Video Article

Detection of Viruses from Bioaerosols Using Anion Exchange Resin

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Abstract

This protocol demonstrates a customized bioaerosol sampling method for viruses. In this system, anion exchange resin is coupled with liquid impingement-based air sampling devices for efficacious concentration of negatively-charged viruses from bioaerosols. Thus, the resin serves as an additional concentration step in the bioaerosol sampling workflow. Nucleic acid extraction of the viral particles is then performed directly from the anion exchange resin, with the resulting sample suitable for molecular analyses. Further, this protocol describes a custom-built bioaerosol chamber capable of generating virus-laden bioaerosols under a variety of environmental conditions and allowing for continuous monitoring of environmental variables such as temperature, humidity, wind speed, and aerosol mass concentration. The main advantage of using this protocol is increased sensitivity of viral detection, as assessed via direct comparison to an unmodified conventional liquid impinger. Other advantages include the potential to concentrate diverse negatively-charged viruses, the low cost of anion exchange resin (~$0.14 per sample), and ease of use. Disadvantages include the inability of this protocol to assess infectivity of resin-adsorbed viral particles, and potentially the need for the optimization of the liquid sampling buffer used within the impinger.

Video Link

The video component of this article can be found at https://www.jove.com/video/58111/

Introduction

The purpose of this method is to provide a highly sensitive bioaerosol sampling platform to facilitate molecular detection of negatively-charged viruses from bioaerosols. Microorganisms, including viral particles, can survive in bioaerosols for extended periods of time[1]. Bioaerosols can travel over relatively long distances and maintain viability and infectivity, as evidenced by an outbreak of Legionnaires' disease that originated from industrial cooling towers located at a distance of 6 km from the affected individuals and resulted in 18 fatalities[2]. Indirect transmission of viruses to humans mediated by bioaerosols can occur in multiple settings and has been demonstrated for norovirus outbreaks in schools and restaurants[3,4]. Similarly, bioaerosol transmission of viruses can occur in agricultural settings such as in swine and poultry farms, with this transmission route being considered as a major factor in the movement of viruses between production facilities[5,6,7,8,9].

Effective sampling of virus-laden bioaerosols allows for improvement in rapid diagnostics and preparedness for outbreak prevention, as shown in demonstrations in which H5 influenza A viruses were detected from bioaerosols in live animal markets in China and the United States[10,11]. Current bioaerosol sampling technologies involve a number of different particle capture principles, and can be broadly categorized into impingers, cyclones, impactors, and filters[12]. It is beyond the scope of this protocol to exhaustively cover all advantages and disadvantages of these platforms for sampling of viruses from bioaerosols; however, it can be stated that the majority of these sampling devices have not been optimized for the collection of viruses and bacteriophages[13]. Furthermore, infectivity of viral particles is often negatively affected, with liquid impingers considered to maintain viral infectivity more effectively than sampling devices such as solid impactors or filters[14]. However, one disadvantage of liquid impingement is the target dilution effect, which occurs because viruses are collected in relatively large volumes (typically ≥20 mL) of liquid in the collection vessel. Another important disadvantage involves the suboptimal efficiency of liquid impingers to concentrate particles <0.5 µM in size[15]. However, capture efficiency of these devices can be improved by immobilization on solid matrices, as immobilization can enhance preservation of viral nucleic acids and viral infectivity[16,17].

We have previously demonstrated that anion exchange resin is an effective tool for the capture and concentration of viruses from liquid matrices, including F-RNA bacteriophages, hepatitis A virus, human adenovirus, and rotavirus[18,19,20]. As defined by the manufacturer, the anion exchange resin utilized in this work is a macroreticular polystyrene strong base anion exchange resin in which functionalized quaternary amine groups mediate attraction and capture of anions in a liquid medium[21]. Consequently, the anion exchange resin is expected to capture viruses with net-negative surface charges, including many enteric viruses, influenza viruses and other viruses relevant to public and animal health.

The current protocol involves the addition of anion exchange resin to a liquid impinger. In this system, the resin serves as a secondary concentration step for viral particles captured in the impinger liquid. Nucleic acids can then be directly eluted in small volumes, providing a concentrated sample for molecular analyses. Thus, the main advantage of this method is the improvement in viral detection sensitivity, primarily through reduction in sample volume. Additionally, due to the inherent non-specific capture of negatively-charged viruses, the method is likely applicable for detection of a large number of viruses of interest. Here, the method is demonstrated for vaccine strains of type A and type B influenza viruses and the FRNA coliphage MS2 (MS2). These viruses are subsequently detected using standard qRT-PCR assays as previously described[22]. The end-point user should not expect to encounter difficulties in performing this method, because modifications to currently existing equipment do not constitute major disruptions to the conventional flow of bioaerosol sampling and analysis.

Protocol

1. Setup of the Bioaerosol Chamber (See Figure 2)

1. **Pre-load the liquid impingers with 20 mL of 0.01 M phosphate buffered saline, pH 7.5 (PBS).**
    1. Add 0.5 g of anion exchange resin and suspend within the PBS of one of the liquid impingers, with another liquid impinger serving as a control.

2. Position liquid impingers in parallel inside the bioaerosol chamber using clamp stands with aerosol inlets facing the nebulizer.
   NOTE: See **Figure 2** for additional detail.
3. Co-locate a direct-read aerosol monitor near the liquid impingers to measure mass concentration (mg/m^3) of the bioaerosol.
4. Co-locate additional direct-read instrumentation (indoor air quality monitor and thermal anemometer) near the liquid impingers to monitor environmental variables, such as temperature, relative humidity and wind speed, within the bioaerosol chamber.
5. Run axial fans in the corners of the bioaerosol chamber to facilitate mixing of aerosol.
   NOTE: Axial fans run at a fixed speed and are not adjustable.

2. Model Bioaerosolization Experiments (see Figure 3)

1. Perform serial, 10-fold dilutions of MS2 bacteriophage and influenza vaccine to desired concentrations in phosphate-buffered saline (PBS).
   NOTE: To demonstrate the method here, $10^{3.5}$ FFU/mL of the type A and B influenza virus vaccine strains and 10^5 PFU/mL of the MS2 bacteriophage are utilized. To determine titers for the inocula, bacteriophages are propagated in *Escherichia coli* HS (pFamp)R and enumerated as previously described[20]. The commercial vaccine contains known quantities of four live attenuated influenza virus strains, two type A influenza viruses and two type B influenza viruses, expressed in fluorescent focus units or FFU.
2. Prepare inocula within the glass vessel of a 6-jet collision nebulizer by creating suspensions of desired concentrations of viral particles in 100 mL of PBS and install within the custom bioaerosol chamber.
3. Initiate instrumentation to measure variables within the bioaerosol chamber, such as temperature, % relative humidity, carbon dioxide concentration, and wind speed (generated using an axial fan). Use a direct-read aerosol monitor to track mass concentration within the bioaerosol chamber and to establish consistency within and between trials.
4. Seal the bioaerosol chamber and purge for 10 min with HEPA-filtered air.
5. Deliver filtered, dried air to the nebulizer operating at 6.9 kPa.
6. Generate a target concentration of viral bioaerosols (via nebulizer) in each bioaerosol chamber trial. For this demonstration, bioaerosols with a concentration of 5 mg/m^3 are generated.
7. Once the target mass concentration of the aerosol has been reached, stop nebulization.
8. Operate an air sampling pump for each liquid impinger that has been calibrated to a flow rate of 12.5 L/min. Conduct calibration using a primary flow standard before and after each sampling event to ensure consistency in sampling volume. Supply dilution air using a HEPA-filtered line located near the nebulizer and maintain constant pressure in the chamber.
9. Actively sample the bioaerosol chamber atmosphere for a period of 40 min to achieve a sampling volume of 500 L per sample.
   NOTE: Sampling for 40 min at 12.5 L/min with two pumps in parallel allows for sampling of 1,000 L of the bioaerosol. Thus, at the completion of the experiment, it is expected that the majority of the air present in the bioaerosol chamber has been sampled and released into the environment via a HEPA-filtered system. This is evidenced by the decrease in particle concentration. Post-experiment, surfaces are decontaminated with 10% household bleach and 70% ethanol. Indicators are used in this demonstration; however, if known pathogenic organisms are used in this system, additional biosafety measures may be implemented as needed.

3. Nucleic Acid Extraction and qRT-PCR Detection

1. **Isolate nucleic acids directly from anion exchange resin and, for comparative purposes, from the liquid used within the liquid impingers. In this example, a RNA isolation procedure is described, but a similar protocol could be used for DNA viruses.**
    1. Nucleic acid isolation from the anion exchange resin.
        1. Transfer all of the anion exchange resin-containing liquid of the liquid impinger to a sterile 50 mL conical tube.
        2. Allow the resin to settle, and slowly decant the liquid sample from the anion exchange resin. Use a 1 mL pipette tip to remove all of the remaining liquid from the anion exchange resin.

3. From a viral RNA isolation kit, add 560 µL of viral lysis buffer containing carrier RNA directly to the anion exchange resin and incubate for 10 min at room temperature with periodic mixing.
4. Using a 1 mL pipette tip, transfer the viral lysate (the viral lysis buffer) to a sterile 1.5 mL conical tube and proceed with RNA isolation using a viral RNA isolation kit (see **Table of Materials**) in accordance with the manufacturer's instructions, except that RNA is eluted in a total volume of 60 µL of elution buffer.

2. Nucleic acid isolation from liquid impingers.
   1. Pipette 140 µL of the liquid sample into a sterile 1.5 mL conical tube and perform RNA isolation using the viral RNA isolation kit in accordance with the manufacturer's instructions, with the exception of eluting RNA in a total volume of 60 µL of elution buffer. NOTE: 140 µL is the maximum sample volume that can be processed with the specific viral RNA isolation kit employed here in a single-step preparation.

2. Perform qRT-PCR for detection of MS2 and influenza A and B viruses as previously described[23,24]. In this demonstration, 5 µL of nucleic acid eluates are used for qRT-PCR.

Representative Results

Figure 1 demonstrates the principle behind charge-based capture of viruses from bioaerosols via inclusion of resin in liquid-based impingers. **Figure 2** shows the setup of the custom-built bioaerosol chamber. **Figure 3** describes the steps involved in setting up the aerosolization experiment and measures to ensure quality control. **Figure 4** shows amplification curves for qRT-PCR detection of negatively-charged viruses captured from bioaerosols. **Table 1** shows the primers and probes used for qRT-PCR for the example viruses used in this study.

Using MS2 and influenza viruses contained within the influenza vaccine as models in the custom bioaerosol chamber, the liquid impingers modified with anion exchange resin allowed for approximately 3x to 9x improvement in viral detection, depending on the specific virus, compared to direct testing of impingement liquid[22]. As shown in representative qRT-PCR amplification curves (**Figure 4**), detection of type A influenza viruses could be achieved up to 3.26 cycles sooner when anion exchange resin was used compared to direct testing of the impingement liquid. Considering that in a qRT-PCR that is 100% efficient, a gain of one Ct corresponds to a 2-fold increase in target concentration, this difference is equivalent to a 9.58x improvement in detection.

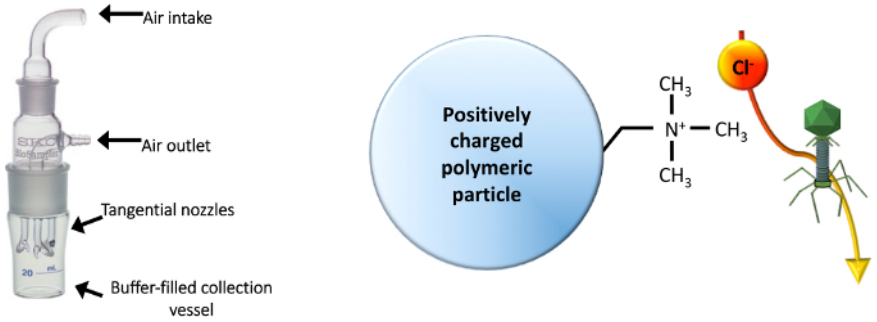

Figure 1: Liquid impingement coupled with anion exchange resin. Each of the modified bioaerosol sampling devices incorporates an anion exchange resin into its design. Each resin bead is a 0.5 to 1.0 mm sphere with a porous surface, a characteristic that increases the anion exchange surface area. Functional quaternary ammonium groups allow for capture of ions in liquid media, mediating capture of viruses with net-negative surface charges, including MS2 coliphages and influenza viruses, which are used in this protocol as model organisms (a tailed bacteriophage is drawn here as an example for captured viruses). Nucleic acid isolation is then performed directly from the resin (using commercially available kits), with the sample suitable for most molecular detection strategies (qRT-PCR employed here). Please click here to view a larger version of this figure.

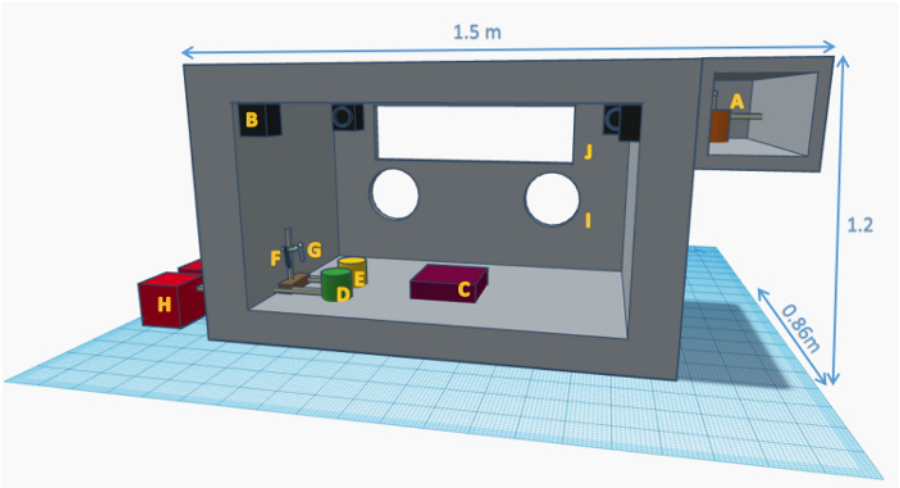

Figure 2: Custom bioaerosol chamber schematic. A: 6-jet nebulizer supplied with filtered and dried air at a generating pressure of 6.9 kPa; B: Axial fans to facilitate uniform bioaaerosol mixing; C: Aerosol monitor to identify the relative bioaerosol concentrations; D: Liquid impinger with resin; E: Liquid impinger without resin; F: Thermal anemometer to measure wind speed; G: Air quality monitor to measure other environmental variables including temperature, carbon dioxide, and percent relative humidity; H: Active sampling pumps calibrated to 12.5 L/min; I: Pneumatically sealed glove ports for positioning sampling equipment within the bioaerosol chamber; J: Observation window. This figure was reused from previous work with permission from the publisher[22]. Please click here to view a larger version of this figure.

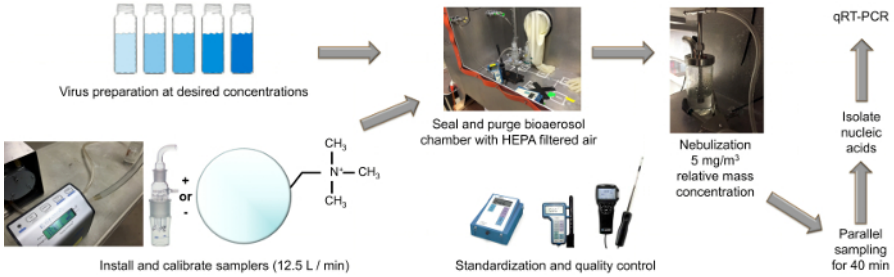

Figure 3: Workflow of the bioaerosolization experiment. Please click here to view a larger version of this figure.

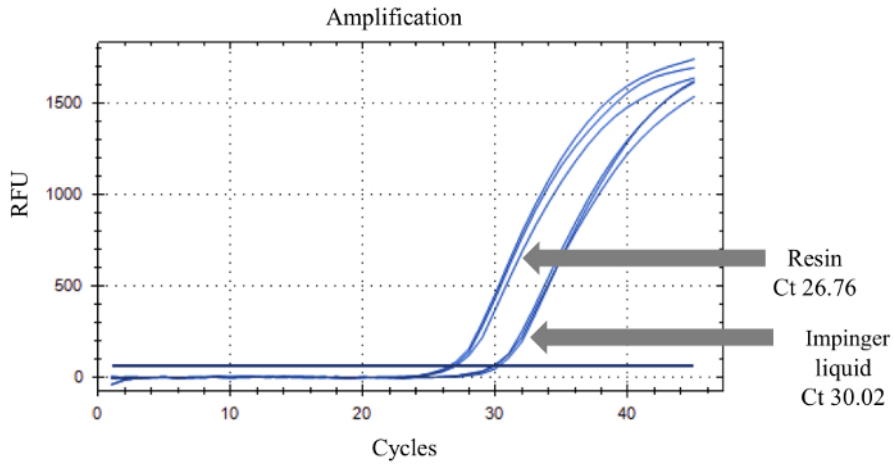

Figure 4: Anion exchange resin improves qRT-PCR based detection of negatively-charged viruses in bioaerosols. In this example, type A influenza viruses at an inoculum concentration of $10^{3.5}$ FFU/mL were aerosolized into the custom bioaerosol chamber until the bioaerosol mass concentration reached 5 mg/m^3. Liquid impingers with and without resin were simultaneously engaged to each sample (500 L) of the bioaerosol. Viral RNA was obtained from the anion exchange resin or from the liquid of the impinger and assayed by qRT-PCR. The experiment was repeated in triplicate. Nucleic acids obtained from resin samples were detected at an average Ct of 26.76, whereas impinger liquid samples were detected at an average Ct of 30.02 (ΔCt of 3.26). This difference in Ct is equivalent to a 9.58x improvement in detection using the anion exchange resin. Please click here to view a larger version of this figure.

Viruses	Primer name	Primer sequence	Reference
MS2	GI Fwd	5'-ATCCATTTTGGTAACGCCG-3'	23
	GI Probe	5'-(6-FAM)-TAGGCATCTACGGGGACGA-(BBQ)-3'	
	GI Rev	5'-TGCAATCTCACTGGGACATAT-3'	
	IAC Fwd (46F)	5'-GACATCGATATGGGTGCCG-3'	
	IAC Probe	5'-(Cy5)-CACATTCACCAGGGAGACGCATGAGA-(BBQ)-3'	
	IAC Rev (186R)	5'-CGAGACGATGCAGCCATTC-3'	
type A influenza viruses	CDC universal influenza A virus forward primer	5'-GAC CRA TCC TGT CAC CTC TGA C-3'	24
	CDC universal influenza A virus reverse primer	5'-AGG GCA TTY TGG ACA AAK CGT CTA-3'	
	CDC universal influenza A virus probe	5'-(FAM)-TGC AGT CCT CGC TCA CTG GGC ACG-(BHQ1)-3'	
type B influenza viruses	CDC universal influenza B virus forward primer	5'-TCCTCAACTCACTCTTCGAGCG-3'	24
	CDC universal influenza B virus reverse primer	5'-CGGTGCTCTTGACCAAATTGG-3'	
	CDC universal influenza B virus probe	5'-(JOE)-CCAATTCGAGCAGCTGAAACTGCGGTG-(BHQ1)-3'	

Table 1: Primers and probes used in this study. All primers and probes were from Friedman *et al.* 2011[23] and Selvaraju & Selvarangan 2010[24].

Discussion

This protocol outlines a method for sensitive viral capture from bioaerosols using modified liquid impingers. The method is optimized for detection and quantification of the viral load in bioaerosols. The specific modification demonstrated here involves the addition of anion exchange resin to liquid contained within a common liquid impinger. This method was developed for its simplicity in downstream sample processing, whereas other sample processing techniques such as centrifugation, filtration, and precipitation-based methods do not offer such an advantage. Extraction of nucleic acids is performed directly from the resin, obviating the need for high-volume elutions and multiple sample preparation steps, ultimately contributing to improved detection sensitivity of the method via reduction in the effective sample volume. Furthermore, it has been shown that immobilization of viral particles on solid surfaces can contribute to increased stability of viral nucleic acid or retention of viral infectivity[17,25]. Immobilization may further contribute to increased capture efficiency by reducing losses due to reaerosolization[16]. The method can be easily coupled with downstream molecular analyses (qRT-PCR demonstrated here). The anion exchange resin may be field-extracted and shipped to a reference laboratory for analysis, while air-sampling equipment can stay dedicated to monitoring in the sampling area. As the principle behind the procedure involves the non-specific capture of viruses carrying a net-negative surface charge, the method is expected to be amenable to the detection of multiple pathogenic viruses of importance to human health and veterinary medicine. As reviewed by Michen and Graule[26], a large number of viruses important to public and animal health have net-negative surface charges, with few known exceptions, such as certain strains of rotaviruses and polioviruses.

This protocol does not directly evaluate the effect of environmental conditions on the sensitivity of detection; however, when air sampling is conducted in field settings, it is conceivable that variables such as relative humidity and temperature may affect collection efficiencies across multiple types of air samplers[12]. Thus, conditions within the bioaerosol chamber may be modified to suit these environmental parameters as needed. The anion exchange resin method has been shown to effectively concentrate multiple viruses from water samples with a wide range of physicochemical properties, indicating that interference from particulate matter, bacteria, and other environmental contaminants is expected to be minimal[18]. Additionally, collection buffers may require optimization for specific viruses, as in some cases it has been shown that optimizing the collection buffer can lead to improved retention of viral stability[27]. A main disadvantage of the method as presented is the fact that it cannot determine viral infectivity. Another disadvantage of this protocol is the inability to assess potentially negative effects of nebulization and air sampling on viral stability, including desiccation, shearing, and non-specific adsorption to bioaerosol chamber surfaces[22].

The modified bioaerosol sampling devices could be amenable to field-based sampling in multiple settings, including hospitals, schools, agricultural operations, and other venues. Because modifications are made to existing air sampling equipment, it is expected the modified devices would be easily adoptable by operators currently sampling bioaerosols for viruses. The protocol is demonstrated here in a custom-built bioaerosol chamber using MS2 and influenza viruses, which are useful targets for method development due to the fact that they represent examples of non-enveloped and enveloped viruses, MS2 is recognized as a surrogate of enteric viruses and are commonly employed in evaluation of impingers[22,26,27,28,29].

Future work will involve optimization of sampling conditions (*i.e.,* air sampling velocity, collection buffers) and inclusion of additional relevant viruses in testing protocols.

Disclosures

The authors have nothing to disclose.

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