U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services

U.S. Government Publication

Journal of Virological Methods 246 (2017) 38–41

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

A method for the improved detection of aerosolized influenza viruses and the male-specific (F+) RNA coliphage MS2

J.C. Chandler[a], J.W. Schaeffer[b], M. Davidson[b,c], S.L. Magzamen[b], A. Pérez-Méndez[d], S.J. Reynolds[b], L.D. Goodridge[e], J. Volckens[f], A.B. Franklin[a], S.A. Shriner[a], B. Bisha[g,*]

[a] National Wildlife Research Center, Wildlife Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Fort Collins, CO, USA
[b] High Plains Intermountain Center for Agricultural Health and Safety, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA
[c] Western Sydney University, Richmond, NSW, Australia
[d] Leprino Foods, Inc., Denver, CO, USA
[e] Department of Food Science and Agricultural Chemistry, McGill University, Ste. Anne de Bellevue, QC, Canada
[f] Department of Mechanical Engineering, Colorado State University, Fort Collins, CO, USA
[g] Department of Animal Science, University of Wyoming, Laramie, WY, USA

ARTICLE INFO

Keywords:
Virus concentration methods
Bioaerosols
Anion exchange resin
Influenza

ABSTRACT

The detection of aerosolized viruses can serve as an important surveillance and control tool in agriculture, human health, and environmental settings. Here, we adapted an anion exchange resin-based method, initially developed to concentrate negatively charged viruses from water, to liquid impingement-based bioaerosol sampling. In this method, aerosolized viruses are collected in a 20 ml liquid sample contained within widely used impingers, BioSamplers (SKC Inc., Eighty Four, PA), and further concentrated via adsorption to an anion exchange resin that is suspended within this liquid. Viral nucleic acids are then extracted from the resin to facilitate molecular analyses through a reduction in the effective sample volume. For this study, various quantities of two negatively charged viruses, type A and type B influenza viruses (FluMist Quadrivalent vaccine) and the male-specific (F+) RNA coliphage MS2 (MS2), were nebulized into a custom-built bioaerosolization chamber, and sampled using BioSamplers with and without anion exchange resin. Compared to direct testing of the BioSampler liquid, detection was improved by 6.77× and 3.33× for type A and type B influenza viruses, respectively, by using the anion exchange resin. For MS2, the anion exchange resin method allowed for an average improvement in detection of 8.26×.

Bioaerosols serve as a transmission vehicle for numerous and diverse types of viruses, including influenza viruses, severe acute respiratory syndrome viruses, and porcine epidemic diarrhea viruses (Alonso et al., 2014; Cowling et al., 2013; Li et al., 2005). The important role of bioaerosols in viral transmission is highlighted by recent events, such as the 2014–2015 outbreak of highly pathogenic avian influenza in U.S. poultry production. In this outbreak, multiple independent lines of epidemiological evidence suggested perpetuation within and between poultry production facilities by influenza virus-laden bioaerosols (United States Department of Agriculture, 2015). Ultimately, 48 million birds were euthanized with economic losses estimated at $3.3 billion (Greene, 2015). Accordingly, active sampling of bioaerosols has been suggested as a tool to help mitigate viral transmission (Anderson et al., 2016; Cowling et al., 2013). Therefore, highly efficacious bioaerosol sampling strategies are needed.

The collection of airborne viruses is generally accomplished using a variety of inertia-based samplers. These samplers include cyclones, impactors, filter-based devices, and impingers (Haig et al., 2016; Verreault et al., 2008). Cyclones and impactors are typically used to size-segregate aerosols based on particle diameter while filter-based devices will collect an integrated sample across a defined range of particle sizes. The most widely used sampling strategy for viruses is liquid impingement. This technique purportedly reduces sampling-related stresses (e.g. impaction force, desiccation) compared to the other methods described above (Verreault et al., 2008). With liquid impingement, bioaerosols are drawn through nozzles to produce an impinging jet that deposits particles into a liquid-filled chamber. This liquid can then be processed using culture or molecular-based methods for viral identification and characterization. However, approximately 20 ml of liquid is required for the effective operation of many liquid

* Corresponding author at: 1000 E. University Ave, Laramie, WY 82071, USA.
E-mail address: bbisha@uwyo.edu (B. Bisha).

http://dx.doi.org/10.1016/j.jviromet.2017.04.004
Received 8 March 2017; Received in revised form 12 April 2017; Accepted 14 April 2017
Available online 24 April 2017

impingers (Pepper et al., 2015), resulting in an undesirable dilutive effect that ultimately limits the sensitivity of downstream detection (Gendron et al., 2010; Hermann et al., 2006). Thus, strategies which enable additional concentration of viruses within liquid impingers would improve the overall performance of these systems.

We previously demonstrated that IRA-900 anion exchange resin was useful for concentrating male-specific (F+) RNA (FRNA) coliphages and several enteric viruses from water (Chandler et al., 2017; Perez-Mendez et al., 2014a,b). Similar to batch chromatography, anion exchange resin was suspended and mixed with aqueous samples to adsorb negatively charged viruses. The liquid was then decantated so that nucleic acids could be directly isolated from the resin in a small volume (60–80 µl), and viruses were detected by quantitative real-time PCR (qPCR). Compared to direct testing of the water sample by qPCR, the anion exchange resin method allowed for improved detection of all viruses tested. Specifically, detection was improved 50× to 200× for FRNA coliphages, 4300× for human adenovirus 40, 128× for rotavirus, and 20× for hepatitis A virus (Perez-Mendez et al., 2014a). Additionally, we observed that the anion exchange resin method was functional in water samples with diverse physicochemical properties. Therefore, we hypothesized that the anion exchange resin method may be applied to air sampling methods, such as liquid impingement, to improve the detection of negatively-charged viruses.

The objective of this study was to test the ability of anion exchange resin to improve the detection sensitivity of airborne type A and type B influenza viruses and FRNA coliphage MS2 (MS2) impinged in the collection liquid of a widely used bioaerosol sampling device, the BioSampler (SKC Inc., Eighty Four, PA). Thus, the anion exchange resin would serve as a second virus concentration step within the overall sampling system. MS2 was selected for evaluation given the precedence for effective anion exchange resin-based concentration in water (Perez-Mendez et al., 2014b). Further, MS2 is considered to be a surrogate of negatively charged enteric viruses (United States Environmental Protection Agency, 2000; Michen and Graule, 2010; Vergara et al., 2015) and is used as a model organism for the evaluation of liquid impingers (Tung-Thompson et al., 2015; Turgeon et al., 2014). Similarly, influenza viruses are reported to have negative charges (Michen and Graule, 2010) and have been used in the evaluation of liquid impinger-based bioaerosol sampling (Turgeon et al., 2014).

The influenza viruses utilized were contained within the FluMist Quadrivalent vaccine 2015–2016 formulation (FluMist) (MedImmune, Gaithersburg, MD) which contains four live attenuated strains: A/Bolivia/559/2013 (H1N1), A/Switzerland/9715293/2013 (H3N2), B/Phuket/3073/2013 (B/Yamagata/16/88 lineage), and B/Brisbane/60/2008 (B/Victoria/2/87 lineage). A single 0.2 ml dose of FluMist is declared to contain between $10^{6.5}$ and $10^{7.5}$ fluorescent focus units (FFU) of virus reassortants for each of the four strains. For this study, the concentration of each type A and type B viruses within FluMist was assumed to be $1 \times 10^{6.5}$ FFU/ml. MS2 (ATCC 15597-B1) was obtained from the American Type Culture Collection (Manassas, VA), and was propagated and enumerated as described previously (Perez-Mendez et al., 2014b).

In preparation for viral aerosolization, two sterile BioSamplers were installed into a custom designed 1.4 m^3 bioaerosol chamber (Fig. 1). The BioSamplers were pre-loaded with 20 ml of 0.01 M phosphate buffered saline, pH 7.5 (PBS) and were co-located inside the bioaerosol chamber using retort stands. One of the samplers also contained 0.5 g of IRA-900 anion exchange resin (Polysciences, Warrington, PA, USA) suspended within the PBS. A SidePak™ AM510 Personal Aerosol Monitor (TSI Inc., Shoreview, MN) was centrally positioned inside the chamber to measure mass concentration (mg/m^3) of the bioaerosols. A Q-TRAK Indoor Air Quality Monitor 8554 (TSI Inc.) and Alnor Velometer Thermal Anemometer AVM440-A (TSI Inc.) were co-located near the samplers to measure and ensure consistency of environmental variables within the bioaerosol chamber, which included temperature, % relative humidity, carbon dioxide concentration, and wind speed (generated using axial fans). The chamber was then sealed and purged with HEPA-filtered air. The average per-trial temperature was 24.4 °C (instantaneous range: 22.7–25.7 °C); average relative humidity was 42.8% (instantaneous range: 27.6–55.2%); average carbon dioxide was 380 ppm (instantaneous range: 58–796 ppm); average per-trial wind speed was 0.06 m/s (instantaneous range: 0.01–0.12 m/s).

For each virus, 10-fold serial dilutions ($10^{-2.5}$ to $10^{3.5}$ FFU/ml for influenza viruses; 10^{-2} to 10^{6} pfu/ml for MS2) were prepared in 100 ml PBS to create the inocula used for aerosolization. Inocula were prepared within the glass vessel of a 6-jet collision nebulizer (BGI Incorporated, Waltham, MA). Filtered, dried air was delivered to the nebulizer, which operated at 6.9 kPa. Using the SidePak, 5 mg/m^3 of viral bioaerosols was generated (via nebulizer) in each chamber trial. The SidePak is calibrated using the respirable fraction of the ISO 12103 A1 test dust, which contains a particle size distribution ranging from 0 to 10 µm. Thus, the 5 mg/m^3 relative mass concentration only serves to establish consistency within and between trials. After the target concentration was reached, nebulization was stopped. An air sampling pump (Vac-U-Go, SKC Inc.) for each BioSampler was then operated at a flow rate of 12.5 L/min to actively sample the chamber atmosphere over 40 min. Bioaerosolization experiments were performed in triplicate for each virus and titer tested.

Nucleic acid isolation from the anion exchange resin and from 140 µl liquid samples (PBS contained within the BioSamplers with and without resin as well as the viral inocula used for bioaerosolization, both pre- and post-experiment) was achieved as reported previously (Perez-Mendez et al., 2014b). Quantitative real-time reverse transcription PCR (qRT-PCR) detection of influenza viruses (using CDC universal influenza A and influenza B oligonucleotides in multiplex) (Selvaraju and Selvarangan, 2010) and MS2 (Perez-Mendez et al., 2014b) was performed as previously described using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad laboratories, Hercules, CA, USA). The methods used for qRT-PCR data interpretation are reported in (Perez-Mendez et al., 2014b). qRT-PCR standard curves for each of the assays were generated using ten-fold serial dilutions of viral stocks ($10^{0.5}$–$10^{5.5}$ FFU/reaction and 10^{0}–10^{5} pfu/reaction for influenza viruses and MS2, respectively). Standard curves for the MS2 qRT-PCR were obtained from our earlier studies (Perez-Mendez et al., 2014b). Quantitative standard curves for type A and type B influenza viruses were generated using ten-fold serial dilutions of the FluMist Quadrivalent vaccine. Cycle thresholds (Cq) were plotted against the logarithm of FFU/reaction to conduct linear regression analysis ($y = mx + b$) in order to determine the correlation coefficient and slope. The standard curves for type A and type B influenza viruses are represented by the equations $y = -0.2777x + 7.8657$ and $y = -0.2748x + 7.7585$, respectively. Amplification efficiency (E) of the reaction was calculated using the formula $E = 10^{(-1/m)} - 1$, where m = slope of the regression lines of the standard curves. The amplification efficiency of the qRT-PCR for type A and type B influenza viruses was 90% and 88%, respectively. The correlation coefficient was ≥0.995 for all three qRT-PCR assays.

To assess the improvement in detection sensitivity afforded by the anion exchange resin within the BioSamplers' liquid, the abundance of RNA collected from BioSampler resin samples was directly compared to the corresponding BioSampler liquid samples (Table 1). These data are reported in Cq, where the difference in Cq (ΔCq) between these two samples indicates the improvement in detection sensitivity. In a qRT-PCR that is 100% efficient, a gain of one Cq corresponds to a 2-fold increase in target concentration. Thus, improvements in detection sensitivity facilitated by the anion exchange resin were estimated using the equation $2^{\Delta Cq}$. The average Cq of three experimental replicates for each condition was utilized in the calculation of ΔCq.

Compared to testing of the BioSampler liquid samples, the use of the anion exchange resin method improved qRT-PCR detection of MS2 by an average of 8.26×. The resin method allowed for reliable detection of MS2 when the inoculum concentration for nebulization was 10^{2} pfu/

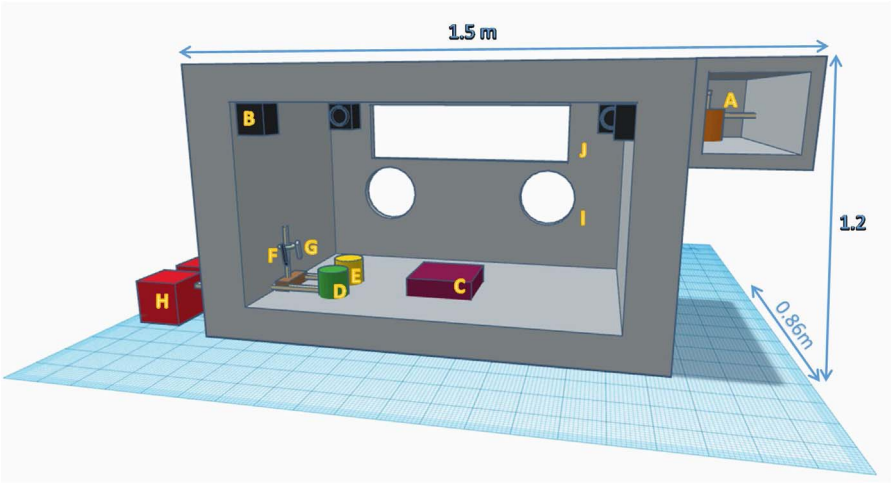

Fig. 1. Schematic and general operation of the custom bioaerosol chamber. (A) 6-jet Collison nebulizer was supplied with filtered and dried air at a generating pressure of 6.9 kPa (source not shown); (B) mixing fans were positioned in each corner of the chamber to facilitate aerosol dispersal and uniform mixing of the aerosol within the atmosphere of the chamber; (C) an aerosol monitor (SidePak AM510, TSI Inc.) was centrally located in the chamber to identify the relative concentrations of the aerosols specific to each trial; (D and E) BioSamplers (SKC Inc.) with and without resin; (F) thermal anemometer (Alnor® Velometer®, TSI, Inc.) was used to measure wind speed and (G) air quality monitor (Qtrak, TSI, Inc.) to measure other environmental variables (temperature, carbon dioxide, and percent relative humidity) inside the chamber; (H) active sampling pumps calibrated to 12.5 L/min; (I) pneumatically sealed glove ports for positioning sampling equipment within the chamber; and (J) an observation window.

ml, whereas detection in the corresponding BioSampler liquid samples was only achieved sporadically at this inoculum concentration. The improvement in MS2 detection afforded by the resin was generally consistent for all inocula concentrations in which viruses were detected in both the resin and liquid samples (10^3–10^6 pfu/ml).

Although MS2 was detected with 8.26× increased sensitivity in bioaerosols using the resin method, our previous experiments using resin for water sampling suggested that the improvement in sensitivity could approach 51× (Perez-Mendez et al., 2014b). The disparity in sensitivities between these two systems is partially explained by the fact that MS2 adsorption in PBS, which is recommended for impingement by the BioSampler manufacturer, is less efficient than in dechlorinated tap water (decreased sensitivity of approximately 2 Cq). A second consideration that may account for decreased sensitivity in the bioaerosol sampling system is that the integrity of MS2 could have been compromised by factors intrinsic to the nebulization or sampling

Table 1
Comparison of the mean viral detection achieved in BioSamplers with and without resin by qRT-PCR.

Viruses	Viral concentration (pfu/ml for MS2 and FFU/ml for influenza viruses) used in the inoculum of the nebulizer	BioSampler resin		BioSampler liquid		$2^{\Delta Cq}$ fold change in detection improvement afforded by anion-exchange resin
		Cq	SD	Cq	SD	
MS2	0	ND	NA	ND	NA	NA
	10^{-2}	ND	NA	ND	NA	NA
	10^{-1}	ND	NA	ND	NA	NA
	10^{0}	[a]39.88	NA	ND	NA	NA
	10^{1}	ND	NA	ND	NA	NA
	10^{2}	36.85	0.46	[a]38.26	NA	NA
	10^{3}	35.76	1.16	38.83	1.18	8.40
	10^{4}	30.05	0.56	33.10	0.03	8.28
	10^{5}	27.80	0.84	30.76	0.25	7.80
	10^{6}	24.28	0.44	27.38	0.16	8.57
Type A influenza viruses	0	ND	NA	ND	NA	NA
	$10^{-2.5}$	ND	NA	ND	NA	NA
	$10^{-1.5}$	ND	NA	ND	NA	NA
	$10^{-0.5}$	ND	NA	ND	NA	NA
	$10^{0.5}$	ND	NA	ND	NA	NA
	$10^{1.5}$	34.77	0.15	36.79	0.29	4.06
	$10^{2.5}$	31.01	0.33	33.75	0.47	6.69
	$10^{3.5}$	26.77	0.22	30.02	0.19	9.55
Type B influenza viruses	0	ND	NA	ND	NA	NA
	$10^{-2.5}$	ND	NA	ND	NA	NA
	$10^{-1.5}$	ND	NA	ND	NA	NA
	$10^{-0.5}$	ND	NA	ND	NA	NA
	$10^{0.5}$	[a]39.96	NA	ND	NA	NA
	$10^{1.5}$	36.80	0.34	38.15	0.72	2.56
	$10^{2.5}$	32.37	0.20	34.44	0.41	4.20
	$10^{3.5}$	28.16	0.04	30.96	0.32	6.98

ND, not detected; NA, not applicable; SD, standard deviation.
[a] One of three samples detected.

processes implemented here. Using a modified double agar overlay plaque assay described previously (Kropinski et al., 2009), the integrity of MS2 was assessed in the inocula used for nebulization and BioSampler liquid samples. Bacteriophage titers in both pre- and post-nebulized inocula were generally unchanged and were in agreement with qRT-PCR results. In contrast, only sporadic detection of MS2 was achieved in BioSampler liquid samples when the inocula concentrations were 10^6 pfu/ml or 10^5 pfu/ml, despite that qRT-PCR measurements indicated that approximately 10^4 and 10^3 pfu/ml of MS2, respectively, were present within these samples. Nevertheless, results obtained by Turgeon et al. (2014) indicated that the relative recovery of aerosolized MS2 collected within BioSamplers was on the same order of magnitude by both qRT-PCR and plaque assay when an impingement buffer consisting of 20 mM Tris–HCl, pH 7.5, 100 mM NaCl, 10 mM $MgSO_4$ was utilized. Thus, the formulation of an optimized impingement buffering system will be critical to achieve maximum sensitivity of the anion-exchange resin-based bioaerosol sampling method.

The anion exchange resin method also allowed for the improved qRT-PCR detection of type A and type B influenza viruses within BioSamplers compared to direct testing of the corresponding 140 µl liquid BioSampler aliquots. On average, the resin improved detection by 6.77× and 3.33× for type A and type B influenza viruses, respectively. In contrast to MS2, both type A and type B influenza viruses were detected more effectively at higher inoculum concentrations. Detection was improved by 9.55× and 6.98× for type A and type B influenza viruses, respectively, when the viral inoculum used for nebulization was at a concentration of $10^{3.5}$ FFU/ml. Each 10-fold dilution of the inocula decreased the detection sensitivity by an average of 2× for both type A and type B influenza viruses. Viral adsorption to surfaces has been suggested to function in a concentration-dependent manner, where the frequencies of impacts of viruses to adsorbent surfaces are more probable at higher viral concentrations (Gerba, 1984). However, in experiments to adsorb influenza viruses spiked directly into 50 ml of dechlorinated tap water, increased viral concentrations were correlated with diminished detection improvement across all concentrations tested ($10^{1.5}$–$10^{3.5}$ FFU/ml) (Table S2). Therefore, concentration-dependent changes in influenza detection sensitivity in the bioaerosol system are potentially an artifact of nebulization and/or the sampling system.

Viruses were detected from inocula samples both pre- and post-experiment with nearly equal efficiency (Table S1). While this metric indicated little change in viral RNA concentration over the course of experiments, it does not offer a direct evaluation of the nebulization process on viral stability. Nebulization is reported to have an impact on virus integrity. For example, decreases in viral titer between 100× and 1000× were reported for bacteriophage ϕ6 (a surrogate used for influenza viruses) following nebulization, whereas the impact of nebulization on MS2 is reportedly less substantial (Turgeon et al., 2014). Our experimental system was not directly capable of monitoring viral survival or stability following nebulization. Factors such as desiccation, impaction on the axial fans, adsorption to the bioaerosol chamber surface, or shearing during sampling may have had a deleterious impact on viral sampling or detection. An additional limitation to this study is that environmental conditions within the bioaerosol chamber was subject to some fluctuations. Despite these environmental fluctuations, only minor variations in the Cq between experimental replicates were observed (see Tables 1 and S1).

In conclusion, we have developed an anion exchange resin-based method for viral capture from bioaerosols that increased detection sensitivity for type A and type B influenza viruses as well as the bacteriophage MS2 (surrogate of enteric viruses). The method is simple to perform, can be adapted to existing aerosol sampling methods, is cost-effective, and allows for molecular detection of viral nucleic acids directly extracted from the anion exchange resin. Future work will focus on optimizing sampling parameters to minimize potentially adverse factors affecting lower than expected concentration/recovery efficiencies.

Acknowledgements

This work was supported by funding from the CDC/NIOSH High Plains Intermountain Center for Agricultural Health and Safety (5U54OH008085) and the Colorado Bioscience Discovery Evaluation Grant Program (14BGF-16).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jviromet.2017.04.004.

References

Alonso, C., Goede, D.P., Morrison, R.B., Davies, P.R., Rovira, A., Marthaler, D.G., Torremorell, M., 2014. Evidence of infectivity of airborne porcine epidemic diarrhea virus and detection of airborne viral RNA at long distances from infected herds. Vet. Res. 45, 73.

Anderson, B.D., Ma, M., Xia, Y., Wang, T., Shu, B., Lednicky, J.A., Ma, M.J., Lu, J., Gray, G.C., 2016. Bioaerosol sampling in modern agriculture: a novel approach for emerging pathogen surveillance? J. Infect. Dis. 214, 537–545.

Chandler, J.C., Perez-Mendez, A., Paar, J.R., Doolittle, M.M., Bisha, B., Goodridge, L.D., 2017. Field-based evaluation of a male-specific (F+) RNA coliphage concentration method. J. Virol. Methods 239, 9–16.

Cowling, B.J., Ip, D.K., Fang, V.J., Suntarattiwong, P., Olsen, S.J., Levy, J., Uyeki, T.M., Leung, G.M., Malik Peiris, J.S., Chotpitayasunondh, T., Nishiura, H., Mark Simmerman, J., 2013. Aerosol transmission is an important mode of influenza A virus spread. Nat. Commun. 4, 1935.

Gendron, L., Verreault, D., Veillette, M., Moineau, S., Duchaine, C., 2010. Evaluation of filters for the sampling and quantification of RNA phage aerosols. Aerosol Sci. Technol. 44, 893–901.

Gerba, C.P., 1984. Applied and theoretical aspects of virus adsorption to surfaces. Adv. Appl. Microbiol. 30, 133–168.

Greene, J.L., 2015. Update on the Highly-Pathogenic Avian Influenza Outbreak of 2014–2015. Congressional Research Service.

Haig, C.W., Mackay, W.G., Walker, J.T., Williams, C., 2016. Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies. J. Hosp. Infect. 93, 242–255.

Hermann, J.R., Hoff, S.J., Yoon, K.J., Burkhardt, A.C., Evans, R.B., Zimmerman, J.J., 2006. Optimization of a sampling system for recovery and detection of airborne porcine reproductive and respiratory syndrome virus and swine influenza virus. Appl. Environ. Microbiol. 72, 4811–4818.

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., Johnson, R.P., 2009. Enumeration of bacteriophages by double agar overlay plaque assay. Methods Mol. Biol. 501, 69–76.

Li, Y., Huang, X., Yu, I.T., Wong, T.W., Qian, H., 2005. Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. Indoor Air 15, 83–95.

Michen, B., Graule, T., 2010. Isoelectric points of viruses. J. Appl. Microbiol. 109, 388–397.

Pepper, I.L., Gerba, C.P., Gentry, T.J., 2015. Environmental Microbiology, 3rd ed. Elsevier/AP, Academic Press is an imprint of Elsevier, Amsterdam.

Perez-Mendez, A., Chandler, J.C., Bisha, B., Goodridge, L.D., 2014a. Concentration of enteric viruses from tap water using an anion exchange resin-based method. J. Virol. Methods 206, 95–98.

Perez-Mendez, A., Chandler, J.C., Bisha, B., Goodridge, L.D., 2014b. Evaluation of an anion exchange resin-based method for concentration of F-RNA coliphages (enteric virus indicators) from water samples. J. Virol. Methods 204, 109–115.

Selvaraju, S.B., Selvarangan, R., 2010. Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. J. Clin. Microbiol. 48, 3870–3875.

Tung-Thompson, G., Libera, D.A., Koch, K.L., de Los Reyes 3rd, F.L., Jaykus, L.A., 2015. Aerosolization of a human norovirus surrogate, bacteriophage MS2, during simulated vomiting. PLoS ONE 10, e0134277.

Turgeon, N., Toulouse, M.J., Martel, B., Moineau, S., Duchaine, C., 2014. Comparison of five bacteriophages as models for viral aerosol studies. Appl. Environ. Microbiol. 80, 4242–4250.

United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, 2015. Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: July 15, 2015 .

United States Environmental Protection Agency, 2000. Proposed Ground Water Rule .

Vergara, G.G., Goh, S.G., Rezaeinejad, S., Chang, S.Y., Sobsey, M.D., Gin, K.Y., 2015. Evaluation of FRNA coliphages as indicators of human enteric viruses in a tropical urban freshwater catchment. Water Res. 79, 39–47.

Verreault, D., Moineau, S., Duchaine, C., 2008. Methods for sampling of airborne viruses. Microbiol. Mol. Biol. Rev. 72, 413–444.