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A safe and effective sample collection method for assessment of SARS-CoV-2 in aerosol samples

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17.1 Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that appeared in November 2019 in Wuhan province in China, rapidly spread and has been declared as pandemic (WHO, 2020a). This pandemic has affected every country on this planet with over 120,417,290 confirmed cases and 2,665,249 deaths worldwide as of March 15,2021. A significant volume of literature has been generated on various aspects of SARS-CoV-2 in this short span of 15 months; the spread of SARS-CoV-2 is largely attributed to respiratory droplets, produced by coughing, sneezing; hence use of masks and social distancing has been recommended as ways to prevention of spread of this virus.

Recent evidence of existence of SARS-CoV-2 in hospital air in China (Liu et al., 2020a), the United States (Santarpia et al., 2020), and Kuwait (Habibi et al., 2021) suggests the probability of transmission via person to person contact, contact with contaminated surfaces as well as aerosol. Aerosol transmission of SARS-CoV-2 was also demonstrated in *Mesocricetus auratus* (Golden Syrian hamster), where a 100% efficient aerosol transmission among animals was observed both from direct contact and aerosol transfer (Sia et al., 2020). On the other hand indoor air samples collected from hospital in Iran using midget impingers showed these samples were coronavirus disease of 2019 (COVID-19) negative at 2–5 m from patients bed (Faridi et al., 2020).

Some studies have indicated the higher efficacy of the small aerosol particles in transmission of infections, since they are directly inhalable (Siegel et al., 2007). Small aerosols are more susceptible to be inhaled deep into the lung, which causes infection in the alveolar tissues of the lower respiratory tract (Jayaweera et al., 2020; Thomas, 2013). It was reported in a recent study that aerosol-mediated transmission of COVID-19 virus has been shown only in confined spaces mainly in hospitals and near COVID-19 positive cases (Lane et al., 2020). In order to protect health care personnel and uninfected people involved in patient care, it is important to understand the attributes of aerosols containing COVID-19. There is very limited information available on the characteristics of airborne transmission of SARS-CoV-2 and its concentration. This is mainly due to the challenges associated with sampling virus-laden aerosols and their quantification at low concentration. Such a lack of understanding limits the effective risk assessment, prevention, and control of COVID-19 disease outbreaks (Liu et al., 2020b).

Considering this presumption and limited information on COVID-19 transmission, many research groups have started collection of aerosol samples to determine aerosol transfer of SARS-CoV-2. Such assessments become more relevant since a huge number of patients are admitted to the hospitals and at the quarantine facilities; A very large number of health care workers take care of them increasing the chances of infection transmission, as a cumulative inhaled dose. The diagnosis of viruses is mainly based on polymerase chain reaction (PCR) and advanced molecular biology applications such as whole viral sequencing (Hsih et al., 2020; WHO, 2020a). The fundamental requirement of these biotechnological applications is a good quality noninfectious viral ribonucleic acid (RNA). This makes it immensely important to design a sampling method to extract good quality inactive (noninfectious) RNA that can be used for lower levels of biocontainment, minimizing the risk of infection transmission among the laboratory professionals. Therefore, simplified and safe aerosol collection procedures are urgently needed.

17.2 Novel aerosol sampling method

With the growing interest of scientists in studying SARS-CoV-2, there is a need to ensure biosafety. Most laboratories that lack the biosafety level (BSL) 3 and 4 containments to deal with live and infectious virus samples are prone to cross contamination and therefore can be hazardous. Most of the current studies on SARS-CoV-2 employ a collection of swabs from hospital facilities (WHO, 2020a), some have collected aerosol samples using midget impinger (Faridi et al., 2020), others have collected samples on a gelatin substrate using a suction pump and four-stage cascade impactor (Guan et al., 2020; Liu et al., 2020a). All of these techniques have their own limitations, i.e., the size cutoff is not fine enough to capture the finest droplets (Behbehani et al., 2020). We have designed a simplified and safe sample collection approach for assessing the level of SARS-CoV-2 in the aerosol. This sampling device utilizes a variable speed suction pump that is connected to three gas wash bottles (Duran) of 500 mL in series using a Teflon tubing (Fig. 17.1). Each of the gas wash bottle is filled with 100 mL of collection fluid and the known volume of air passes through these bottles. This sampling setup captures the aerosol load irrespective of the particle size.

17.3. Trizol versus phosphate buffer solution as collection medium

The guanidinium thiocyanate–phenol–chloroform extraction reagent sold as TRIzol (referred to as Trizol hereafter) has been proved to be useful for the extraction and inactivation of nucleic acids of a wide group of viral families such as *Togaviridae*, *Arenaviridae*, *Bunyaviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, and *Paramyxoviridae* (Kochel et al., 2017). It was also reported as the best collection media for the Ebola virus rendering chemical inactivation of the virus from serum and viral cell cultures (Alfson and Griffiths, 2018). Recently, a laboratory in Florida, USA reported Trizol as a simple, low-cost reagent for the collection and transportation of COVID-19 samples (Paz et al., 2020). Trizol was also used for lysis and inactivation of SARS-CoV-2 in the two hospitals of Wuhan (Liu et al., 2020a).

We have also tested the efficacy of the sampling for collecting microbial–viral load in the aerosol samples by using both the Trizol and a sterile phosphate buffer saline (PBS). From various runs, we have found a sampling
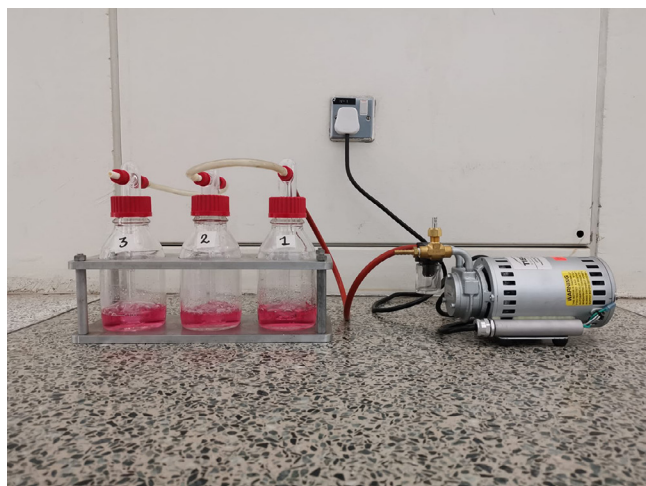

FIG. 17.1 **The novel aerosol sampling setup.** The three gas wash bottles filled with 100 mL of Trizol.

period of 120 minutes to be sufficient in capturing enough aerosol sample for RNA isolation. Our observations suggested that both Trizol and PBS are excellent collection medium. However, an additional lysis step is required prior to deoxyribonucleic acid (DNA) or RNA isolation in case of PBS, which requires a BSL-3 laboratory. It should be noted here that the present methodology can also be used to collect viable microbes when the objective is to isolate particular strains to study the whole genomic composition.

The possibility of occurrence of SARS-CoV-2 in indoor settings is most likely through discharges from symptomatic and nonsymptomatic carriers. In a very recent study, it was observed that levels of airborne SARS-CoV-2 in aerosols were more in crowded areas (Liu et al., 2020b).

In the present study, we intend to demonstrate the effectiveness of the sampling device and medium. The aerosol samples were collected from main campus of Kuwait Institute for Scientific Research (KISR) located at 29°20′15.14″N and 47°57′19.71″E. Aerosol samples from the reception area of KISR were collected during 900–1100 hours. This is the first area of contact in KISR main campus and with highest footfall as these were the peak working hours that bears maximum human traffic. Two samplers were co-deployed one with three gas glass wash bottles filled with 100 mL Trizol (Invitrogen, CA) and the other with 100 mL of sterile PBS near the entrance and the staircase where the staff registers their attendance, so everyone passes through these samplers. Air was pumped through the experimental setup for 120 minutes @ 30 L/min. The experiment was conducted on two consecutive days which are referred to as Day 1 and Day 2.

The Center for Diseases Control and Prevention has recommended the adenosine triphosphate (ATP) testing as a basis of monitoring cleanliness in health care settings (Aycicek et al., 2006). We have adopted ATP testing for these samples for assessing the viability status. The samples were subjected to ATP testing immediately after collection (Nante et al., 2017). Each of the gas glass wash bottle were marked as 1, 2, and 3 depending on their position of attachment with the pump, where 1 was the first bottle and 3 was the last bottle, these were tested individually (Table 17.1). The QuenchGone Aqueous (QGA) Kit from Lumin Ultra (Canada) was used for ATP testing. Standard protocol as per the manufacturer's instructions was followed to calculate the luminescence. All the measurements were done on the PhotonMaster (Lumin Ultra, CA) luminometer at the Biotechnology Laboratory, KISR. The relative luminescence units (RLU) were recorded for the calibration and background prior to each estimation, this information was used for reporting results. The RLU were recorded for each sample and converted to the cellular ATP (cATP) concentration in pg/mL (Nante et al., 2017). The cATP values were then converted to microbial equivalents (ME cells/mL) by multiplying with factor of 1000 considering, that one *E. coli* cell consists of 0.001 pg/mL of ATP (Alvarez et al., 2017).

The results highlight that only the microbial load collected in PBS was viable and averaged up to 1100 cells/mL. On the contrary, only 100 cells/mL remained viable in the first two bottles of Trizol, while in the bottle 3 it was zero (Table 17.1). Our results indicates the lysis efficiency of Trizol was 90–100% on both sampling days. It is prudent to mention that these samples were immediately processed, if the processing is delayed of 30 min entire microbial load will be lysed, suggesting the suitability and safety of the reagent as a safe and cost-effective collection medium over PBS.

It is the DNA or RNA that forms the basis of molecular identification of microbes, whether it is bacteria, fungi, or viruses. The concentration and yield of extracted DNA or RNA should be in enough quantities to be used for downstream applications such as PCR or sequencing. The novel coronavirus consists of a single positive RNA strand (Mousavizadeh et al., 2020) that is normally amplified during a PCR reaction to register its presence or absence in a particular sample. Although spread of coronavirus in bioaerosols has been predicted, no reports are currently available on effectively isolating sufficient quantities of viral RNA from aerosols. In the present study, RNA was

TABLE 17.1 Adenosine triphosphate (ATP)-based viability testing in sterile PBS and Trizol.

	Day 1			Day 2		
	RLU	cATP (pg/mL)	ME (cells/mL)	RLU	cATP (pg/mL)	ME (cells/mL)
PBS 1	13	1.1	1.10e+03	2	0.45	4.50e+02
PBS 2	15	1.2	1.30e+03	6	0.20	2.00e+02
PBS 3	12	1.0	1.00e+03	17	1.28	1.28e+03
Trizol 1	3	0.10	1.00e+02	2	0.09	9.00e+01
Trizol 2	5	0.10	1.00e+02	1	0.0	0.00e+00
Trizol 3	2	0.00	0.00e+00	1	0.0	0.00e+00

cATP, cellular ATP; ME, microbial equivalents; PBS, phosphate buffered saline; RLU, relative luminescence units.
Numbers 1–3 represent the bottle number.

TABLE 17.2   RNA yield in 3.6 m³ of aerosol samples collected in Trizol and PBS.

	Day 1		Day 2	
	RNA conc. (ng/µL)	Expected total yield (µg/3.6 m³)	RNA conc. (ng/µL)	Expected total yield (µg/3.6 m³)
PBS 1	27.27	1.3	19.23	1.0
PBS 2	35.40	1.7	20.35	1.0
PBS 3	11.54	0.5	15.41	0.8
Trizol 1	51.00	2.5	5.61	0.3
Trizol 2	11.56	0.6	24.72	1.2
Trizol 3	21.45	1.1	23.62	1.2

isolated from the aerosols and significant quantities of usable RNA (5.61–51 ng/µL) could be recovered from samples collected in Trizol (Table 17.2). The total concentration of RNA was measured through the NanoDrop 2000 spectrophotometer (Thermo Scientific, SA) following the recommended protocol. We expect to recover over 1 µg of total microbial RNA including bacterial, fungal, and viral RNA from 3.6 m³ of aerosols collected in Trizol or PBS. The isolated RNA was stored at −70°C until further processing.

RNA was isolated from Trizol using the standard procedure recomended by the manufacturer. The RNA was quantified employing the NanoDrop 2000 spectrophotometer (Thermofisher Scientific, CA) and the purity (A260/230) was within the acceptable range.

This sampling strategy was applied to collect aerosol samples from two major hospitals dealing with COVID-19 patients in Kuwait with an objective to detect and quantify the viral communities through quantitative PCR and next generation sequencing (NGS). Reasonable quantities of RNA were recovered (2–6 ng/µL) from all the three sampling bottles, demonstrating further the effectiveness of this sampling protocol and safety of this collection method. The more advanced fluorometric method was used for RNA quantification (Simbolo et al., 2013) as both these downstream applications are based on highly precise estimations of nucleic acids. The high sensitivity Qubit HS ss RNA kit was used for this purpose and all the measurements were done on the Qubit 4 fluorometer (Thermo Scientific, Paisley, UK).

To further test the utility of isolated RNA, the PCR was conducted with universal bacterial (16s gene; Mustafa et al., 2017), fungal (ITS 1 and ITS 2 region; Al Salameen et al., 2020a, 2020b), and viral primers (Influenza A and B; WHO, 2020b). The total RNA was converted to cDNA prior to the PCR employing the iScript Reverse Transcriptase kit (BioRad, Germany). Thereafter, the PCR reaction was setup by adding 2 µL of cDNA to 4 µL of 5× HOT FIREPol master mix (Solis Biodyne, Estonia). The forward and reverse primers were added at the concentration of 10 µM. The total volume was made up to 20 µL by adding sterile nuclease-free water. The reaction was run on Veriti 96-Well Thermal Cycler (Applied Biosystems, SA). Initial deactivation was done at 95°C for 5 minutes followed by 35 cycles of denaturation (95°C for 20 seconds), annealing (60°C for bacterial; 50°C for fungal; 65°C for viral primers for 60 seconds) and extension (72°C for 4 minutes). The final elongation step was set at 72°C for 10 minutes. The PCR products were run on an agarose gel (2%) at 10 V/cm for 1 hour and visualized on a Gel Doc (BioRad, CA).

We successfully amplified the RNA (cDNA) with clear and visible bands (Fig. 17.2) observed with bacterial (500 bp) and fungal primers (150 bp) and very faint bands with Influenza A and B primers (~100 bp) showing the
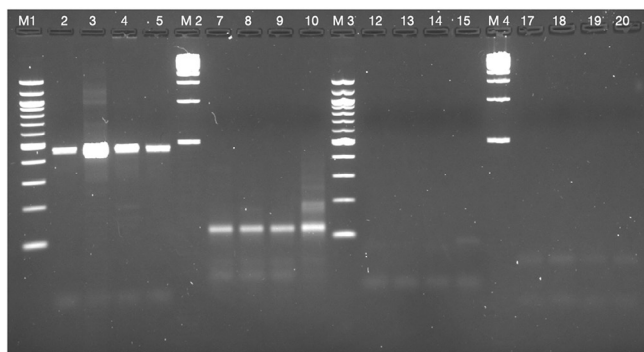

FIG. 17.2 **PCR with cDNA of air samples collected from KISR reception area.** Lane M1 and M3: 100 bp marker; Lane M2 and M4: I kb marker; Lane 2–3: RNA (recovered from PBS) amplified with universal bacterial primers; Lane 4–5: RNA (recovered from Trizol) amplified with universal bacterial primers; Lane 7–8: RNA (recovered from PBS) amplified with universal fungal primers; Lane 9–10: RNA (recovered from Trizol) amplified with universal fungal primers; Lane 12–13: RNA (recovered from PBS) amplified with Influenza A primers; Lane 14–15: RNA (recovered from Trizol) amplified with Influenza A primers; Lane 17–18: RNA (recovered from PBS) amplified with Influenza B primers; Lane 19–20: RNA (recovered from Trizol) amplified with Influenza B primers.

validity of technique to be effectively used for assessing all the microbial communities.

We tested these samples against a panel of respiratory viruses (Coronavirus CoV-229E, HKU1, OC43; Parainfluenza 1, 2, 3, 4; human rhinoviruses and human enteroviruses). The extracted RNA was added to real-time PCR master mix consisting of specific primers and fluorescent reporter dye probes and amplified on a real-time PCR (BioRad, Germany) for 45 cycles. Our samples tested positive for CoV HKU1, Parainfluenza 4, human rhinovirus, and human enterovirus. The cycle threshold cutoff for these samples was set at ≤40. Positive amplification of CoV HKU1 indicates that the present sampling strategy was able to collect detectable quantities of coronavirus communities in aerosols of indoor environments. It further informs us that this methodology will effectively pick SARS-CoV-2 RNA from aerosols and subsequently amplify it with high specificity and precision levels as low as 10 copies per cubic meter of air.

17.4 Next generation-based applications

Taken together, the existence of other viruses along with SARS-CoV-2 virus in the air microbiome can induce life-threatening symptoms in COVID-19 patients. In a recent study (Qu et al., 2020), it was hypothesized that the extent to which the COVID-19 virus induces respiratory stress in infected individuals may also be influenced by the extent to which an individual's respiratory system is already compromised due to the presence of other viruses. The Middle East respiratory syndrome can provide important parallels: one of the risk factors is the presence of other coinfections that require management and treatment. This may also be the case with COVID-19 (Moore et al., 2020). The simultaneous inhalation of other viral communities along with the COVID-19 virus is assumed to exacerbate the level of COVID-19 infection. This is due to the fact that SARS-CoV-2 rapidly undergoes recombination with other human coronaviruses and mutates. This has hampered both vaccine development and antiviral efficacy testing. With the advent of NGS technology, the whole infectome could be simultaneously revealed that is (RNA viruses, DNA viruses, bacteria, and eukaryotes) present in an environmental sample (Hish et al., 2020). NGS in field settings proved critical in the development of countermeasures for the 2014–2016 Ebola virus epidemics in West Africa (Gire et al., 2014; WHO, 2020b). Recently, this approach has been used as a surveillance strategy in Cambodia for COVID-19 (Hoenen et al., 2016). Amplicon and metagenomic MinION-based sequencing were used to rapidly (within 10 hours) identify SARS-CoV-2 and assess the microbiome in nasopharyngeal swabs obtained from patients with COVID-19 by the ISARIC 4C consortium (Moore et al., 2020). In this context also the proposed methodology would serve the purpose as it will allow the extraction of both RNA and DNA viruses. The protocol of RNA extraction mediated through Trizol integrates both RNA and DNA. A simple and standardized protocol based on Trizol was reported for viral metagenome study from complex biological and environmental samples (Temmam et al., 2015).

17.5 Conclusions

We have found that 100 mL of media in each of the three bottles is sufficient to capture the microbial load when pumped @ 30 L/min. The results show that Trizol was very efficient media for sampling microbial load, it was observed that 90%–100% of microbial load was lysed immediately. The sampling device is easy to deploy, use of Trizol over PBS is safe since the entire microbial load was lysed in former. Hence, use of this sampling technique with Trizol as collection medium is safe for use in laboratories that don't meet BSL-3 specifications or other stringent safety requirements of a virology laboratory. Crosscontamination is also minimized in this procedure as the entire setup is autoclavable after each use.

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