory and clinical staff involved in testing for SARS-CoV-2 became a top priority. Given the highly virulent nature of SARS-CoV-2 and the lack of knowledge and treatments we had available, it was essential to neutralize the virus during laboratory testing while preserving the diagnostic capacity of all assays [5]. Employing viral neutralization techniques in the diagnostic workflow was a critical step in increasing the number of facilities available to process COVID-19 patient samples, supporting increased public health testing efforts.

Techniques involving thermal inactivation, chemical neutralization or degradation, enzymatic digestion, and mechanical disruption of samples were all proposed as potential solutions to laboratory safety when handling potential COVID-19 positive patient samples [5, 6]. However, given the global strain on the plastics and chemical reagents needed to complete many of these neutralization steps, the authors felt it was prudent to examine the potential of mechanical sample dissociation in the form of homogenization and its effect on virus neutralization [2, 3, 8]. Ultimately, it was shown that following 30 seconds of homogenization, 98% of the
virus in any given sample was inactivated, while still preserving the genetic material for adequate PCR detection [2, 3]. This finding supported expanding the implementation of homogenization in the COVID-19 diagnostic workflow because it could be done both in the laboratory setting, as well as the location of sample collection provided the homogenized sample would be properly refrigerated and transferred for PCR detection within the next 12 hours [2–4, 15].

The mechanical lysis of the SARS-CoV-2 particles in a potentially infectious sample permitted these samples to be processed in a BSL-2 facility, supporting the expansion of laboratory testing facilities equipped to process COVID-19 samples [2, 3, 8]. Without a proven neutralization step, such as mechanical homogenization, all COVID-19 samples would have to be processed in BSL-3 facilities due to the potential risk of exposure to infectious virus. While it is still recommended that the homogenization procedure occur in a biosafety cabinet within a BSL-2 facility, the procedure provides sufficient viral lysis to improve safety when handling potentially infected patient samples and allows additional laboratories to assist with testing in a cost-effective manner [2, 3, 8, 15].

6. Conclusion

Mechanical homogenization has proven its utility in the response to COVID-19 through shaker mill and bead beating technologies implemented in a variety of diagnostic workflows [2–4, 8, 15]. These innovations made possible through effective and efficient viral lysis of SARS-CoV-2 are proving to improve access, speed, and safety while processing patient samples [2–4, 8, 15]. As the global community continues to push innovation to combat COVID-19, mechanical homogenization should be viewed as one of the many repurposed technologies adapted to assist with the response through improving the safety and efficacy of diagnostic testing in a cost-effective manner.

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Conflict of interest

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**Acronyms and abbreviations**

- PCR: polymerase chain reaction.
- RT-PCR: reverse transcriptase polymerase chain reaction.
- RT-qPCR: quantitative reverse transcriptase polymerase chain reaction.
- WHO: World Health Organization.
- FDA: Food and Drug Administration.

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