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CHAPTER 2

Virus-sampling technologies in different environments

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2.1 Introduction

Biological airborne particles such as viruses are commonly found in the environment. Any respiratory virus that is able to survive and transmit in the air has a potential risk of disease. The three ways of direct contact, inhalable droplets, and airborne transmission can spread respiratory viral diseases. Hand washing and face masking can substantially reduce the first two paths of the viral transmission.¹ An infectious sneeze can spread 40,000 aerosolized droplets in the air up to approximately 2 m distance. As a result, the droplet nucleus can suspend in the gases environment for a long time (up to 30 h) and results in respiratory tract infections.^{2,3}

Bioaerosol sampling has been researched for over 200 years.⁴ Three key points associated to sampling performance include sampling efficiency for inhaled bioaerosol particles, efficiency of particulate capture in filtration or laboratory culture, and biological recovery efficiency for bioaerosol particle viability.^{5,6} Various physical mechanisms are applied to remove particles from the gas media by these samplers. The impaction force can simply remove particles with greater aerodynamic diameters from the air. The smaller particles (less than 100 nm) can be enlarged using higher diffusivity or by size density, and finally, can be collected by impaction.⁷ There are several methods for sampling airborne viruses. Although the primary studies were performed on cyclone sampling, most studies have been conducted by the liquid impingers.⁷

Most air sampling methods are a function of the aerodynamic diameter, Brownian motion, inertia and adhesion properties of the airborne particles

and the temperature gradient of the environment. The aerosolized particles adhere to any surface that comes in contact with it.⁷

The SARS-CoV-2 has a sphere-shaped structure with an aerodynamic diameter of approximately 120 nm.⁸ Coronaviruses such as SARS-CoV-2 can be moved via airborne transmission. People become infected when viruses enter their body through the respiratory system, eyes, nose, and mouth. Other media that can transmit SARS-CoV-2 include water and wastewater, urine, stool, contaminated surface, food, solid waste, etc. The SARS-CoV-2 has a sphere-shaped structure with a single-segmented RNA in the 80-nm-diameter lumen.⁹ In this study, the important procedures for environmental sampling and analysis of the virus were investigated.

2.2 Methods of SARS-CoV-2 air sampling

Various sampling principles like impaction, impingement, electrostatic precipitation, filtration, and cyclone have been applied to viral bio-aerosol.^{7,10,11} In a study, the collection performance of four devices including all-glass impinger (AGI), impinger (AGI-30), one-stage Anderson impactor (1-STG), gelatin filter, and nucleoporous polycarbonate filter were employed for four bacteriophages. The results showed that about 95% of the cultured phages with a particle diameter of less than 2.1 μm were collected by the Anderson 6-STG impactor.¹²

The recovery efficiency of heating, ventilation, and air conditioning (HVAC) filters and two types of impingers (AGI-30 and biosampler) using transmissible gastroenteritis virus (TGEV) were investigated by other research. The researchers found that by increasing the relative humidity, the collection performance of the samplers declined. However, the biosamplers had a higher efficiency than the AGI-30 impinger.¹³

In an experimental project on the survival of the airborne viral organisms HCoV-229E, the researchers investigated the effect of the nebulization phenomenon on the survival of the virus and the lack of viral infection at 20°C and various relative humidity (RH). At 20°C and moderate RH, HCoV-229E indicated the highest virus retrieval (87%–91%), while increasing the RH had a detrimental effect. At 6°C and higher RH, a recovery rate was observed 100%.¹⁴

2.2.1 Filtration

Filtration procedure is commonly applied to catch airborne bioaerosols. The bioaerosols trapped on the filter can be analyzed directly by an electron

microscope or by agar culture. Based on the analytical method types, polytetrafluoroethylene (PTFE, Teflon), mixed cellulose ester, polycarbonate, polyvinyl chloride (PVC), nylon, gelatin, and other filters can be applied for bioaerosol sampling.^{15,16} When flat-bed filters such as nucleopores were used, the accumulated particles were specifically suitable for microscopic analysis. The washable filters such as gelatin filters have been extended for the better preservation of the culturable sample (like bacteria and virus) and it can be dissolved in a buffer solution or cultured directly on agar for analysis.¹⁶

The collection devices such as impactors and impingers have lower collection efficiency for particles smaller than 500 nm. Therefore, various filters have been commonly employed for aerosolized viruses sampling. PTFE and cellulose filters have been used for the sampling of aerosols containing virus.^{17,18} Because the filtration process dehydrates the viruses during sampling, it can inactivate a noteworthy part of the viruses.⁷ In a sampling procedure, it was found that only 22% and 10% of influenza viruses was recovered alive using Teflon and gelatin filters, respectively, compared to biosamplers.¹⁹

An approach was performed to compare the extraction efficiency for bacteriophage MS2 phage using alumina nanofiber against fiberglass filters. The efficiency of nanofiber filter was less than 10%, while fiberglass filter ranged from 32.3% to 162%.²⁰

Among conventional filters, the gelatin type can be readily dissolved in a liquid for molecular counting or cell culture without significant changes in virus tissue. In Zhao et al. study, four six-stage samplers ACI, AGI-30, OMNI-3000, and MD8 were explored with gelatin filter for airborne Brucella virus infection. The results showed that the gelatin filters had the highest collection efficiency (100%) without significant dehydration effects on the virus.²¹ The collection efficiency of gelatin filters toward the nucleoporous polycarbonate filters for hydrophilic viruses was about 10 times.¹²

2.2.2 Impactor

Impactors such as a slotted-vessel sampler, six-step Anderson sampler and cyclones are applied for airborne viruses sampling. These sampling methods require a suction pump to suck air into the device. Then, the aerosols with higher inertia force hit the internal surface of the device.²²

During the SARS prevalence in 2003, two air sampling procedures, including a high-resolution modified slotted-vessel sampler and PTFE membrane filters (0.3 μm) were applied.²³ The findings showed that all the

medium cultures were negative, although 2 of 10 reverse-transcription polymerase chain reaction (RT-PCR) samples were positive in terms of the virus. The practical limitation of the conventional impactors is the smallest cut-off size for 0.2–0.3 μm particles.²³

2.2.3 Impinger

The air sampling devices such as liquid impingers present a little information about the aerosol particle size as a significant parameter that influences on airborne particles movement.²⁴ Liquid impingers are the most frequently devices that are applied for the collection of viral aerosols. Because the liquid medium helps to survive the viruses and subsequently it can be directly applied for virus extraction analysis.²⁵ AGIs and biosamplers are extensively used as liquid impregnators. During the sampling, the produced bubbles into the liquid phase can enhance the collection performance for finer particles via the diffusion process, but it can resuspend the collected viruses.²⁶

AGI-4, AGI-30, and biosampler were employed as reference samplers. The liquid medium can be straightly used for molecular analysis techniques such as PCR. In Hogan et al. study, the collection performance of AGI-30, biosamplers, and frit bubblers for MS2 and T3 bacteriophages for particles size of 20–100 nm was found to be less than 10%.²⁷ The high centrifugal force of the biosampler can inactivate a significant proportion of influenza viruses.²⁸

2.2.4 Cyclone

This device has not been designed as a high performance sampler (>95%) for the particles size of greater than 10 nm. The collection performance for a typical single-stage cyclone for PM_{10} and $\text{PM}_{2.5}$ is approximately 30%–90% and 0%–40%, respectively.²⁹ However, viral particles because of their size (>100 nm) are not easily trapped by this procedure.³⁰

The National Institute for Occupational Safety and Health (NIOSH) improved a multistage cyclone with air rate of 3.5 L/min. The performance of this sampler for viral aerosols is similar to the standard midjet impinger (SKC) biosampler for 15 min of sampling. The results showed that only 34% of the viral aerosols trapped by the NIOSH cyclone, because of the dryness of the viruses, were infectious.³¹ The liquid cyclone collectors have been used for the sampling of viral aerosols.^{32,33}

The result of a project showed that cyclone performance cannot meet the sampling needs of small particle-containing viruses and the number of

live viruses collected by this device is less than the actual ones.⁷ However, ACI has a high capability to collect viral particles in various size ranges and it can also be used to trap particles downstream the impactor sampler.³⁴

2.2.5 Electrostatic precipitator

Electrostatic precipitator (ESP) is another sampler for the collection of airborne particles. Few studies have been performed on virus behavior in ESPs that focus solely on sampling efficiency.³⁵ One of the most important characteristics of electrostatic sampler is the lower particle settling velocity rate compared to inertial based methods, which leads to less damage to microorganisms.³⁶ The collection efficiency of ESP reached to 99.3%–99.8% for polystyrene latex (PSL) particles with a size range of 0.5–0.5 μm .³⁷

2.2.6 MD-8 airscan

This simple system consists of MD-8 portable air sampler attached to a gelatin filter (diameter of 80 mm and pore size of 3 μm). MD-8 airscan was applied for the influenza viruses sampling in air.³⁸ Lower relative humidity can lead to drying the virus and higher relative moisture can cause to the liquidation of gelatin filters.⁷ These types of filters should be employed in a short time (less than 15 min), because they are quickly dried and melted at higher temperatures.¹⁹ MD-8 airscan was used for MERS-CoV and SARS-CoV sampling in hospital indoor air.³⁹

In Liu et al. study, MD-8 airscan sampler and PTFE filter in a cassette were used for sampling and identification of SARS-CoV-2 virus. The application of gelatin filter in MD-8 airscan showed good performance for coronavirus sampling.⁴⁰

2.3 Literature review for SARS-CoV-2 sampling in air

In a study for aerosolized SARS-CoV-2 in hospital, portable pump (APEX2, Casella) equipped with the sterilized gelatin filters (pore size of 3 μm and diameter of 25 mm) was used at air rate of 5 L/min for 1 h. The results showed that the samples were positive.⁴⁰

Guo et al. research on the SARS-CoV-2 in a hospital air through wetted wall cyclone sampler (SAAA 2300) with flow rate of 300 L/min for 30 min showed that the existence of the virus in the air samples were positive.⁴¹

Cheng et al. explored SARS-CoV-2 in the indoor air of COVID-19 Hospitals. Sartorius MD-8 airscan with sterile gelatin filters (diameter of 80 mm and a pore size of 3 μm) was applied for sampling. The sampler was vertically installed at 10 cm from the patients' heads. The sampling was performed at flow rate of 50 L/min for 20 min while patients with or without surgical mask. After sampling, each filter was immersed in 5 mL of viral transport medium (VTM) and placed at 37°C for 10 min. The results showed that out of 377 air samples close the patients, 19 samples were positive using RT-PCR assay.⁴²

Ong et al. examined the personal protective equipment and air of the symptomatic SARS-CoV-2 patients. The air sampling was performed in the indoor environment for 2 days through SKC Universal pumps (with 37 mm filter cassettes and 0.3 μm polytetrafluoroethylene filters at flow rate of 5 L/min for 4 h). The results showed that none of the samples contained the virus.⁴³

In various studies, PTFE, gelatin, and polycarbonate filters have been used to sample viruses, of which PTFE and gelatin filters showed higher collection efficiency.⁴⁴ However, the NIOSH has suggested that PTFE can be used for sampling of airborne pathogens for immunological assay and PCR.⁴⁵ Some studies have reported that the use of filters for the viral sampling is not suitable.⁴⁶

Cyclone samplers were also employed for sampling of SARS-like viruses. These devices were used to collect viruses at high air flow rate for 8 h. The NIOSH 0600 method has suggested that the cyclone can be used as a prefilter to remove nonrespirable particles from bioaerosols.⁴⁵ Chia et al. used a NIOSH BC 251 bioaerosol sampler for SARS-CoV-2 in patient's rooms of hospital air. The samplers were equipped with SKC AirCheck TOUCH pumps or SKC Universal air sampling pumps with air rate of 3.5 L/min for 4 h.⁴⁷

In Faridi et al. study on SARS-CoV-2 in hospital air, a setup consisted of a pump with air rate of 1.5 L/min and SKC was used. The sampling device was installed at a height of 1.5–1.8 m from the floor and about 2–5 m away from the patient's beds. The air was collected by a sterile impregnator containing 20 mL of Dulbecco's Modified Eagle Medium (DMEM), 100 $\mu\text{g}/\text{mL}$ streptomycin, 100 U/mL penicillin, and 1% anti-foam reagent (isoamyl alcohol) for 1 h. The specimens were placed on ice and instantaneously transferred to laboratory. The finding showed that the samples were not positive in terms of SARS-CoV-2.⁴⁸

Razzini et al. research was performed for the sampling of SARS-CoV-2 in a hospital air. The samples were taken by an MD-8 Airport portable air

sampler with gelatin filters. The sampling time was 40 min with air rate of 50 L/min. The positive samples were detected from the ICU ward and corridor.⁴⁹

2.4 Surface detection for SARS-CoV-2

Sterile synthetic fiber swabs with plastic shafts have been mainly used to collect the environmental surface samples in many studies.^{41,49,50} The swabs were premoistened with VTM and rubbed over the surfaces of the target for a few seconds. Then, swabs were immediately positioned in the sterile tubes containing 2–3 mL of VTM. Each swab was separately taken to curb cross-contamination. Finally, all the samples were transferred to the laboratory for SARS-CoV-2 within 4 h under cool conditions (2–8°C).^{41,49,50}

2.4.1 Practical tips before sampling

- The sealing plastic bags should be cleaned with 70% ethanol solution, 80% isopropyl alcohol, or 5% NaClO solution before inserted to the transport container.⁵⁰
- To achieve the best results, sampling must be carefully duplicated at the same time and treated as separate samples to determine the precision.⁵⁰
- Use only commercially available laboratory fiber swabs with plastic or wire shafts.⁵¹

2.4.2 Surface sampling area

The swabbed surface area depends on the equipment size. The World Health Organization (WHO) recommended that normal swab surface area is 25 cm².⁵¹ In this regard, the area (25 cm²) was swabbed by applying pressure to the swab and rotating stick it.⁵² For the larger surfaces, develop the surface area up to 50 cm² and do not allow the swap to dehydrate entirely.⁵³

According to the Yang et al. method, if the surface area of the equipment is greater than 100 cm², the sampling area of the object surface is limited to 100 cm², otherwise all surface area would be sampled. In their study, a standard specification board with a size 5 × 5 cm was positioned on the object surface, afterward a premoistened sterile swab with VTM was applied to wipe the specification disk five times.⁵⁴ Also, entire surfaces of small objects like faucets, pipettes, and door handles were rubbed by a sterile swab soaked with VTM.⁵⁴

2.4.3 Swab types

Acceptable swab types for SARS-CoV-2 sampling are flocked tapered swabs,⁵⁵ Dacron swabs,⁵⁶ spun polyester swabs,⁵¹ and cotton and foam swabs.⁵⁷

In Santarpia et al., surface sampling of small objects was performed by premoistened sterile gauze pads (with a size 3×3 cm) with 3 mL of phosphate buffered saline (PBS). The larger area surfaces were sampled by “S” pattern wiping in two paths to cover the accessible surface as much as probable. Furthermore, the smaller objects (e.g., mobile phones, TV remote controls) were swabbed in one path on every accessible surface.⁵⁸ For sampling of the facemasks of COVID-19 patients, it should be cut into small pieces and immediately placed in VTM.⁵⁹

2.5 Municipal wastewater for SARS-CoV-2 sampling

Many studies showed that municipal wastewaters contain SARS-CoV-2. The amount of virus in sewage is much lower than feces due to factors such as solution pH, temperature, and the presence of disturbing factors.⁶⁰ Different methods including precipitation, ultracentrifugation, electronegative membrane, and ultrafiltration have been used to prepare samples of sewage, feces, and urine for RNA virus extraction, which are listed later in the chapter.

2.5.1 Sampling procedures for SARS-CoV-2 in municipal wastewater

In Randazzo et al. project on municipal wastewater treatment plants, 500–1000 mL of wastewater was collected by a grab sampling method in high-density polyethylene (HDPE) sterile containers. The specimens were transferred to the laboratory on ice and kept at 4°C. Then, 200 mL of the sewage was processed by precipitation-thickening method as follows.⁶¹ For precipitation, 200 mL of the sample was poured into a 250 mL centrifugation tube and the pH was regulated to 6. Then, to produce aluminum hydroxide precipitate, 2 mL of aluminum chloride (0.9 N) was mixed with the sample. The solution pH was readjusted to six and mixing was performed by a rotary shaker (150 rpm for 15 min) at room temperature. The thickening process was then carried out by centrifugation (1700 g for 20 min). The residue was mixed with 10 mL of beef extract (pH = 7.4) into a 50 mL centrifugation tube by an orbital shaker (150 rpm for 10 min)

and centrifuged (1900 g for 30 min). The precipitation was finally resuspended into 1 mL of phosphate-buffered saline (PBS) and 150 μ L of the suspension was applied for RNA extraction.⁶¹

In Zhang et al. research on septic tank effluent, 2 L of wastewater was collected using a sterile plastic bag and it was immediately transferred to the laboratory at 4°C. The sample was then mixed with sodium chloride (0.3 M) and PEG-6000 and it was stayed overnight at 4°C for precipitation. Finally, the mixture was centrifuged (10,000 g for 30 min) and the virus RNA was tested from the precipitate.⁶²

A composite sampling of raw wastewater for SARS-CoV-2 was taken by Wu et al. and after transfer of the sample to the laboratory, it was pasteurized (90 min at 60°C) to inactivate the viruses. The pasteurized sample was then filtered (0.22 micron) to separate large solids and bacterial cells. The filter was discarded because the analysis showed that the filter lacked RNA virus. Then, 4 g of PEG-8000 (8% by weight/volume) and 0.9 g of sodium chloride (0.3 M) were added to 40 mL of the filtrate and centrifuged at 12,000 g for 2 h (or centrifuged until the pellet became observable). Finally, the pellets were suspended in Trizol (Thermofisher) and prepared for the RT-qPCR analysis.⁶³

To detect the viral RNA, raw wastewater and treated samples were gathered by combined technique. The samples were transferred to the laboratory by sterile bottles via cold chain and kept at 4°C. The specimens were first centrifuged (4500 g for 30 min) and then passed through a cellulose ester syringe filter (0.22 micron). The filtrate was then thickened using the polyethylene glycol method as follows: 25 mL of the filtrate, polyethylene glycol-9000 (80 g/L) and sodium chloride (17.5 g/L) were poured into a tube. The mixture was shaken overnight at 17°C at 100 rpm. Finally, the mixture was centrifuged (1000 g for 90 min) and the pellet was suspended again in 300 μ L of RNase-free water for PCR analysis.⁶⁴

On wastewater from the municipal treatment plant, a composite sampling was performed. Initially, 0.25–1 L of sewage was centrifuged to separate bulky particles. The centrifuged sample was then treated with polyethylene glycol or aluminum sulfate (20 mg/L) and centrifuged again. The sample was shaken at 100 rpm for 12 h at 4°C and the sample was then centrifuged (14,000 g for 45 min) at 4°C to produce a viral residue. The sediments were eventually suspended in PBS. The viral suspension was finally filtered (0.22 micron) and Ultra-15 centrifugal tubes with cut-off of 30 kDa were applied to thicken the sample volume to 1 mL. The sample was kept at –20°C to –80°C to extract the viral RNA.⁶⁵

In a project on municipal wastewater, the sampling was performed and kept in the laboratory at 4°C. Firstly, 11 mL of the samples were centrifuged (200,000 g for 1 h) at 4°C. Then, the residue was mixed into 400 µL of PBS 1×. Finally, 200 µL of the suspension was used for viral analysis.⁶⁶

For determination of SARS-CoV-2 in domestic wastewater, 1.9 L of wastewater was taken by composite sampling and carried to the laboratory on ice packs and placed at 4°C. After homogenization, 20 mL of the sample was poured into 38.5 mL ultracentrifuge bottles. Then, 12 mL sucrose cushion (50% sucrose in Tris-NACL-EDT buffer (TNE) buffer [20 Mm Tris-HCl (pH 7.0), 100 mM NaCl, 2 mM EDTA]) was sensibly injected beneath the surface of sewage with pipette so that two separate layers were created. Finally, the mixture was centrifuged (50,000 g for 45–90 min) at 4°C and the residue was homogenized into 200 µL of 1× PBS. The suspension was stored at –20°C to extract virus RNA.⁶⁷

In an approach for direct extraction of virus RNA from raw municipal wastewater, firstly, the pH of 100–200 mL of raw wastewater was adjusted to about 3.5–5 using HCl (2N). The sample was then filtered by an electronegative filter (9 cm diameter with a pore size of 0.45 microns) using a glass funnel. The filter was placed in a 5 mL bead tube from the RNeasy PowerWater Kit and homogenized at 3 × 20 s at 8000 rpm at 10 s interval. Finally, the virus RNA was tested based on the manufacturer's direction of the RNeasy Power Microbiome kit.⁶⁸

In an experimental procedure for the extraction of virus RNA from raw municipal wastewater, 100–200 mL of the sample was centrifuged (4750 g for 30 min). The clear solution was removed from the tube without any disturbance for the residuals and centrifuged (3500 g for 15 min) by Centricon Plus-70 centrifugal filter (cut-off of 10 kDa). The concentrate cup was inversely installed on the top of the sample filter cup. The instrument was then centrifuged (1000 g for 2 min). The concentrated sample (~250 µL) was finally gathered from the collection cup for RNA analysis.⁶⁸

Kocamemi et al. survey on wastewater treatment plant, 250 mL of raw wastewater was collected and large particles and bacteria were centrifuged at 3200 g for 45 min. The clear solution was then filtrated by ultrafiltration (3200 g for 25–40 min). Finally, 200–600 µL of the viral particles on the filter were used to extract RNA.⁶⁹

In another exploration on municipal wastewater, wastewater samples were transported to the laboratory along with an ice pack. First, in order to remove coarse materials and bacteria, the samples were centrifuged in

funnel containers of 250–50 mL at 4654 rpm for 30 min. Then, the ultrafiltration of 100–200 mL of the clear solution was performed using Centricon Plus-70 centrifugal ultrafilter with a cut-off of 100 kDa using centrifugation (1500 g for 15 min). The centricon concentrate was 0.44–1.79 g which used to PCR analysis.⁷⁰

In an investigation on municipal wastewater, the samples were received by the laboratory at 4°C and it was immediately centrifuged (4500 g for 30 min) at 4°C. Then, suspended particles were removed from the centrifuged sample by filtration (pore size of 40 µm). Finally, the filtrate was frozen at –20°C and stored for PCR. Then, 50 mL of the sample was filtered by a 50 KDa membrane and 500 µL of the residue on the filter was used for RNA analysis.⁷¹

To extract of the virus, wastewater samples were firstly collected into 500 mL polypropylene containers and immediately transferred to the laboratory under cold chain for filtration. The samples were then filtered by fiberglass filter (0.7 micron with a diameter of 145 mm). Then, 200 µL of the filtrates were employed to extract viral RNA.⁷²

In another study, 200 mL and 5 L of raw and treated wastewaters were taken from the treatment plant, respectively. The samples was mixed with 25 mM MgCl₂ and filtered by mixed cellulose ester filter (pore size of 0.8 micron, diameter of 90 mm). Then, the virus RNA was straightly collected from one-fourth of the filter that placed into 5-mL of PowerWater Bead Tube of a RNeasy PowerWater Kit. A volume of 50 µL of the concentrate was used for viral RNA analysis.⁷³

2.6 SARS-CoV-2 in urine and stool

Some people with COVID-19 have gastrointestinal symptoms such as diarrhea.⁶⁰ Cheung et al. examination also expressed that 17.6% of the patients had gastrointestinal symptoms and 48.1% of them had positive stool tests.⁷⁴ Fecal-oral spread of SARS-CoV-2, due to gastrointestinal symptoms in some COVID-19 patients, is possible.⁷⁵ Previous studies have shown that large amounts of the virus RNA were present in the urine and feces of the infected animals.⁷⁶ The findings of Jeong et al. survey showed that the virus RNA level of COVID-19 during the 8–30 days of their clinical period in the urine and feces of patients was equal or even more than nasopharyngeal. The stool test for SARS-CoV-2 in patients with negative respiratory symptoms may be positive.⁶⁰ Therefore, the stool tests are very essential to diagnose COVID-19.^{60,75}

2.6.1 Sampling method for SARS-CoV-2 in urine and stool

In an analysis to examine the virus in feces, the patient's feces were diluted (10 times) in 2 mL of phosphate saline solution and then the debris was separated by centrifugation (12,000 rpm for 20 min). The clear liquid was employed for culture and qRT-PCR.⁷⁵

2.7 SARS-CoV-2 sampling in food

Because the global attention during the COVID-19 pandemic is paid on human health, the impact of the disease on foodstuffs, food processing, and food packaging is still not recognized. Therefore, it is crucial to develop the detection methods for SARS-CoV-2 in food products. Because of the low viral load, the heterogeneous distribution of the viral particles and nonoptimal tedious isolation, the SARS-CoV-2 detection in foodstuffs remains a challenge.⁷⁷

In this regard, several methods have been suggested for SARS-CoV-2, such as RT-qPCR, enzyme-free immunosorbent assay, and nanoparticles and enzyme-linked immunosorbent assays (Nano-ELISA).^{78,79} In spite of the COVID-19 pandemic, according to the FDA, there is no documentation about SARS-CoV-2 transmission via food products to date.^{80–82} The existence of SARS-CoV-2 in refrigerated and iced meat and salmon was evaluated in Fisher et al. study. In this study, the individual portions of salmon, chicken, and pork provided from food stores were sliced (500 mm³), then 200 μ L of 3×10^6 TCID₅₀/ml SARS-CoV-2 was mixed with each cube. All specimens were placed at 4, -20, and -80°C. The samples were then harvested for 1, 2, 5, 7, 14, and 21 days after inoculation. After that, an aliquot of 150 μ L of the virus inoculum was poured into a new tube and frozen at -80°C until titration. Each condition was conducted in triplicates. The cell-free virus titer (TCID₅₀/mL) for the samples was defined by limited dilution. The limit of detection (LOD) was 5×10^1 TCID₅₀/mL.⁸³

2.8 SARS-CoV-2 detection techniques in air and surface samples

Presently, SARS-CoV-2 detection is mainly conducted via RT-qPCR. However, other available methods for the virus assays can be explained briefly as follows.

2.8.1 RT-PCR

Most of the literature employed PCR analysis for SARS-CoV-2 in air. Real-time PCR, due to high sensitivity, ease of use, and concurrent detection of more respiratory agents, have been extensively applied to diagnosis of viral respiratory infections.⁸⁴

Presently, RT-PCR and RT-LAMP are the most used procedures for SARS-CoV-2. Until today, the RT-PCR method has been broadly applied to detect SARS-CoV-2 in various media like air and surfaces and biological samples such as sputum, urine, blood, saliva, and stool.^{85,86}

2.8.2 Droplet digital PCR (ddPCR)

The droplet digital PCR (ddPCR) with a high LOD was recently used for SARS-CoV-2 in surface media. However, this approach has not yet been used for SARS-CoV-2 in air, and more research is required to evaluate the method efficiency for the virus detection in such samples.^{87,107}

2.8.3 Sensor-based method

The sensor-based method for the fast analysis of SARS-CoV-2 which presented by Seo et al.³² can be used for air samples. They introduced a field-effect transistor (FET)-based biosensing device to detect the virus. Due to this fact, graphene sheets of the FET were covered with a specific antibody against SARS-CoV-2 spike protein to produce the sensor. The efficiency of the mentioned procedure is not required to sample pretreatment and a high sensitivity compared to the conventional detection methods.⁸⁸ Due to its high sensitivity, this recognition procedure has the ability to distinguish the low burden of SARS-CoV-2 in air.⁸⁸ To our literature review, no study has been conducted by this technique for SARS-CoV-2 in air. The available primers applied for RT-qPCR of SARS-CoV-2 RNA in the air and surface media are summarized in [Table 2.1](#).

2.9 SARS-CoV-2 recognition in water

The RT-qPCR assay, which shows an ALOD of b10 copies per reaction, can be suitable for wastewaters containing SARS-CoV-2.^{92,93} It is extremely recommended that positive RT-qPCR signals should be validated for environmental samples by sequencing analysis, because the RT-qPCR assay was established for clinical diagnosis.^{94,95} However, the available primers that were used for the recognition of SARS-CoV-2 RNA by RT-qPCR and nested RT-PCR techniques in the river water and wastewater are demonstrated in [Table 2.2](#).

Table 2.1 The primers used for RT-qPCR assays to detect SARS-CoV-2 in air and surface media.

Study	Target	Primer name	Sequence (5'–3')	Type of sample
Chia et al. ⁵⁷	Envelope gene ORF1ab	E_Sarbeco_F1 E_Sarbeco_R2 E_Sarbeco_P1 Wu-BNI-F Wu-BNI-R WU-BNI-P-FAM	ACAGGTACGTTAATAGTTAATAGCGT ATATTGCAGCAGTACGCACACA ACACTAGCCATCCTTACTGCGCTTCGBHQ1 CTAACATGTTTATCACCCGCG CTCTAGTAGCATGACACCCCTC TAAGACATGTACGTGCATGGATTGGCTTBHQ1	Air and surface
Santarpia et al. ⁸⁹	E gene	F1 R2 P1	ATATTGCAGCAGTACGCACACA ACAGGTACGTTAATAGTTAATAGCGT FAM/ACACTAAGCC/ZEN/ ATCCTTACTGCGCTTCG/3AIBkFG	Air and surface
Ryu et al. ⁹⁰	E gene, RdRp gene, and N gene	Based on the producer's protocols		Surface
Kenarkoochi et al. ⁹¹	ORF1ab and N genes	Based on the producer's protocols		Air
Wu et al. ⁵⁵	RdRp, N and E gene	Based on the producer's protocols		Air and surface
Peyrony et al. ⁵³	ORF1a/b and E genes	Based on the producer's protocols		Surface
Tan et al. ⁵⁹	ORF1a/b	Based on the producer's protocols		Air and surface
Ye et al. ⁵⁶ and Lv et al. ⁵⁴	ORF1ab and N genes	Based on the producer's protocols		Surface
Guo et al. ⁴¹	ORF1ab and nucleocapsid protein (NP)	Based on the producer's protocols		Air and surface

Table 2.2 The primers used for RT-qPCR assays for SARS-CoV-2 in water and wastewater.

Study	Target	Primer name	Sequence (5'–3')	Product length (bp)
Corman et al. ⁹²	N_Sarbeco	N_Sarbeco_F1 N_Sarbeco_R1 N_Sarbeco_P1	CACATTGGCACCCGCAATC GAGGAACGAGAAGAGGCTTG FAMACTTCCTCAAGGAACAACATTGCCA-BHQ1	128
Nao et al. ⁹⁶	S	WuhanCoV-spk1-f	TTGGCAAATTC AAGACTCACTTT	547
		WuhanCoV-spk2-r NIID_WH-1_F24381 (Nested) NIID_WH-1_R24873 (Nested)	TGTGGTTCATAAAAATTCCTTTGTG TCAAGACTCACTTTCTTCCAC ATTTGAAACAAAGACACCTTCAC	493
WHO ⁹⁷	ORF1b-nonstructural protein 14	HKU-ORF1b-nsp14F HKU- ORF1b-nsp14R HKU-ORF1b-nsp141P	TGGGGYTTTACRGGTAACCT AACRCGCTTAACAAAGCACTC FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA	132
CDC ⁹⁸	CDC-N1	2019-nCoV_N1-F 2019-nCoV_N1-R 2019-nCoV_N1-P	GACCCCAAAATCAGCGAAAT TCTGGTTACTGCCAGTTGAATCTG FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1	72
Rosa et al. ⁹⁹	ORF1ab	2274 - CO-FW1	GTGCTAAACCACCGCCTG	368
		2275 - CO-REV1 2276 - CO-FW2 (Nested) 2277 - CO-REV2 (Nested)	CAGATCATGGTTGCTTTGTAGGT CGCCTGGAGATCAATTTAAACAC ACCTGTAAAACCCCATTTGTTGA	332

Table 2.2 The primers used for RT-qPCR assays for SARS-CoV-2 in water and wastewater.—cont'd

Study	Target	Primer name	Sequence (5'–3')	Product length (bp)
Zhou et al. ¹⁰⁰	S protein	RBD-qF1	CAATGGTTTAACAGGCACAGG	121
		RBD-qR1	CTCAAGTGTCTGTGGATCACG	
Shirato et al. ⁹³	NIID_2019-nCoV_N	NIID_2019-nCoV_N_F2	AAATTTTGGGGACCAGGAAC	158
		NIID_2019-nCoV_N_R2ver3	TGGCACCTGTGTAGGTCAAC	
		NIID_2019-nCoV_N_P2	FAM-ATGTCGCGCATTGGCATGGA-BHQ1	
Haramoto et al. ⁹⁵	ORF1a	NIID_WH-1_F501	TTCGGATGCTCGAACTGCACC	413
		NIID_WH-1_R913	CTTACCAGCACGTGCTAGAAGG	
		NIID_WH-1_F509	CTCGAACTGCACCTCATGG	346
		NIID_WH-1_R854	CAGAAGTTGTTATCGACATAGC	
Medema et al. ⁷⁰	(N) gene	IC-F	ATGACAGCCACTCCTCCG	412
		IC-R	GGAACGAACCAAACAGTCTTC	
		IC-P	AGCAGAGACCCATTCCCTCAGAGC	

2.10 SARS-CoV-2 detection in solid wastes

To our knowledge, no experimental research has been previously carried out to detect the SARS-CoV-2 in domestic solid wastes. However, few scientific papers were found about this topic. It can be said that the most infectious wastes are generated in hospitals and other health care facilities, which incinerated in place or autoclaved before landfilling. Therefore, no study has been conducted in this regard so far.¹⁰¹ However, insufficient waste management, improper use of personal protective equipment (PPE), and other unfavorable conditions, especially in developing countries, could potentially increase the spread of SARS-CoV-2. Therefore, management and monitoring of the solid wastes of the self-quarantined patients is a vital key for controlling the virus. Treating patients at home can produce infected wastes, which possibly discarded along with domestic waste; subsequently, it can potentially result in risks to workers, other people, and the environment, depending on the transportation and disposal conditions.¹⁰²

2.11 Analysis techniques for SARS-CoV-2

The PCR and antibody-based methods are currently applied for SARS-CoV-2. But other processes, including LAMP, reverse transcription-loop-mediated isothermal amplification (RT-LAMP),^{84,103} and CRISPR, are under investigation and may be used soon to diagnose the SARS-CoV-2.^{104,105}

Current studies are focused on SARS-Cov-2 RNA to improve the detection methods. Target genes are spiked (S) protein genes, nucleocapsid protein (N1,N2,N3) genes, open reading frame protein (ORF1, ORF1ab, ORF1b) genes, envelop protein (E) gene, and RNA-dependent RNA polymerase (RdRp) gene.¹⁰⁶

The PCR-based detection methods are generally used and research is indicating many developments in this method. RT-PCR or qPCR is a strategy for measuring of gene expression at the transcript level.¹⁹ The strategy is based on the following ways: (1) isolation of virus RNA from the samples; (2) synthesis of cDNA through reverse transcription kit, and (3) amplification and detection of cDNA. The PCR is set by mixing of the buffer, dNTPs, primers of the target gene, Taq polymerase, cDNA template, and SYBR green dye. The PCR mix is incubated in a PCR machine and the fluorescence created by PCR amplification is determined to present Cycle threshold (Ct) values. The Ct values of the controls are

compared with the experimental samples and the relative expression is estimated. Also a key point for the detection of SARS-CoV-2 in the RT-PCR method is the specimen type selection and time of taking it.^{19,106}

However, all of the following steps are performed on the basis of the manufacturer's protocol of RNA kit to perceive SARS-CoV-2 in different samples: primers and probes, nucleic acid extraction, mix preparation for all separate primer/probe combinations, controls, amplification cycles (light cycler system), sensitivity, specificity, and positive control for SARS-CoV-2 RT-PCR.^{19,105,106}

2.12 Conclusion

SARS-CoV-2 has been detected in a various environments including air, sewage, urine, and feces. There are different procedures such as filtration, impactor, impinger, cyclone, electrostatic separator, and MD-8 airscan are applied for sampling and measuring viruses from air. Among filters, the gelatin type can be readily dissolved in a liquid for molecular counting or cell culture without significant changes in virus tissue. Liquid impingers are the most frequently used devices that are applied for the collection of viral aerosols. Sterile synthetic fiber swabs with plastic shafts have been mainly used to collect the environmental surface samples in many studies. The swabs were premoistened with VTM and rubbed over the surfaces of the target for a few seconds. The amount of virus in sewage is much lower than feces due to factors such as solution pH, temperature, and the presence of disturbing factors. Also, many methods including precipitation, ultracentrifugation, electronegative membrane, and ultrafiltration have been used to prepare samples of food, wastewater, feces, urine, and surfaces. In many studies, the aforementioned methods have been employed for the sampling of the coronaviruses such as SARS-CoV-2 in various environments. Also, PCR procedures have been commonly used to identify the virus from the environmental samples.

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