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Speeding Up COVID-19 Detection Using Shaker-Mill Homogenization and a Direct-to-PCR Workflow

Zachary P. Morehouse[1,2,**], Caleb M Proctor[2,3], Gabriella L Ryan[2,3], Rodney J Nash[2,3,4,*]

1. Michigan State University College of Osteopathic Medicine, East Lansing MI, USA; moreho17@msu.edu (Z.P.M.)
2. Omni International Inc, Kennesaw GA, USA; moreho17@msu.edu (Z.P.M.), cproctor@omni-inc.com (C.M.P.), gryan@omni-inc.com (G.L.R.), rnash@omni-inc.com (R.J.N.)
3. Georgia State University, Department of Biology, Atlanta GA, USA; cproctor@omni-inc.com (C.M.P.), gryan@omni-inc.com (G.L.R.), rnash@omni-inc.com (R.J.N.)
4. JeevanBiosciences, Tucker GA, USA; rnash@omni-inc.com (R.J.N.)

* Corresponding Author, rnash@omni-inc.com, tel: +1 770-421-0058
** Co-Corresponding Author, moreho17@msu.edu, tel: +1 813-476-5661

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Abstract

Accurate and timely testing has become an essential measure in combatting the COVID-19 global pandemic. Currently, polymerase chain reaction (PCR) based assays are the most relied on methods for SARS-CoV-2 detection. This traditional workflow involves a viral RNA extraction from the viral transport media storing nasopharyngeal swabs collected from patients, followed by PCR based detection. While accurate, this methodology is time consuming and resource heavy, causing for delays in receiving results or limited access to testing. Herein, we demonstrate a validated method for SARS-CoV-2 detection from viral transport media using a two-step, direct-to-PCR workflow revolving around shaker-mill homogenization. This method completely bypasses the extraction steps of the traditional workflow, replacing it with 30 seconds of mechanical disruption sufficient to allow for COVID-19 detection with a 96.43% sensitivity and 100% specificity when compared to traditional extraction to PCR based methods.

Key Words (3 – 10):

COVID-19, Virus Detection, Diagnostics, SARS-CoV-2, Coronavirus, Viral Diagnostics, PCR

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1. Introduction

Amid the COVID-19 pandemic, the critical need for efficient and cost-effective viral detection methods has been amplified globally [1,2]. Currently, the most relied on method for SARS-CoV-2 detection is a standard viral RNA extraction procedure followed by a polymerase chain reaction (PCR) based assay looking to detect a specific gene product from replicating viruses off patient acquired nasopharyngeal swabs [2,3,4]. This methodology has been proven time and time again to be effective in respiratory virus detection from patients actively infected with everything from influenza to SARS-CoV-2 [3,4,5]. However, this process of extraction followed by PCR based detection requires significant amounts of chemical reagents, consumable plastics, and laboratory man-hours to complete. These costs of both capital and time, potentially cause delays in patients receiving their results, and the possibility of degradation of viral transcripts within the storage media impairing detection [6,7]. Shortages in the supplies required for adequate viral detection and significant delays in patient notifications following nasopharyngeal swab collections have both been attributed to the exponential growth of COVID-19 cases in the United States in June and July of 2020 [8].

Herein, we are proposing a validated methodology for SARS-CoV-2 detection from viral transport media (VTM) storing patient derived nasopharyngeal swabs, which completely bypasses the extraction portion of the classic workflow described above. This novel process employs shaker-mill homogenization to mechanically lyse the virus particles in the transport media, permitting the lysate to be directly transferred into a PCR based assay for detection. Taking a total of 30 seconds of processing prior to the sample being added to PCR detection assays, this method allows patient samples to be processed directly in the viral transport media (VTM) they are stored in following patient sampling, significantly reducing the resources invested and time spent on processing each sample for virus detection.

2. Materials and Methods

2.1 Samples Used for Method Validation

The samples used for this method validation were obtained via a materials transfer agreement with Emory University's School of Medicine and their Clinical Virology Research Laboratory (Atlanta, GA, USA). These samples were obtained with informed patient consent under and following the protocols approved through the Emory University Institutional Review Board (IRB) (IRB Contract 000001082) and conducted following the rules of the Declaration of Helsinki of 1975.

Each patient provided two nasopharyngeal swab samples. One of the samples was validated as COVID-19 positive or negative by the CLIA approved laboratory at Emory University's Clinical Virology Research Laboratory following the traditional, US Centers for Disease Control and Prevention approved extraction and PCR based detection protocols. The second sample from the same patient was then deidentified and labeled as only COVID-19 positive or negative prior to transfer to our laboratory. 58 samples were transferred, 30 confirmed COVID-19 and 28 confirmed COVID-19 positive patient samples. The samples remained stored in their original viral transport media (Fisher, Cat. No. 23-001-726) and collection tube which they were placed in at the time of acquisition and were frozen at -80°C for storage prior to transfer.

2.2 Patient Sample Processing

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Patient samples stored in viral transport media were thawed in their original collection tubes at 24°C for 1 hr, or until VTM had completely thawed. The swabs and 1 mL of VTM were then transferred to a 2 mL screw cap tube (Omni International Inc., Cat. No. 19-647) and sealed [9]. 24 samples were loaded into the Omni Bead Ruptor Elite (Cat. No. 19-040E) for processing. The samples were run at 4.2 m/s for 30 seconds and removed from the device [9]. The samples were permitted to sit for 1 minute after processing to allow for any froth that formed in the tubes during shaker-mill homogenization to settle [9].

2.3 RT-qPCR Detection of SARS-CoV-2

1 µL of lysate was transferred into a premixed RT-qPCR plate from the 2 mL screw cap tube containing the nasopharyngeal swab in VTM following shaker-mill homogenization. The RT-qPCR reaction plate was premixed with New England Biolab's Luna Universal Probe Based One-Step RT-qPCR Kit (Cat. No. E3006) and US CDC approved primers and probes for the nucleocapsid (N) gene purchased from approved vendor, Integrated DNA Technologies (Coralville, IL, USA). The 2019-nCoV_N1-F primer (GAC-CCC-AAA-ATC-AGC-GAA-AT), the 2019-nCoV_N1-R primer (TCT-GGT-TAC-TGC-CAG-TTG-AAT-CTG), and the 2019-nCoV_N1-P probe (FAM-ACC-CCG-CAT-TAC-GTT-TGG-TGG-ACC-BHQ1) sequences were all obtained directly from the US CDC website [10,11]. The RT-qPCR reaction was premixed following vendor guidelines and 1 µL of lysate was added to bring the reaction to a final volume of 20 µL. The reaction was then loaded into the CFX Connect Real Time PCR Detection System (BioRad, Cat. No. 1855200) and run with the vendor recommended temperature and cycle timing for a total of 45 amplification cycles. Cq values were recorded and any sample with a Cq value less than or equal to 40 was labeled as positive for COVID-19 detection based on the US CDC recommended analysis of COVID-19 probe based RT-qPCR results [10,11] (Figure 1, Table 1).

Sample Number	Traditional Testing Status	Cq Value	Mean	Standard Deviation
1	Positive	24.93	29.94	5.44
2	Positive	38.94		
3	Positive	37.25		
4	Positive	22.11		
5	Positive	36.57		
6	Positive	37.78		
7	Positive	29.18		
8	Positive	27.44		
9	Positive	32.05		
10	Positive	22.50		
11	Positive	31.33		
12	Positive	37.60		
13	Positive	28.78		
14	Positive	36.51		
15	Positive	22.58		
16	Positive	35.46		
17	Positive	34.70		
18	Positive	31.20		
19	Positive	31.26		
20	Positive	N/A		

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21	Positive	22.79		
22	Positive	24.37		
23	Positive	23.10		
24	Positive	24.15		
25	Positive	32.84		
26	Positive	26.11		
27	Positive	27.84		
28	Positive	29.04		

Table 1. RT-qPCR results following shaker-mill homogenization represented as Cq values from VTM containing patient samples that tested positive for COVID-19 when tested with the traditional extraction to PCR methodology. Only one sample, sample 20, was not detected as COVID-19 positive after shaker-mill homogenization processing from a previously confirmed COVID-19 positive patient. The 30 COVID-19 negative samples are not shown because 0 of the 30 produced a Cq value following RT-qPCR.

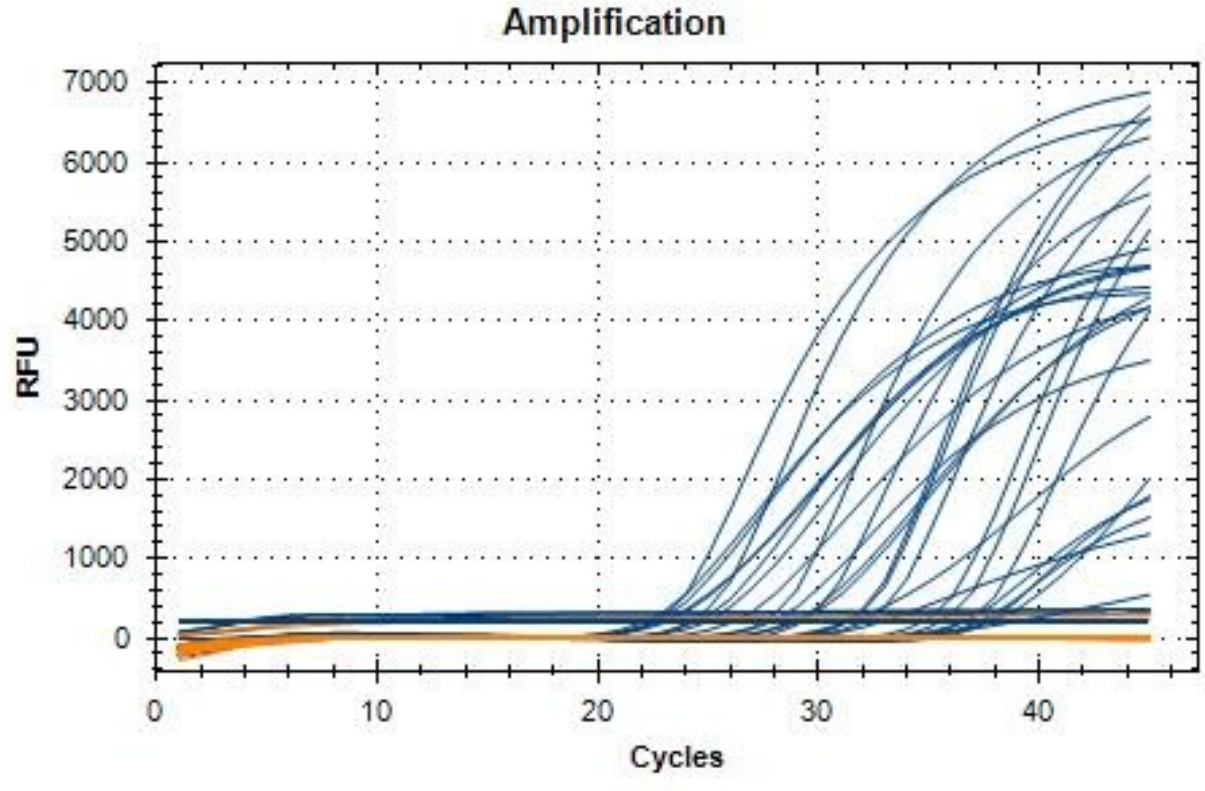

Figure 1. RT-qPCR results detecting the SARS-CoV-2 nucleocapsid gene off nasopharyngeal swabs processed via shaker-mill homogenization. Blue lines, VTM containing patient samples from COVID-19 positive patients processed via shaker-mill homogenization. Orange lines, VTM containing patient samples from COVID-19 negative patients processed via shaker-mill homogenization.

2.4 Statistical Comparison of COVID-19 Detection Methodologies

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The positive or negative COVID-19 status of each sample as determined by the traditional processing method used by the Emory University laboratory was used as the baseline for our analysis of each sample. Following sample processing conducted via our novel methodology, we compared the positive or negative status obtained on each sample to the predetermined status in order to analyze the sensitivity and specificity when compared to the current extraction to PCR based testing model.

3. Results

With the use of shaker-mill homogenization on the Omni Bead Ruptor Elite, we were able to sufficiently lyse SARS-CoV-2 out of patient samples stored in VTM with the resulting lysate providing sufficient genetic material for RT-qPCR detection of the viral N gene. This data validates our novel two-step, direct-to-PCR approach for detecting COVID-19 off nasopharyngeal samples when run using the parameters described in this manuscript. With 27 of 28 COVID-19 positive samples being detected as positive following our methodology, and 0 of 30 COVID-10 negative samples detecting as positive, this detection method provides a 96.43% sensitivity and 100% specificity when compared to traditional extraction to PCR based methods (Figure 1, Table 1).

The 27 COVID-19 positive samples had an average Cq value of 29.94, with a standard deviation of 5.44 (Table 1). This average Cq, falls 10 cycles below the US CDC cut-off Cq of 40 recommended for a COVID-19 positive detection using these N gene primers [10,11,12]. The wide standard deviation associated with these samples can be attributed to a variety of factors such as the variability of each patient's viral load, the quality of the swabbing procedure when obtaining each sample, and the inhibitors present in the VTM when going directly to RT-qPCR reactions [12]. This wide standard deviation in Cq values should not be viewed a prominent error in this methodology, but rather a byproduct of using patient samples, and should in no way diminish the statistical validity of the sensitivity and specificity reported for this novel process [12].

4. Discussion

Throughout the course of the COVID-19 pandemic, we have seen the need for efficient, effective, and timely viral testing procedures dramatically increase [1,4]. As the demand for these tests rise, the market availability of the reagents and consumable plastics required to complete the traditional extraction to PCR workflow have become increasingly scarce. This lack of availability in reagents and consumables needed for adequate testing is hindering the global efforts in disease surveillance and combatting of the SARS-CoV-2 pandemic. Herein, we believe our novel two-step, direct-to-PCR workflow is a potential solution to the resource pitfalls currently delaying testing in many parts of the world. While we acknowledge that this may not be the perfect solution for all instances, the authors feel that through bypassing the traditional extraction steps requiring multiple reagents and consumable plastics that this method has the potential to fill critical gaps in testing in resource challenged areas. Additionally, with this validation using COVID-19 patient samples, resource challenged areas will be able to implement this technology with the reassurance that it has already proven a 100% positive predictive value and a 96.77% negative predictive value when compared to traditional extraction to PCR based methodologies in the first round of testing.

Aside from supplementing testing in resource challenged areas, this shaker-mill based detection workflow significantly reduces the time required to process each sample through bypassing the extraction steps, while increasing throughput of the process in preparing samples for PCR based

detection. Using the Omni Bead Ruptor Elite, 24 swabs can be processed in 30 seconds, allowing for a full 384 well PCR plate to be prepared in as little as 30 minutes. This is in comparison to the hours of processing that a traditional viral RNA extraction kit requires [4,7]. As seen in the United States in July of 2020 when waiting periods for results after being swabbed have taken up to 8 days, any ability to reduce the processing time surrounding COVID-19 testing is currently of critical need in order to provide public health officials with the accurate data they need to advise patients and track infections [1,2,4,7].

While additional, larger scale testing is still needed to gain a more robust statistical interpretation on the sensitivity and specificity of this methodology; we feel that after testing 58 sample resulting in a 96.43% sensitivity and 100% specificity, this technology is validated and viable for implementation in the arsenal of testing strategies currently employed surrounding COVID-19. It is our hope that this efficient and cost-effective measure for COVID-19 PCR based testing can work to fill the void in testing in many areas of the world or can be implemented for increasing the throughput of current testing sites, while reducing the time to obtain results.

Author Contributions

Conceptualization, Z.P.M, C.M.P., and R.J.N.; Methodology, Z.P.M. and C.M.P.; Validation, C.M.P., G.A.R., and R.J.N.; Writing – Original Draft Preparation, Z.P.M.; Writing – Review and Editing, Z.P.M., C.M.P, G.A.R., and R.J.N.; Supervision, R.J.N.; Funding Acquisition, Z.P.M. and R.J.N. All authors have read and agree to the published version of the manuscript.

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Conflicts of Interest

Z.P.M., C.M.P., G.A.R., and R.J.N. disclose that they are all currently employed by Omni International Inc in some capacity, however, none of the authors have any personal financial interests in the success or failure of this privately owned company. R.J.N. also discloses that he is the founder and CEO of JeevanBiosciences with personal financial interests in the success or failure of this privately owned company.

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